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Differential Fos Expression Following Aspiration, Electrolytic, or Excitotoxic Lesions of the Perirhinal Cortex in Rats

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The authors explored the possibility that there are different neural consequences, beyond the primary site of brain damage, following perirhinal cortex (PRh) lesions made in different ways. Fos expression was used as a marker for neuronal activation and compared across the forebrains of rats that underwent the different types of surgery. Electrolytic and excitotoxic PRh lesions produced dramatic increases in Fos expression in the cortex, and excitotoxic and aspiration PRh lesions increased Fos expression in the dentate gyrus. These data are consistent with the hypothesis that different lesion methods have separable effects on neural function in regions outside the lesion site that could account for inconsistencies in the literature regarding the behavioral effects of PRh lesions on tests of spatial memory.

Keywords: medial temporal lobe, immediate early genes, c-*fos*, neuronal activation, brain damage

Behavioral neuroscientists have made a tremendous contribution to the understanding of brain and behavior relations by making extensive use of the lesion method. Important advances in the knowledge base have been enriched and guided by observations of human patients that have sustained neural injury through disease, surgery, or trauma. However, only by selectively and repeatedly targeting specific brain areas in animals have scientists been able to clearly ascertain the neural substrates for a wide range of behaviors. Techniques used to ablate brain structures in rodents and nonhuman primates have undergone considerable transformation, and in recent decades technological advances have led to the development of more sophisticated, and allegedly more precise, tools with which to target discrete brain regions. For example, some newer methods entail the infusion of small amounts of excitotoxic substances into target regions, leading to the selective destruction of cell bodies. Contrast this technique with early methods of tissue aspiration or electrolytic surgery. These two latter examples could be used to target a particular brain region with some precision in the hands of a skilled user, but they both produce widespread tissue loss, destroying cell bodies and fibers of passage in the region damaged.

Recent research aimed at identifying the effects of lesions to the perirhinal cortex (PRh) on spatial memory in rats has revealed a somewhat surprising distinction between the behavioral outcome of aspiration and electrolytic lesions: Electrolytic lesions of the PRh impair performance on tasks that require spatial learning and memory (Liu & Bilkey, 1998a, 1998b; Mumby & Glenn, 2000; Wiig & Bilkey, 1994a, 1994b), whereas aspiration lesions of the PRh do not (Glenn & Mumby, 1996, 1998; Glenn, Nesbitt, & Mumby, 2003; Mumby & Glenn, 2000). This is surprising for two reasons. First, as stated above, both techniques produce gross tissue damage. Second, the aspiration lesions in these studies tended to be larger than the electrolytic lesions and more likely to include collateral damage to regions adjacent to the PRh. Thus, these two surgical methods can lead to differing amounts of brain damage, but this fails to account for the discrepant behavioral outcomes, as the rats with the larger aspiration lesions fared better on spatial tasks than the rats with the smaller, more discrete electrolytic lesions (see Glenn et al., 2003, for further discussion of this issue).

Despite numerous attempts to identify differences in experimental procedures that may account for discrepant findings in the literature, no critical features have been identified so far. For example, Glenn and Mumby have tested rats with aspiration PRh lesions on working and reference memory tasks (see Glenn & Mumby, 1998, vs. Mumby & Glenn, 2000), with and without presurgery training (Glenn & Mumby, 1998), and they have also used different intertrial intervals and compared massed and distributed training (Glenn & Mumby, 1997). In each of the cases described above, aspiration PRh lesions have not impaired spatial memory. In addition, Liu and Bilkey (2001) have suggested that differences in delay intervals or habituation procedures may contribute to the various effects of PRh lesions on spatial memory, but the impact of these variables on discrepancies in the literature is not yet clear.

In the present experiment, we investigated the possibility that different surgical techniques have neural consequences outside the primary site of brain damage. Both electrolytic and aspiration lesions produce widespread damage to brain tissue, but it is possible that they differ in the degree to which they disrupt processes elsewhere in the brain. The application of electrical current (1.5 mA for 10 s at five sites per hemisphere) along the extent of the PRh could produce an abnormal cascade of activation in structures

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efferent to the lesion site, such as the entorhinal cortex or hippocampal formation. These secondary effects, in addition to or even instead of the primary PRh damage, may be critical to the deficits in spatial memory seen in animals sustaining this type of injury. Aspiration lesions would not be expected to produce such widespread excitation, and the absence of such secondary effects may account for normal spatial learning observed following PRh aspiration lesions.

To address our hypothesis that electrolytic, but not aspiration, PRh lesions affect neural function beyond the site of injury, we compared the expression of the protein products of the immediate early gene, c-*fos*, as a marker of neuronal activation in rats that underwent electrolytic or aspiration PRh lesions. We also considered it judicious to include a group of rats with excitotoxic lesions of the PRh because this method is now commonly used and because a study by Liu and Bilkey (1998a) found that both electrolytic and excitotoxic lesions of the PRh in rats produce comparable deficits in spatial memory. We expected that the surgical techniques characterized by the application of either electrical current or excitotoxins would lead to hyperexcitation in brain regions efferent or adjacent to the target site. Such a finding would support the notion that electrolytic and excitotoxic surgeries have consequences for normal brain function, and subsequently behavior, beyond those resulting from the brain damage alone.

Table 1
Coordinates (in Millimeters) Relative to Bregma for Each of the Five Sites at Which Current Was Delivered During the Electrolytic Lesions of the PRh (PRh-Elec) and IML (Con-Elec) and the Five Sites at Which NMDA Was Infused During the Excitotoxic Lesions of the PRh (PRh-NMDA)

Lesion type	Anterior–posterior	Medial–lateral	Dorsal–ventral
PRh-Elec	3.5	8.5	9.2
	4.5	8.5	9.2
	5.5	8.5	9.2
	6.5	8.5	9.2
	7.5	8.5	8.4
Con-Elec	2.0	1.1	6.8
	2.8	1.1	7.2
	2.8	1.1	7.3
	3.0	1.1	7.3
	3.6	1.1	7.4
PRh-NMDA	3.3	5.4	9.2
	4.3	5.4	9.2
	5.3	5.4	9.2
	6.3	5.4	9.2
	7.3	5.4	8.2

Note. PRh = perirhinal cortex; IML = internal medullary lamina; NMDA = *N*-methyl-D-aspartate.

Method

Subjects

Twenty-one experimentally naive rats weighing between 300 and 350 g served as subjects in this experiment. Rats were singly housed in opaque cages and had free access to food and water throughout the experiment. The colony was maintained at 21 °C with a 12:12 light–dark cycle (lights on at 8 a.m.). All procedures were conducted during the light phase of the cycle.

Procedure

Surgery. Rats were assigned to one of five surgical groups: (a) bilateral aspiration lesions of the PRh ($n = 5$), (b) bilateral electrolytic lesions of the PRh ($n = 5$), (c) bilateral excitotoxic lesions of the PRh ($n = 3$), (d) bilateral electrolytic lesions of the internal medullary lamina (IML; $n = 3$; this group served as an electrolytic control group), or (e) sham surgery ($n = 5$).

Rats were anesthetized with pentobarbital (65 mg/kg). For the aspiration and electrolytic lesions of the PRh, a scalp incision was made, and the muscle overlying the temporal skull was displaced. A portion of skull overlying the PRh was removed using a hand-held dental drill. For the aspiration PRh lesion, tissue was aspirated using a glass pipette attached to a vacuum pump. For the electrolytic PRh lesion, a bipolar stainless steel electrode insulated with Teflon except for approximately 1 mm at the tip and angled at 10° to the vertical plane was used to deliver electric current (1.5 mA for 10 s) to five sites per hemisphere through the PRh. The coordinates for these sites are shown in Table 1.

The excitotoxic lesions of the PRh were made by infusing 0.4 μl *N*-methyl-D-aspartate (NMDA; Sigma Chemical, St. Louis, MO; 5.1 M in 0.1 M phosphate buffered saline, pH 7.4) at five sites through the PRh in each hemisphere, and the infusion cannulas were lowered from the dorsal surface and angled at 10° to the vertical plane. Infusions were made at a flow rate of 0.15 μl/min, and the coordinates for each of the five injection sites are shown in Table 1.

The electrolytic lesions of the IML were made using the same materials as described for the electrolytic PRh surgery. Current was applied at five sites per hemisphere, but the electrode was not angled. The coordinates for this surgery are shown in Table 1. Sham rats received a scalp incision only.

Immunocytochemistry. One hour after the completion of surgery, rats were given a supplemental dose of sodium pentobarbital (50% of the original 65 mg/kg dose) and were transcardially perfused with ice-cold saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were extracted and immersed in a 30% sucrose-paraformaldehyde solution for 48 hr. They were sectioned using a cryostat, and every fourth 30-μm coronal section from approximately 1 mm anterior to bregma to 8 mm posterior to bregma was retained.

Tissue sections were placed in Trizma-buffered saline (TBS; pH 7.3) and 24 hr later were processed for Fos-like immunoreactivity (Fos-lir). Each treatment group was represented in all assays to eliminate the confounding effects of interassay variability. The sections were first incubated for 30 min in a 3% H_2O_2 solution in TBS to reduce nonspecific staining. Following this, the sections were washed in TBS and incubated for 90 min in blocking serum consisting of 0.3% Triton X (Sigma Chemical) and 3% normal goat serum (Vector Laboratories, Burlington, Ontario, Canada) in TBS. Following further washing with TBS, the sections were incubated in a primary antibody solution for 48 hr. This solution consisted of a polyclonal antibody (Ab-5, PC-38, Oncogene Research Products, San Diego, CA) diluted 1:130,000. This antibody recognizes amino acids 4–17 of the human c-*fos* epitope. After this incubation, sections were washed in TBS and incubated in a secondary antibody solution (biotinylated rabbit anti-goat, Vector Laboratories) for 1 hr. The sections were then washed again in TBS and incubated in a tertiary, avidin-biotin complex solution (Vector Laboratories) overnight. Diaminobenzidine (nickel intensified) was used for visualization of Fos-lir. Stained sections were mounted on gelatin-coated slides and coverslipped for microscopic analysis.

Image analysis. Per the exploratory nature of this study, each section of brain tissue was examined microscopically for patterns of Fos-lir. It was immediately evident that the brains of rats that underwent electrolytic and NMDA PRh surgery displayed a substantial amount of Fos-lir cells in many areas. However, to maintain the manageability of the analysis, seven regions were selected for closer examination. According to our hypothesis

we were primarily interested in the hippocampal formation and entorhinal cortex. However, our initial examination of tissue revealed marginal Fos-lir within the hippocampal formation. There were detectable Fos-lir cells in the dentate gyrus, but few or no Fos-lir cells were observed in the Ammon's horn CA cell fields of the hippocampus proper. For this reason, only the dentate gyrus was selected for further analysis. The preliminary examination also revealed a substantial number of Fos-lir cells in cortical regions. Thus, the remaining six areas examined were the entorhinal, temporal, parietal, retrosplenial agranular and granular, and frontal cortices. Figures 1 and 2 (top left and top panels, respectively) show the location of each of the seven areas investigated on coronal sections of the rat brain.

To obtain an estimate of the number of Fos-lir cells, a rectangular 600 × 300 μm selection was made within each region (see Figures 1 and 2) and the numbers of cells expressing Fos-lir within that area were counted using National Institutes of Health Image public domain software (Scion Image Beta 3b, Scion Corporation, Frederick, MD). Three sections through each region were analyzed, and the rectangular selection was made in approximately the same position for all sections, within and across experimental conditions. The numbers from each section were averaged for each region for each rat. To adjust for differences in background levels, a density criterion was established by first examining sections from each rat and obtaining density values for all cells judged to be showing Fos-lir. The average of those density values then became the density criterion used for all sections across all animals. Unfortunately, because of the tissue damage it was not possible for cell counters to be blind to the group assignment. However, the differences between groups were usually so large that it is unlikely that experimenter expectations could have influenced the results.

Statistical analyses. The final averages were used to calculate the mean and *SEM* for each of the five lesion groups. These values are displayed in Figures 1 and 2. One-way between-subjects analyses of variance (ANOVAs) were used to compare the average numbers of Fos-lir cells between the groups for each region examined. Follow-up analyses to significant ANOVAs consisted of pairwise comparisons using Tukey's honestly significant difference tests. In one instance (dentate gyrus analysis), we obtained a nonsignificant ANOVA result and instead conducted planned comparisons of each experimental group with the sham group only. Significance level for all statistical tests was set at .05.

Results

Figure 3 shows the extent of damage produced by the different surgeries. In Figures 1–2 and 4, and in each of the following sections, letter designations from A to G are used to denote each of the seven regions examined for Fos-lir. Figure 4 shows photomicrographs of a representative section from each of the five surgical conditions for each of the seven regions examined and described below. Figure 1 shows the approximate location of the region analyzed in the hippocampal formation and entorhinal and

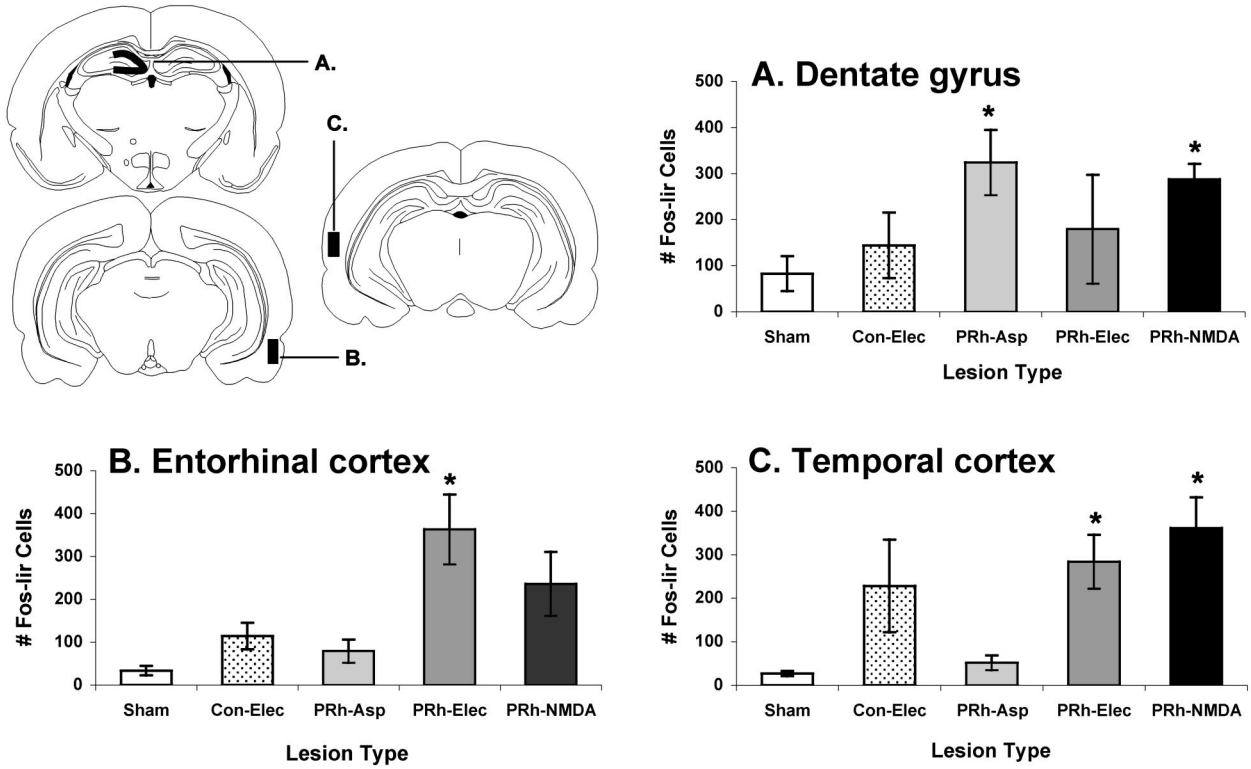

Figure 1. The top left-hand panel shows the region of analysis for each of the figures shown. Graphs show the mean numbers of cells showing Fos-like immunoreactivity (Fos-lir) for each of the five surgical conditions. Error bars represent *SEM*. * $p < .05$. A. dentate gyrus (* compared with sham surgery [Sham]). B. entorhinal cortex (* compared with Sham; bilateral aspiration lesions of the perirhinal cortex [PRh-Asp]; and control, electrolytic lesions of the internal medullary lamina [Con-Elec]). C. temporal cortex (* compared with Sham and PRh-Asp). PRh-Elec = electrolytic lesions of the perirhinal cortex; PRh-NMDA = excitotoxic lesions of the perirhinal cortex. Regions of analysis reprinted from *The Rat Brain in Stereotaxic Coordinates*, 4th ed., G. Paxinos and C. Watson, Copyright 1998, with permission from Elsevier.

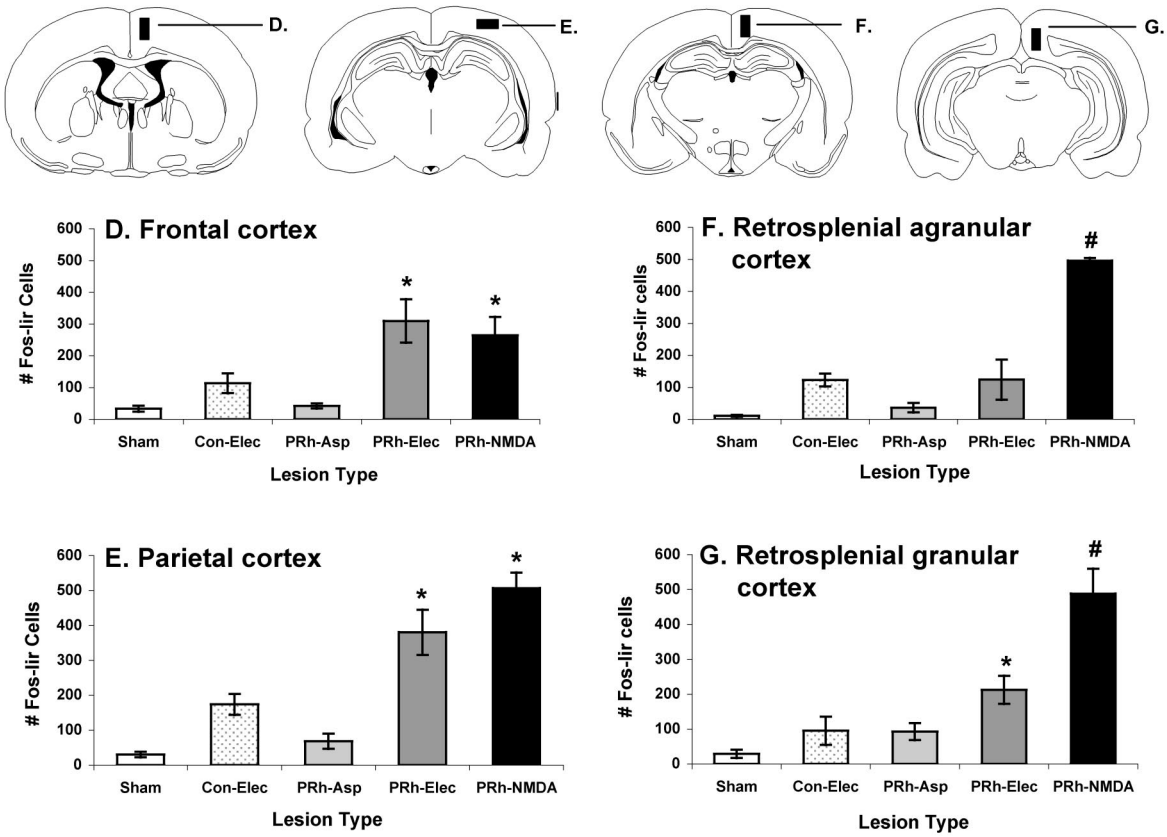

Figure 2. The top panel shows the regions of analysis for each of the figures shown. Graphs show the mean numbers of cells showing Fos-like immunoreactivity (Fos-lir) for each of the five surgical conditions. Error bars represent *SEM*. * $p < .05$. D. frontal cortex (* compared with sham surgery [Sham] and bilateral aspiration lesions of the perirhinal cortex [PRh-Asp]). E. parietal cortex (* compared with Sham; PRh-Asp; and control, electrolytic lesions of the internal medullary lamina [Con-Elec]). F. retrosplenial agranular cortex (# compared with all other groups, $p < .05$). G. retrosplenial granular cortex (# compared with all other groups, $p < .05$; * compared with Sham). PRh-Elec = electrolytic lesions of the perirhinal cortex; PRh-NMDA = excitotoxic lesions of the perirhinal cortex. Regions of analysis reprinted from *The Rat Brain in Stereotaxic Coordinates*, 4th ed., G. Paxinos and C. Watson, Copyright 1998, with permission from Elsevier.

temporal cortices and the average numbers of Fos-lir cells detected in these regions. Figure 2 shows the approximate location of the region analyzed in the frontal, parietal, and retrosplenial agranular and granular cortices and the average numbers of Fos-lir cells detected in these regions.

A. Dentate Gyrus

The sections used in this analysis were between 2.5 and 3.5 mm posterior to bregma. For this region only, a rectangular selection was not used. Rather, a free-tool selection around the dentate gyrus was made. The results from the ANOVA indicated that the groups were not significantly different, $F(4, 16) = 2.235$, $p = .111$. However, planned comparisons revealed that rats with PRh aspiration lesions ($p = .014$) and rats with NMDA PRh lesions ($p = .004$) displayed significantly more Fos-lir cells in the dentate gyrus than rats that had sham surgery. The other lesion groups were not significantly different from the sham group (all ps $> .05$).

B. Entorhinal Cortex

The sections used in this analysis were between 5.5 and 6.0 mm posterior to bregma. The results from the ANOVA indicated that there were statistically significant differences among the groups, $F(4, 16) = 7.298$, $p = .002$. Post hoc Tukey's tests revealed that electrolytic PRh lesions induced more Fos-lir in the entorhinal cortex than sham lesions ($p = .002$). The remaining groups were not significantly different from the sham group (ps $> .05$). There were significantly more Fos-lir cells in the entorhinal cortex following electrolytic PRh lesions than following aspiration PRh lesions or electrolytic control lesions (ps = .006 and .043, respectively). No other pairwise comparisons were statistically significant (ps $> .05$).

C. Temporal Cortex

The sections used in this analysis were between 5.5 and 6.0 mm posterior to bregma. The results from the ANOVA indicated that

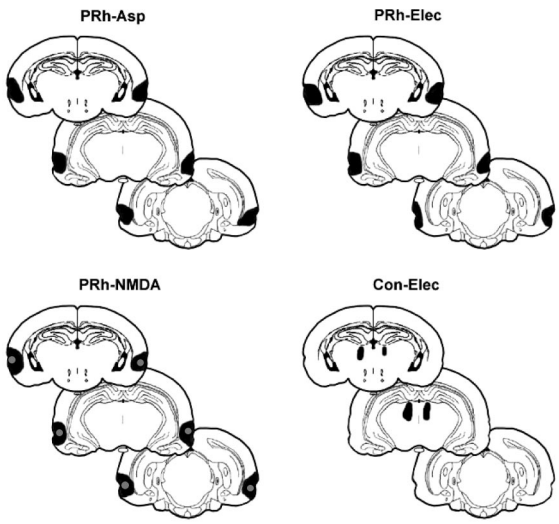

Figure 3. Schematic diagrams showing the extent of tissue damage produced by the aspiration, electrolytic, or excitotoxic lesions of the perirhinal cortex (PRh-Asp, PRh-Elec, and PRh-NMDA, respectively) and the control, electrolytic lesions of the internal medullary lamina (Con-Elec) at each of three coronal planes: 3.8, 5.3, and 6.8 mm behind bregma. For PRh-Elec, the depicted region does not represent total tissue loss but covers the range of damaged tissue that was detected. For PRh-NMDA, the gray circular region shows the approximate location of the end of the cannulas tract that was visible in the tissue. Diagrams are adapted on the basis of *The Rat Brain in Stereotaxic Coordinates*, 4th ed., G. Paxinos and C. Watson, Copyright 1998, with permission from Elsevier.

there were statistically significant differences among the groups, $F(4, 16) = 7.857$, $p = .001$. Tukey's tests revealed that electrolytic and NMDA PRh lesions led to more Fos-lir cells in the temporal association cortex relative to sham lesions (ps = .011 and .003, respectively). In addition, both the electrolytic PRh lesion and the NMDA PRh lesion resulted in more Fos-lir cells than did the aspiration PRh lesion (ps = .024 and .008, respectively). No other pairwise comparisons were statistically significant (ps > .05).

D. Frontal Cortex

The sections used in this analysis were between 0.5 and 1.0 mm posterior to bregma. The results from the ANOVA indicated that there were statistically significant differences among the groups, $F(4, 16) = 9.786$, $p = .001$. Tukey's tests revealed that electrolytic and NMDA PRh lesions led to more Fos-lir cells in the frontal cortex relative to sham lesions (ps = .001 and .016, respectively). In addition, both the electrolytic and NMDA PRh lesions resulted in more Fos-lir cells than did the aspiration PRh lesion (ps = .001 and .021, respectively). Furthermore, the electrolytic PRh lesions resulted in more Fos-lir cells in this region than did the electrolytic control lesions ($p = .048$). No other pairwise comparisons were statistically significant (ps > .05).

E. Parietal Cortex

The sections used in this analysis were between 4.0 and 4.5 mm posterior to bregma. The results from the ANOVA indicated that there were statistically significant differences among the groups, $F(4, 16) = 24.189$, $p = .001$. Tukey's tests revealed that electrolytic and NMDA PRh lesions led to more Fos-lir cells in the parietal cortex relative to sham lesions (ps = .001). In addition, both the electrolytic and NMDA PRh lesions resulted in more Fos-lir cells than did the aspiration PRh lesion (ps = .001) or the electrolytic control lesions (ps = .027 and .001, respectively). No other pairwise comparisons were statistically significant (ps > .05).

F. Retrospenial Agranular Cortex

The sections used in this analysis were between 3.5 and 4.0 mm posterior to bregma. The results from the ANOVA indicated that there were statistically significant differences among the groups, $F(4, 16) = 24.046$, $p = .001$. Tukey's tests revealed that NMDA PRh lesions led to more Fos-lir cells in this region relative to all other groups (all ps < .001). No other pairwise comparisons were statistically significant (ps > .05).

G. Retrospenial Granular Cortex

The sections used in this analysis were between 5.5 and 6.0 mm posterior to bregma. In this case, both the retrosplenial granular a and b cortex were examined. The same pattern of results were obtained for both regions; therefore, cell counts from all six selections were averaged. The results from the ANOVA indicated that there were statistically significant differences among the groups, $F(4, 16) = 24.91$, $p = .001$. Tukey's tests revealed that NMDA PRh lesions led to more Fos-lir cells in this region relative to all other groups (all ps < .001). Electrolytic PRh lesions also led to more Fos-lir cells relative to sham lesions ($p = .004$). No other pairwise comparisons were statistically significant (ps > .05).

Discussion

The main findings of this experiment are that electrolytic and excitotoxic lesions of the PRh led to dramatic increases in Fos-lir throughout the cortex that were not seen following PRh aspiration or control electrolytic lesions or in rats that had sham surgery. Excitotoxic PRh lesions, in particular, increased Fos expression relative to sham lesions in every rat and area examined. Electrolytic PRh lesions increased Fos expression in the cortex but not in the dentate gyrus. Conversely, aspiration PRh lesions increased Fos expression in the dentate gyrus but not in the cortex. In general, there was increased Fos expression following the electrolytic control lesions of the IML relative to the sham surgery. However, the electrolytic lesions of the IML produced much less activation overall than the electrolytic lesions of the PRh. This finding suggests that the location of an electrolytic lesion has an effect on which brain areas are activated and to what extent.

Both electrolytic and aspiration lesions result in gross tissue damage, destroying both cell bodies and fibers of passage. Typically, there are differences in the extent of damage to structures outside of and adjacent to the PRh following the two lesion methods; however, aspiration PRh lesions tend to be larger and include more damage to the adjacent postrhinal and entorhinal cortices, the hippocampal formation, and the amygdala than electrolytic PRh lesions. It seemed unlikely that larger, less selective lesions would produce fewer deficits in learning and memory, as is

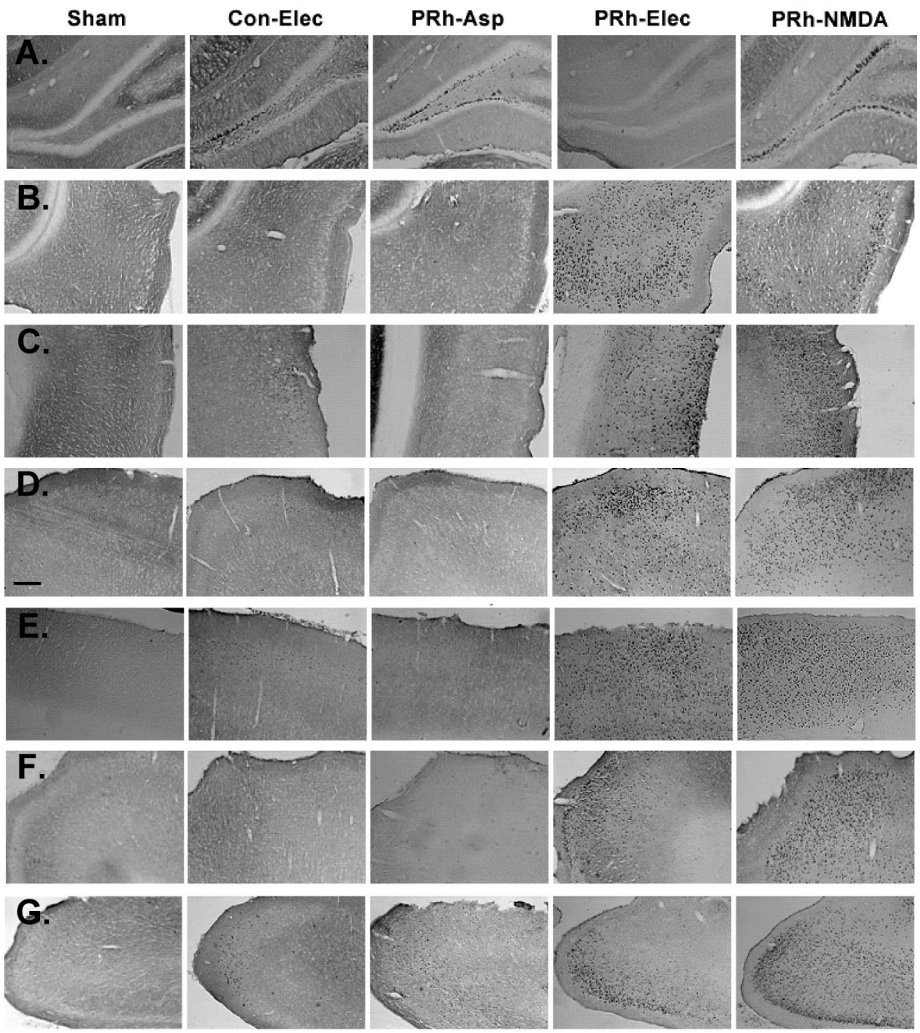

Figure 4. Photomicrographs showing Fos expression in a representative coronal section from each of the five surgical conditions (as indicated, top) in each of the following seven areas analyzed: A. dentate gyrus, B. entorhinal cortex, C. temporal cortex, D. frontal cortex, E. parietal cortex, F. retrosplenial agranular cortex, G. retrosplenial granular cortex. Scale bar shown in D. applies to all images and represents 100 μm.

evident in the literature (see Glenn & Mumby, 1998; Glenn et al., 2003; and Mumby & Glenn, 2000, compared with Liu & Bilkey, 1998a, 1998b). Instead, we hypothesized that the electrolytic technique itself may have adverse consequences for the function of brain regions outside of the PRh (Glenn et al., 2003); applying repetitive electrical currents through the extent of the PRh may initiate an anomalous and possibly detrimental pattern of activation in regions efferent to the lesion site, such as the hippocampal formation or entorhinal cortex.

The results of the present experiment are partially consistent with this hypothesis. Electrolytic PRh lesions increased activation in other brain regions. However, contrary to our expectations, increased activation was not observed in the hippocampal formation, and instead, the activation was present throughout the cortex. Excitotoxic PRh lesions increased activation throughout the cortex as well as in the hippocampal formation. This lesion technique may affect normal function in other brain regions in a manner similar to that of electrolysis, as both techniques are characterized by hyperexcitation of cells in the target region, which could consequently trigger a cascade of activation through nearby regions. Aspiration PRh lesions did not increase activation through the cortex, providing compelling support for this interpretation. In addition, aspiration PRh lesions tend to be larger than electrolytic or excitotoxic lesions, yet, overall, we observed the fewest numbers of Fos-lir cells following the aspiration PRh lesions. This suggests that the degree of Fos activation cannot be attributed to the extent of damage; it is more likely that the surgical method itself contributed to the observed differences in neuronal activation.

Unexpectedly, aspiration PRh lesions did lead to increased activation in the dentate gyrus. It should be noted that PRh lesions, produced using any of the three techniques examined in the present study, do not produce spatial memory deficits that resemble those following lesions of the hippocampal formation (cf. Glenn et al., 2003; Mumby, Astur, Weisend, & Sutherland, 1999; Mumby &

Glenn, 2000; see also Bussey, Duck, Muir, & Aggleton, 2000; Ennaceur, Neave, & Aggleton, 1996; Liu & Bilkey, 1998a, 2001; Winters, Forwood, Cowell, Saksida, & Bussey, 2004). Combined, these findings suggest that PRh damage impairs spatial memory in a manner that is unique from the hippocampal contribution to this ability. Thus, the tendency for PRh aspiration lesions to spare spatial memory and the distinct way in which other types of PRh lesions impair spatial memory suggest abnormal activation of the hippocampal formation is contributing little to the behavioral impact of these lesions. An alternative possibility is that spatial memory deficits observed following PRh lesions made in different ways could result from different types of pathology, not all of which involve hippocampal dysfunction. Accordingly, of the other regions selected for analysis in the present experiment, the retrosplenial and parietal cortices are both considered important to normal place memory (Cooper, Manka, & Mizumori, 2001; DiMattia & Kesner, 1988; Harker & Wishaw, 2002; Vann & Aggleton, 2002). The temporal association cortex has been implicated in visual processing and is thought to be important for feature detection and discrimination (Buffalo et al., 1999; Buffalo, Stefanacci, Squire, & Zola, 1998; see also Murray, Bussey, Hampton, & Saksida, 2000). The entorhinal cortex is the primary source of neocortical input to the hippocampal formation. In addition, these polymodal association cortices are important sites for the integration and processing of sensory information. Thus, disturbing function in any or all of these regions could adversely affect the ability of animals to acquire and recall object and/or place information.

In the present experiment, rats were killed by transcardial perfusion 1 hr after surgery, and the use of Fos as a marker for neuronal activation enabled us to determine which brain structures were activated soon after the different types of surgery. Detecting differential patterns of neuronal activation at this time has implications for studies of retrograde amnesia as certain neurological responses separate from or in addition to the lesion itself could affect consolidation processes that occur within a few days of a learning event. However, these data do not provide information about the longer term impact of the different surgeries that may adversely affect performance on anterograde learning and memory tests that are typically conducted 2 weeks after surgery. For this reason, it would be beneficial for future research to examine other markers of neuronal function to characterize the time course of changes following the different types of surgery. Markers of cell death may provide pertinent information about whether the fate of cells in regions of increased activation is changed following electrolytic and excitotoxic lesions. It may also be informative to assess whether there are long-term changes in neurochemical profiles in other brain regions.

Fos expression as a marker of neuronal activation is a versatile tool for evaluating discrepant responses to a manipulation. However, what is not revealed is the nature and consequence of the response. It is furthermore not possible to use this technique to detect the suppression of neuronal responses. Clearly, there are a variety of undetected effects that could be occurring in any or all of the surgical groups. As c-*fos* is only one of several immediate early genes, it may be possible to obtain a more comprehensive assay of neural changes following different types of surgeries by comparing Fos activation with markers for other, related genes, such as *c-jun* or *zif268*.

The present experiment was a first and necessary step to identify the preliminary effects of various lesion methods, and solely on the basis of this information, it can be concluded that electrolytic and NMDA PRh lesions have neurological consequences that differ from those of aspiration PRh lesions. In general, these findings underscore the importance of remembering that even "selective" brain damage has consequences that may not be readily observable. Thus, they have implications for any study that seeks to attribute postsurgical effects of a lesion to the brain region where the damage is most obvious. More specifically, these findings provide compelling evidence that the use of different lesion techniques may have contributed substantially to inconsistencies in the behavioral effects of PRh lesions that are evident in the literature.

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