The Contribution of the Perirhinal Cortex to Anterograde and Retrograde Memory for Objects and Places in Rats

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ABSTRACT

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The present thesis examined the effects of bilateral perirhinal cortex (PRh) lesions on rats' anterograde and retrograde memory for places and objects. Allocentric place memory was assessed using a standard water maze task and a test of novelty preference, which relies on the ability of rats to detect that an object has moved to a new location. Object-recognition memory was also assessed using a test of novelty preference, which, in this case, relies on the ability of rats to detect that an object is novel. The effects of aspiration and electrolytic PRh lesions were compared on most tests. Findings from the object-recognition tests suggest that the PRh is necessary to support anterograde, but not retrograde, object recognition. By contrast, findings from the place memory tests suggest that the functions of the PRh are important for retrograde, but not anterograde, place memory. Furthermore, on most tests, electrolytic PRh lesions were more effective than aspiration PRh lesions in revealing performance deficits. Evidence that electrolytic, but not aspiration, PRh lesions led to widespread neuronal activation in cortex is also presented and suggests that this lesion method may have adverse consequences for normal brain function that go beyond the functional effects of damage to the PRh. Current views of medial-temporal-lobe function are discussed and it is concluded that few models adequately capture the complex contribution of the PRh to learning and memory that is indicated by the present data.

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For Charlotte

"When you expose your stupidity, you give yourself the chance to have it caught, corrected, and replaced with wisdom."

Ender Wiggin

Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

The ability of all animals to learn and remember information shapes their behaviour and is vital to survival. The neurobiological bases of learning and memory have been extensively investigated, but a clear understanding of how information is acquired and memories are formed remains elusive. Much of our knowledge is derived from case studies of human patients with indiscriminate brain damage and animal experiments in which specific brain structures are ablated. Human cases provide insight into the types of deficiencies in learning and memory that occur following brain damage, but only animal models provide the opportunity to systematically investigate the contribution of specific brain structures to performance on tasks that are designed to mimic some of the abilities that are impaired in humans with brain damage. The main focus of this thesis is the contribution of the perirhinal cortex (PRh) to learning and memory about objects and places in rats.

The PRh is located in the medial temporal lobe (MTL) of the primate brain. The importance of the MTL to learning and memory was revealed when, in 1957, Scoville and Milner reported learning deficits and memory loss in human patients who had undergone surgical removal of this region (Scoville & Milner, 1957). These findings stimulated the development of animal models that relied mostly on a test of object-recognition memory: delayed nonmatching-to-sample (DNMS). Initially, amnesia was modelled in monkeys. Lesion-based studies were also being conducted with rats but were primarily aimed at delineating the neural substrates of memory and, in contrast to the emphasis on object-recognition as an index of memory in monkeys, spatial memory was extensively examined in

rats. These lines of research did not converge until recently and the experiments described in this thesis are a further attempt to integrate them.

The MTL consists of the hippocampal formation (HPC), the amygdala, and the overlying rhinal cortices. HPC in the present thesis refers to the CA cell fields, the dentate gyrus, and the subiculum. The rhinal cortices include the PRh, entorhinal, and postrhinal cortices. In monkeys and humans, large lesions of the MTL that included all these structures resulted in a marked and enduring impairment in DNMS performance (Mahut, Moss, & Zola-Morgan, 1981; Murray & Mishkin, 1984; Squire & Zola-Morgan, 1983). However, lesions restricted to either the HPC and the overlying cortex or the amygdala and the overlying cortex did not produce as severe a DNMS impairment in monkeys as large MTL lesions (Mishkin, 1978; Saunders, Murray, & Mishkin, 1984). These findings led researchers to hypothesize that the functions of the MTL were only fully compromised if both the HPC and amygdala were destroyed (e.g. Mishkin, 1978; 1982).

The rhinal cortices overlying the HPC and amygdala were viewed as necessary conduits through which these structures communicated with the neocortex (Murray & Mishkin, 1986); they were not believed to have memory functions. Therefore, the collateral damage to this region was not considered problematic for interpreting the effects of HPC and amygdala damage. However, subsequent studies revealed that complete PRh damage in the absence of HPC or amygdala damage resulted in DNMS deficits as severe as those following larger MTL lesions (Gaffan & Murray, 1992; Meunier, Bachevalier, Murray, & Mishkin, 1993; Zola-Morgan, Squire, Amaral, & Suzuki, 1989).

It is now widely accepted that the PRh is an essential component in the MTL memory system. However, recent findings that the functions of the PRh and HPC can be doubly dissociated on the basis of the type of information to be processed (e.g. Glenn & Mumby,

1996) are inconsistent with the notion that these structures are components in a unitary MTL memory system. It is possible that the PRh is a vital part of a separate system that has functions distinct from those of a HPC system. Yet, the contribution of the PRh to the type of information processing thought to rely on the HPC (e.g. place memory) is not well understood. Thus, a major aim of the present thesis was to more fully characterize the effects of PRh lesions on place memory. It is also not clear what role the PRh may play in the long-term formation of memories. Therefore, a second major aim was to examine the contribution of the PRh to the acquisition and long-term retention of place and object information.

The following sections describe the emergence of the PRh as key structure in neurobiological research of learning and memory. In Section 1.1, the anatomy and connectivity of the MTL is described. The anatomical features of the MTL region have factored significantly in functional models of learning and memory, and an understanding of the input and output of the PRh is essential to the formulation of predictions regarding its functions. Section 1.2 describes the general features of the human amnesic syndrome. The importance of MTL structures to memory originated with case studies of human patients with damage to this region. Based on these reports, animal models were developed and Section 1.3 reviews the ways in which non-human animals have been used to study memory. Section 1.4 discusses current views of PRh function, highlighting relevant empirical findings from lesion, electrophysiological, and neuronal activation experiments. Section 1.5 states the main questions addressed in this thesis and summarizes the rationale for them.

1.1 Anatomy of the Medial Temporal Lobe

The primary source of neocortical input to the HPC is through the rhinal cortices. Recent anatomical studies have revealed that the patterns of afferent and efferent projections

to and from individual rhinal cortices are distinct. This suggests that these structures may have unique functions and may contribute to learning and memory in ways that are different from each other and the HPC.

1.1.1 Location and Boundaries of the PRh

Figure 1 depicts the location of the PRh and adjacent cortical areas on the lateral surface of the rat brain. The PRh is comprised of Brodmann's areas 35 and 36 (Brodmann, 1909; as cited in Burwell, Witter, & Amaral, 1995. The boundaries of the PRh in the monkey brain are generally well defined (Suzuki, 1996; Suzuki & Amaral, 1994). The boundaries of the PRh in the rat brain are more difficult to identify due to the lack of cytoarchitectural data (Burwell, Witter, & Amaral, 1995; Witter, 1993), but the recent work of Burwell and her colleagues has led to generally accepted boundaries of the PRh and postrhinal cortex in rats which serve as the basis for PRh location and connectivity in the present thesis.

The PRh occupies the dorsal and ventral banks, as well as the fundus of the rhinal sulcus in both rats and monkeys (Burwell et al., 1995; Suzuki & Amaral, 1994; Suzuki, 1996). In rats, the PRh is mainly situated along the caudal portion of the rhinal sulcus (Burwell et al., 1995). Burwell (2001) places the rostral border of the PRh at approximately 2.8 millimetres behind Bregma, and the caudal border at approximately 7.8 millimetres behind Bregma.

The postrhinal cortex is situated at the caudal border of the PRh in rats. This region is considered analogous to areas TF and TH (collectively termed parahippocampal cortex: Burwell et al., 1995; Suzuki, 1996), which occupy the posterior portion of the parahippocampal gyrus in the monkey brain (Amaral, Insausti, & Cowan, 1987; Burwell et al., 1995). The posterior insular cortex is located at the rostral border of the PRh (Burwell, 2001; Burwell et al., 1995) and the entorhinal cortex spans its entire ventral border (Burwell,

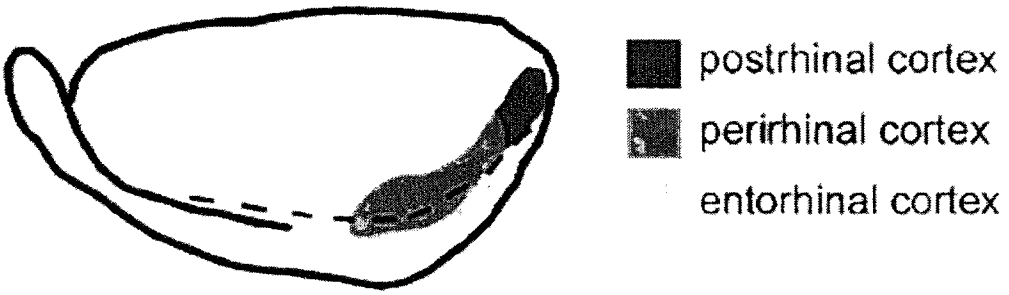


Figure 1. Lateral view of the rat brain showing the location of the PRh. Also shown are the boundaries of the postrhinal and entorhinal cortices (see Legend). Location and boundaries of these structures are adapted from Burwell, 2001.

2001). Polymodal temporal association cortices are situated dorsal to the PRh (Burwell et al., 1995).

Figure 2 shows the location of the PRh and other MTL structures on three coronal sections of the rat brain. The CA1 cell field and the subicular complex of the HPC are situated medial to the rostral portions of the PRh, and the amygdalar complex is situated medial to the caudal portions of the PRh.

Both the PRh and postrhinal cortex are sites of convergence for highly processed sensory information arising from neocortical areas and have substantial efferent projections to the entorhinal cortex. The interconnections between the entorhinal cortex and the HPC are well documented (for review see Witter, 1993). Briefly, projections from the entorhinal cortex travel through the perforant path and target all CA cell fields, the dentate gyrus, and the subiculum (Steward, 1976; for review see Amaral & Witter, 1989; Witter, 1993). Projections to the entorhinal cortex from the HPC originate from both the CA1 cell field and the subiculum; these inputs arise from the entire extent of the HPC and terminate throughout the extent of the entorhinal area (Amaral & Witter, 1989; Witter, 1992).

In the following sections the afferent and efferent projections of the PRh will be described. It is important to describe what is known about the patterns of connectivity in this region to make predictions regarding the mnemonic functions of the PRh. Unless otherwise indicated, all anatomical details reported are based on descriptions by Burwell (2000; 2001) and Burwell and Amaral (1998a; 1998b).

1.1.2 Afferent projections of the PRh

Figure 3 shows the predominant afferent projections of the rhinal cortices in the rat. In rats and monkeys, the PRh, postrhinal and entorhinal cortices receive input from more than

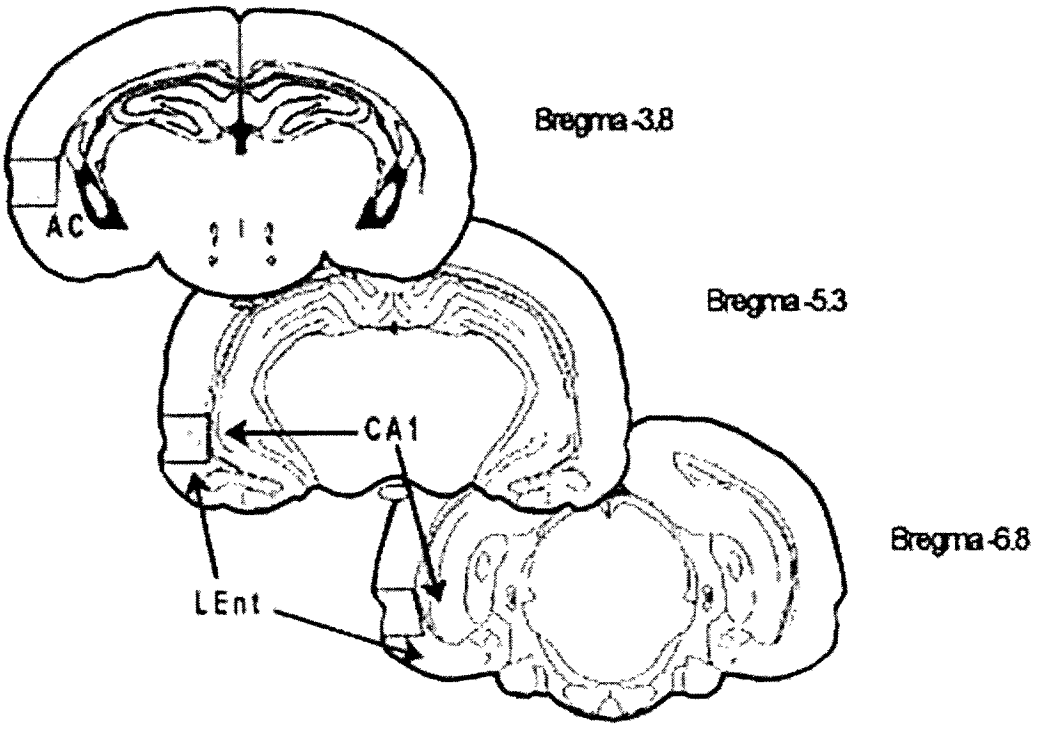


Figure 2. Coronal view of the rat brain at three positions relative to bregma (mm, as indicated). The approximate location of the PRh is shown in grey. The relative position of the amygdaloid complex (AC) is shown in the top section, and the relative locations of the lateral entorhinal cortex (LEnt) and the CA1 cell field of the HPC are indicated on the bottom two sections. Images are adapted from Paxinos and Watson (1986).

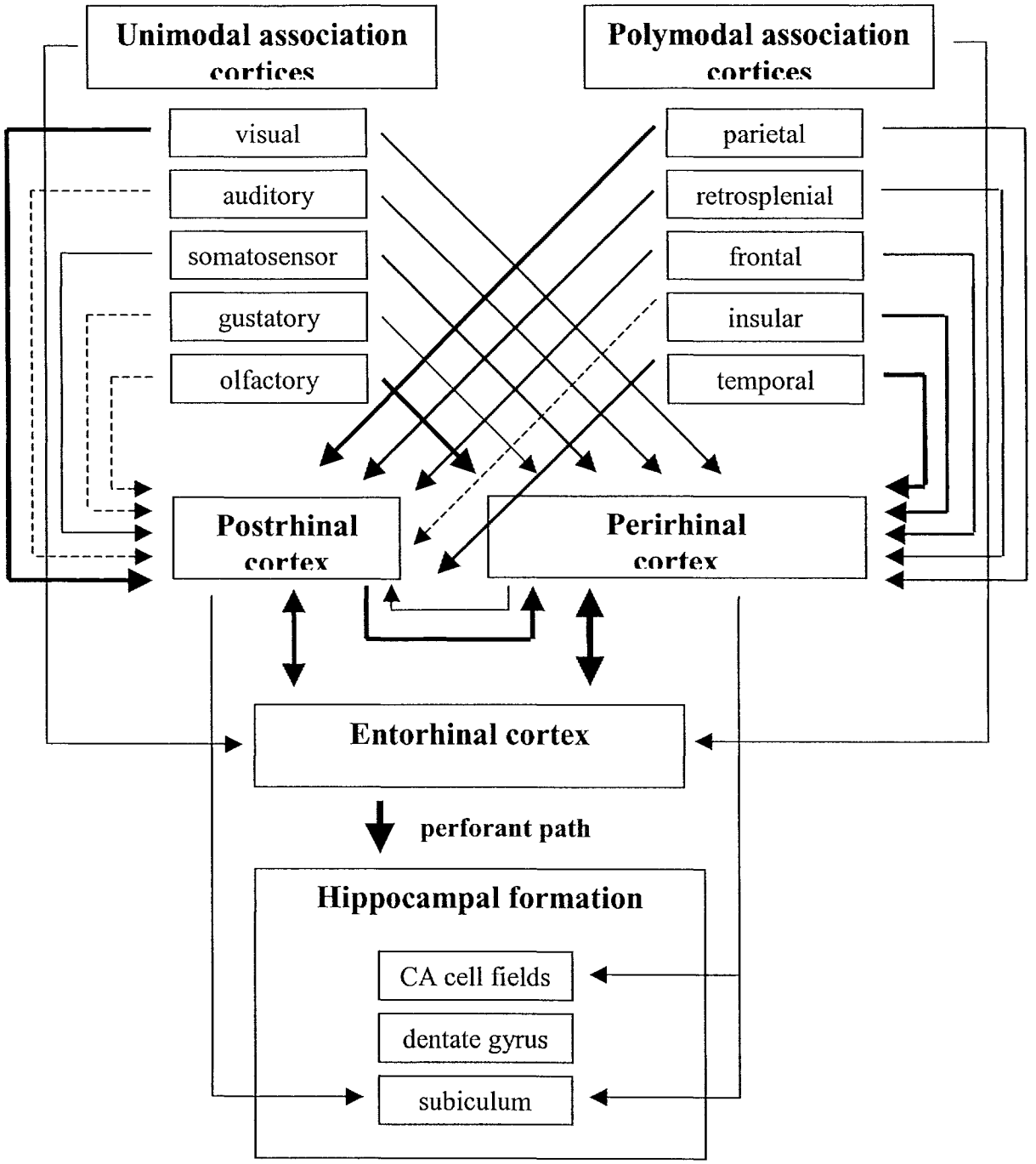


Figure 3. Schematic diagram showing the major afferent and efferent projections of the PRh and related MTL structures. Projection density is approximated by arrow widths.

one unimodal sensory cortical area as well as other polymodal association cortices and thereby satisfy the criterion for polymodal association cortex (Burwell, 2001; Burwell et al., 1995; Suzuki, 1996). Also similar for both species is the pattern of interconnections between the PRh and postrhinal cortex. There is more substantial input of polymodal information to the postrhinal cortex than to the PRh and the postrhinal cortex is a major source of polymodal input to the PRh, but the PRh returns sparse projections to the postrhinal cortex.

A major difference between rats and monkeys is the magnitude and kind of uni- and polymodal input to the PRh (Burwell et al., 1995). In monkeys, the PRh predominantly receives input from primary and secondary visual areas, though there are also afferent projections from somatosensory and auditory association cortices (Burwell et al., 1995; Suzuki, 1996; Suzuki & Amaral, 1994). In rats, the pattern of input is somewhat different and is described in detail in the next section.

The PRh receives substantial input from all sensory modalities, but the greatest unimodal input is of olfactory information arising from both the olfactory association cortex and the periamygdaloid area. Prominent projections from the piriform cortex terminate throughout the PRh. Inputs from somatosensory association cortex also terminate throughout the PRh, but are not as prominent as those from olfactory regions. There is also input from the auditory association cortex. The PRh receives gustatory information from insular cortex and visual information from posterior parietal and occipital cortices.

The two most prominent sources of polymodal input to the PRh in the rat arise from temporal association and postrhinal cortices. Projections from the ventral temporal association area terminate throughout the PRh. The main projection from the postrhinal cortex arises from its rostral portion and terminates in the caudal PRh. The PRh also receives substantial input from orbital, medial, and motor regions of frontal cortex; these

projections terminate throughout the full extent of the PRh. There are also substantial projections from the lateral and medial entorhinal cortex, but the input from the lateral area is more prominent. There are sparse projections to the PRh from the anterior cingulate and retrosplenial cortices.

Other afferents to the PRh include inputs from the HPC, amygdala, thalamus, striatum, and the nucleus accumbens (Burwell et al., 1995). Swanson & Cowan (1977) found projections from the CA1 and subiculum of the HPC to the PRh in the rat. Projections from the amygdala to the PRh come from the basal, accessory basal, central, and lateral nuclei (Burwell et al., 1995; Pikkarainen & Pitkanen, 2001; Shi & Cassell, 1999). Input from the thalamus arises from the perigeniculate region and the midline thalamic nuclei (Burwell et al., 1995).

1.1.3 Efferent projections of the PRh

Figure 3 also shows the primary efferent connections of the rhinal cortices. As mentioned in the preceding section, there is substantial input from the PRh to the entorhinal cortex. In fact, the entorhinal cortex is the primary target of the PRh, comprising approximately 40 % of its total output. These projections terminate mainly in the lateral entorhinal area. Efferent projections from the PRh to the postrhinal cortex are sparse.

Other important targets of projections from the PRh include the HPC, the amygdala, and thalamic nuclei. There are projections from the PRh to the CA1 cell field and subiculum of the HPC (Kosel, Van Hoesen, & Rosene, 1983). The PRh projects to the lateral, central, and basal nuclei of the amygdala (Burwell et al., 1995; Pikkarainen & Pitkanen, 2001; Shi & Cassell, 1999). The PRh also projects to midline, posterior and mediodorsal nuclei of the thalamus, the striatum, caudate nucleus, and the nucleus accumbens.

1.1.4 Functional implications of rhinal cortex connectivity for learning and memory

The rhinal cortices have significant overlap in their patterns of input and output (see Figure 3), but there are also differences. Additionally, careful attention to the density of projections to and from each of the rhinal cortices further distinguishes them. The structures are clearly an important source of neocortical information for the HPC. This is particularly true of the PRh, as it is a primary target of the postrhinal cortex and itself mainly targets the entorhinal cortex. However, given that the rhinal cortices have many connections in common, with the primary difference between them being the density of the projections, it follows that in the absence of the PRh, connections to and from either the postrhinal, entorhinal, or both cortices could serve as compensatory routes for neocortical information to reach the HPC.

The PRh is heavily interconnected with the amygdala, a characteristic that sets it apart from the other rhinal cortices. It also has substantial connections with frontal and temporal association cortices and thalamic nuclei. This places it in a unique position to support information processing in a way that other rhinal cortices and the HPC may not. Overall, the anatomical features of this region are consistent with the varied notions about how MTL structures may contribute to memory processes and highlight the need for detailed behavioural assays to delineate the contributions of individual structures to information processing.

1.2 The Amnesic Syndrome

Current views about the contribution of MTL structures to memory primarily originated from the correlation of brain damage in humans with memory deficits. Key behavioural

tasks that are widely used to evaluate learning and memory in non-human animals are based on the descriptions of amnesia in humans. Therefore, it is important to first outline features of the amnesic syndrome prior to describing the animal tasks designed to model it and the findings from experiments that make use of these models.

Amnesia refers to a persistent and disabling memory loss in the absence of appreciable decrements in intellectual and cognitive abilities and occurs following damage to the MTL or dienchephalon. Amnesia can be divided into two components: an inability to acquire new information and form long-term memories following brain damage is referred to as anterograde amnesia and an inability to recall information acquired prior to brain damage is referred to as retrograde amnesia. In many cases, human amnesic patients show some degree of both types of amnesia, though not always to the same extent and they do not always co-occur. Some patients with retrograde amnesia display a temporally graded loss of memory in which information acquired close to the time of brain damage is lost whereas information acquired remote to the time of damage is spared. This pattern of retrograde memory loss has factored significantly in models of memory formation (e.g. Alvarez & Squire, 1994) and will be discussed in detail in Section 1.4.1.

The types of memories affected in human amnesia are those that require the conscious recollection of information. This kind of memory is referred to as *explicit* (Tulving, 1984) or *declarative* (Squire, 1987) and can be further subdivided into memories for facts, or *semantic* memory, and memories for events, or *episodic* memory. *Implicit*, or *nondeclarative* memories are, by contrast, spared in human amnesia. Implicit memories are those that involve the acquisition of knowledge that is not consciously referred to but can be reflected in changes in behaviour, such as skill learning (*procedural* memory), classical conditioning, and priming (Tulving, 1984; Schacter, 1992; Squire, 1987).

1.2.1 MTL amnesia

In 1957, Scoville and Milner reported the landmark case of patient H.M. who underwent a bilateral MTL resection in a radical attempt to alleviate a debilitating epileptic condition. The surgery was successful in attenuating the epilepsy, however, H.M. thereafter suffered from amnesia. A striking feature of his new condition was a severe anterograde amnesia in which he was unable to acquire certain types of new information. Specifically, detailed analyses of this amnesia revealed that H.M. was unable to form new long-term memories, while his capacity for implicit memory remained intact. H.M. was also observed to have retrograde amnesia, in that he could not recall events or information that he had acquired between 1 and 11 years before the surgery. This memory loss was also specific for explicit information, thus providing evidence that the MTL was also involved in the retrieval of explicit memories, but only for a certain period as memories acquired early in H.M.'s life were not lost. H.M. displayed little or no change in other intellectual abilities.

According to the surgeon's description of the tissue resection, H.M.'s lesion would have included the entire HPC, the amygdala, and the rhinal cortices (Scoville & Milner, 1957). Also, they described memory loss in other patients that underwent less radical procedures in which surgical resections were either unilateral or bilateral, but included less tissue overall than H.M.'s lesion. All patients displaying memory loss also sustained HPC damage. Though the HPC damage never occurred without some damage to the overlying cortices, Scoville and Milner attributed the memory loss to the HPC damage. This continued to be a common tendency of researchers, and still occurs. As will be discussed in detail later, this has drastically skewed the way empirical data have been collected and interpreted. Interestingly, H.M.'s lesion was examined with magnetic resonance imaging (Corkin et al., 1997) and it was discovered that there was, in fact, substantial sparing of the HPC.

In addition to the case of H.M., several other reports confirm that damage to this region results in an inability to form new memories in the absence of other cognitive or implicit learning deficits (for examples and descriptions see Nadel & Moscovitch, 1997; Nadel et al., 2000; Squire, Zola-Morgan, & Chen, 1988; Warrington, 1996). Retrograde amnesia is not always systematically investigated, but most studies report some loss of memory for information acquired prior to brain damage in addition to anterograde amnesia. However, Fast and Fujiwara (2001) describe a number of cases in which temporal lobe damage resulted in 'isolated retrograde amnesia'. In these cases, there was a specific loss of information acquired prior to brain damage in the absence of any appreciable inability to learn new information.

Unfortunately, there are very few reports of circumscribed damage to the PRh in humans; concomitant damage to other MTL structures is typically present. This limits the extent to which conclusions about PRh function can be drawn based solely on data obtained from case studies of human patients with brain damage. However, there are a few reports describing the effects of brain damage that included the PRh, but not the HPC (Hodges & Graham, 1998; Schmolck, 2002). For example, damage to the temporal neocortex that includes the PRh but spares the HPC has been associated with a specific type of memory loss termed 'semantic dementia'. Patients suffering from this disease typically show an impaired ability to recall facts and recognize and name every-day items. Conversely, episodic memory— their ability to recall autobiographical details and events— is relatively unchanged.

The foregoing findings suggest that the functions of PRh can be dissociated from those of the HPC. In addition, Ploner et al. (2000) found that human patients with PRh damage performed normally on spatial tasks, whereas other patients with damage restricted to the

parahippocampal cortex were impaired. This finding suggests that there may also be functional dissociations within the rhinal cortices.

1.2.3 Limitations of memory research with human subjects

A major limitation to using human patients in memory research is that brain damage varies widely from patient to patient, the location and extent of the tissue loss is either difficult or impossible to fully discern, or the lesions includes portions of the many structures. Furthermore, a wide variety of methods have been employed to evaluate the nature and extent of memory loss. A number of neuropsychological profiles have been used to determine the presence and degree of anterograde amnesia. Similar profiles exist for retrograde amnesia but it is frequently difficult to determine what patients knew before surgery, how well they knew it, and when the information was acquired.

Nonetheless, studies of human patients with brain damage provide important clues regarding the contribution of MTL structures to memory function. However, the use of animal models, described in detail in the next section, is vital to a clear understanding of how specific MTL structures contribute, either individually or cooperatively, to learning and the formation of memories. The use of animals enables investigators to exert control over both the location of brain damage and the nature and timing of information acquisition.

1.3 Animal Models of Amnesia

In the decades that followed Scoville and Milner's report (1957) many attempts were made to create both monkey and rat models of the amnesia displayed by H.M. The creation of such models factored prominently in the determination of the critical sites of brain damage in amnesia. As will be evident from this overview, however, there has been a tendency of the models to focus specifically on object-recognition memory— the ability of animals to detect that an object is familiar. In the present thesis, the effects of PRh lesions

on both object and place memory were investigated. Therefore, in this section, relevant place memory tasks are also described.

1.3.1 Monkey models of MTL amnesia

The first animal models of MTL amnesia involved monkeys that displayed delay-dependent deficits on the DNMS test of object-recognition memory. In DNMS, monkeys are first shown an object and are typically required to execute an appropriate response towards it, which leads to a food reward. After a retention delay, monkeys are shown two objects, one is identical to the object they previously saw (*sample* object) and the other is a new object that is not familiar (*novel* object). The monkey makes a nonmatch response by selecting the novel object and avoiding the sample object. A correct response results in a food reward. Successful performance on this task requires object-recognition memory because in order to reliably select the novel object, the subject must be able to discriminate the familiarity of the sample object.

1.3.1.1 Trial-unique DNMS

In 1978, Mishkin revised the trial-unique DNMS procedure which has since served as the benchmark task in animal models of MTL amnesia. In the trial-unique version, different pairs of objects serve as the sample and novel objects on each trial. With trial-unique stimuli, monkeys perform well with retention delay of up to several minutes. This made it possible to test memory for objects that were seen either a few seconds previously (spared in MTL amnesics who have intact short-term memory) or several seconds or minutes previously (impaired in MTL amnesics).

1.3.1.2 Lesion studies in monkeys

Monkeys with combined removals of the HPC, amygdala, and the overlying rhinal cortices displayed severe deficits on trial-unique DNMS (Mishkin; 1978; Murray & Mishkin,

1983; Squire & Zola-Morgan, 1983; Mahut & Moss, 1984). However, lesions restricted to the HPC or the amygdala produced only mild deficits on DNMS (Mishkin, 1978; Murray & Mishkin, 1986; Saunders et al., 1984). At the time it was not possible to make discrete lesions of the HPC or amygdala in monkeys without necessarily damaging the overlying cortex. The appropriate control, to assess DNMS performance in monkeys with damage to just the overlying cortex, was not immediately carried out. Therefore, it was several years before the importance of the rhinal cortices was identified and investigated.

Murray and Mishkin (1986) reported that monkeys with amygdala plus rhinal cortex damage displayed more marked deficits on DNMS than monkeys with HPC plus rhinal cortex damage. However, discrete lesions of the amygdala that spared the overlying cortex did not impair DNMS performance (Zola-Morgan, Squire, & Amaral, 1989). Additionally, Zola-Morgan, Squire, Amaral, & Suzuki (1989) found that lesions restricted to the PRh and parahippocampal cortex resulted in severe DNMS deficits. It was argued that removal of the amygdala produced more damage to the rhinal cortex than removal of the HPC (Murray, 1992).

The finding that lesions of the rhinal cortex produced severe DNMS deficits while lesions of the HPC and subtotal lesions of the rhinal cortex produced only mild DNMS deficits suggested that the rhinal cortex alone was essential for normal object-recognition memory. Furthermore, Zola-Morgan et al. (1989) noted that the PRh was the only region not fully damaged in their previous attempts to make lesions of the HPC, whereas the parahippocampal cortex sustained substantial damage. Thus, damage to the parahippocampal cortex was not sufficient to impair DNMS performance in monkeys.

Meunier et al. (1993) compared DNMS performance in monkeys with lesions of either the PRh or the entorhinal cortex and found that PRh, but not entorhinal cortex, lesions

produced severe DNMS deficits. Meunier et al. (1996) reported that monkeys with lesions of the HPC that included complete or near complete damage to the rhinal cortex were more impaired on DNMS than monkeys with HPC lesions in which the rostral portions of the PRh were spared. These findings were the first to identify the PRh as a critical site of damage within the MTL that alone produced memory deficits as severe as those following large MTL removals. Thus, it was now clear and generally well-accepted that the PRh made a unique contribution to object-recognition memory (Mishkin & Murray, 1994; Baxter & Murray, 2001). Further descriptions of the effects of PRh lesions on memory in monkeys will be discussed in Section 1.4.

1.3.2 Rat models of MTL amnesia

During the period of research described above restricted lesions of HPC and amygdala could be produced in rats, but it remained a challenge to adapt DNMS for use with rats. In the 1980s, there were several attempts to produce a model for use with rats, with mixed success. With these adapted versions of DNMS, a pattern of findings similar to those described in the preceding section concerning the contributions of individual MTL structures to DNMS performance emerged from rat research.

1.3.2.1 Rat DNMS

Aggleton, Hunt, and Rawlins (1986) were the first to develop a version of DNMS for use with rats. Their version was a modified Y-maze in which the ends of two arms could be fitted with changeable goal boxes. Rothblat and Hayes (1987) also developed a rat version of DNMS, but used a straight runway with a large pool of objects as stimuli, which more closely mimicked the monkey version of DNMS. In 1990, Mumby, Pinel, and Wood developed a rat version of DNMS that also used objects as stimuli, but did not require the handling of the rat during a trial or even a session, whereas previous versions did. These

three versions of rodent DNMS contributed substantially to the field as they expanded the comparative basis and afforded the opportunity for more researchers to investigate the neural bases of object recognition memory.

1.3.2.2 Lesion studies in rats

Aggleton et al. (1986) found that rats with hippocampal lesions were not impaired on DNMS, even with retention delays as long as 60 seconds. Similarly, Rothblat and Kromer (1991) reported that rats with fimbria-fornix lesions (destruction of the large fibre bundle that comprises the major subcortical input/output of the HPC) were not impaired on their version of DNMS with delays up to 30 seconds. Mumby, Wood, and Pinel (1992) found that rats with either HPC or amygdala lesions displayed normal DNMS performance at delays of up to 2 minutes, and mild impairments at delays of 10 minutes.

The findings from rat DNMS research were consistent with those from studies in monkeys with damage limited to the HPC and amygdala. Moreover, Mumby and Pinel (1994) reported that rats with lesions restricted to the rhinal cortex were impaired on DNMS. Like monkeys with PRh or rhinal cortex lesions, rats with rhinal cortex lesions displayed a delay-dependent impairment; their performance was not significantly different from that of control rats at very short delays, but as the delay period increased their performance dropped to chance levels. Glenn and Mumby (1996) found that rats with selective lesions of the PRh, but not the HPC, displayed delay-dependent deficits on DNMS, suggesting that the PRh was a crucial area fro mediating object-recognition memory.

1.3.3 Novelty preference

Another task for assessing object-recognition memory in rats was described in detail by Ennaceur and Delacour (1988). This novelty preference task (NPT), originally referred to as spontaneous object recognition, differs from rat DNMS in many ways, but is also designed

to assess the ability of rats to hold information about objects over variable retention delays. A key feature of NPT is that it takes advantage of the rat's natural propensity to explore unfamiliar objects. Rats are placed in an open arena with two identical 'sample' objects for between 3 and 5 minutes. Alternate procedures involve allowing the rat to accumulate some pre-determined amount of exploration time with the sample objects, e.g. 20 seconds, at which point the sample phase is terminated. In either case, the sample phase of the trial is followed by a retention delay, which the rat spends outside of the arena, typically in its home cage. As in DNMS the delay period can be of variable duration. For the test phase of the trial the rat is returned to the arena where it again encounters two objects: a third copy of the sample object and a novel object. Most normal rats will spend significantly more time exploring the novel object, indicating that they recognize the sample object.

1.3.3.1 DNMS and NPT compared

The DNMS procedure is viewed as having several potential problems. First, the ability of animals to successfully solve DNMS requires that they first learn the rule; "always choose the novel object". Therefore, an animal that shows problems learning the task may fail due to an inability to identify and acquire the rule rather than an inability to remember the object over the retention delay. Secondly, there is always a contingency associated with the task; correct responses result in a food reward. This aspect of DNMS relies on a particular motivational state and an animal may fail due to alterations in motivation rather than object-recognition deficits. Third, training and testing rats on DNMS is time-consuming. Rats must first be shaped to displace objects. Many trials are then required to teach rats the nonmatching rule using a brief retention delay. Longer delays are gradually introduced and these are typically followed by mixed-delay sessions in which each delay is presented. There are also issues associated with whether rats (and monkeys) should be trained prior to surgery,

as there is a tendency for deficits to be worse if animals are not presurgically trained (Mumby et al., 1992; Squire & Zola-Morgan, 1991).

NPT attempts to overcome the above difficulties with DNMS: it is not necessary for the animals to learn a rule, there is no explicit contingency associated with the objects, and a single test of object-recognition can be obtained in one day. One major advantage of NPT is that the test session can be adapted in ways that afford the assessment of memory for different types of information about the *same* learning event (Dix & Aggleton, 1999; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002). NPT was originally designed as a test of object recognition; it has since been modified to test place recognition as well. This version of NPT is described in Section 1.3.4.3.

NPT also has its disadvantages. Although there is no rule or reward contingency to learn, it is still possible for non-specific changes in behavior to be mistaken for an inability to recognize the sample object. For example, lesions that increase or decrease neophobia or activity levels may lead to equivalent exploration of the sample and novel objects during the test phase even if object-recognition memory is intact. NPT can be conducted in a much shorter period than DNMS, but fewer samples of the animal's ability are obtained, limiting the conclusions one can make about an animal's stable performance.

Despite these potential difficulties, however, NPT offers an opportunity to examine at least two types of memory abilities while utilizing similar training and testing parameters. For example, the sample session of the place and object versions of NPT is identical. In the retention test of the place version a sample object is moved to a new location, whereas in the object version a sample object is replaced with a novel object. In this way, the ability of rats to recall two different aspects of the same learning event (object location and identity, respectively) can be examined.

NPT can also be used to assess retrograde amnesia in rats. This is done by increasing the number of exposures to the sample objects. Rats that receive five 5-minute sample sessions, one per day for 5 consecutive days, show a preference for the novel object in a test session conducted 6 weeks after the final sample session (Glenn & Mumby, unpublished data; Khoury, 1999). Rats can be familiarized with different objects at different time points prior to surgery, and postsurgery recognition tests can potentially reveal temporal gradients to any retrograde amnesia that is observed.

Retrograde memory testing is not possible using DNMS. DNMS is designed to be a test of working memory for objects. Animals view the sample object for a few seconds and their ability to recognize this object several seconds or minutes later is assessed. Thus, previous studies of retrograde amnesia using the training environment of DNMS primarily involved the teaching of simple object discriminations at different time points prior to surgery. Unfortunately, PRh lesions do not impair simple object discrimination learning in rats (Glenn & Mumby, 1996; Mumby et al., 1992; 1994), nor does this task require the type of memory impaired in human amnesic patients (Gaffan, 1972; Squire et al., 1988).

1.3.3.2 Lesion studies using NPT

The results of several recent lesion studies using NPT are consistent with those obtained with other object-recognition tasks; rats with combined lesions of the PRh and postrhinal cortex did not show a preference for the novel object (Bussey, Muir, & Aggleton, 1999; Bussey, Duck, Muir, & Aggleton, 2000), but rats with fornix transections (Bussey et al., 2000) or HPC lesions (Mumby et al., 2002) did show a preference. These findings confirm a critical role for the PRh, but not the HPC, in object-recognition memory. In addition, the concordance of findings from lesion studies using DNMS or NPT provides evidence that these tasks tax similar abilities in rats.

1.3.4 Spatial Memory

While most lesion-based studies with monkeys sought to model human amnesia, lesion-based studies with rats sought to identify the types of abilities that were affected by damaging specific brain regions. In particular, assessing rodents' ability to navigate in mazes is a hallmark procedure in rodent research that dates back many decades and there is much evidence that the HPC is essential for normal spatial memory (see O'Keefe and Nadel, 1978). In this section, O'Keefe and Nadel's seminal theory is briefly described, followed by descriptions of two place memory tasks relevant to the present thesis.

1.3.4.1 Cognitive map theory of HPC function

O'Keefe and Nadel (1978) proposed that the HPC is central to the formation and retention of 'cognitive maps'. Cognitive maps are neural representations of familiar environments that enable efficient and flexible navigation. Through repeated experience within a particular environment an animal acquires information about its spatial layout, including available visual, auditory, olfactory, and tactile cues and their relative locations. According to O'Keefe and Nadel, this place representation allows the animal to navigate between different parts of the environment in a variable manner. For example, an animal can reach a particular destination from any number of start points. In the absence of a place representation, the animal is unable to navigate flexibly through the environment and must rely on traversing a learned and rigid route between the same two points.

Allocentric place memory refers to the ability of animals to form stable cognitive maps of familiar places. Thus, animals learn and remember the relative locations of available cues and can navigate flexibly to a target location in that place. *Egocentric* navigation can also be used to reach a target location, but it is based solely on orientation cues, it is rigid, it does not require a cognitive map, and it is considered independent of HPC function. The place

memory tasks used in the present thesis, and described in the following sections, are designed to tax allocentric memory.

1.3.4.2 Water Maze

The water maze (Morris et al., 1982) consists of a large pool of water to which nontoxic paint or skim milk powder is added to increase the opacity of the water. A platform is hidden below the surface of the water and in the standard version of the task remains in the same position within the pool throughout training and testing. The task is aversively motivated as the water temperature is kept cool (approximately 21-23 °C) and the platform provides an escape. A rat swimming in the pool is unable to see the platform and must therefore rely on cues outside of the maze to learn its position. When a rat is first placed in the water maze its swim pattern is random, but, after several trials, it becomes apparent that the animal is directing its swim towards the platform's location. Eventually, usually after 10-15 trials, the time it takes the rat to find and escape onto the hidden platform reaches a stable asymptote.

The standard version of the water maze task, in which the platform remains in the same position throughout training and testing, is considered to tax allocentric spatial *reference* memory. Reference memory generally refers to the acquisition of information that remains constant over trials. Thus, on each trial of the standard version of the water maze task the information concerning the platform's location is unchanged. The water maze has been adapted to assess allocentric spatial *working* memory as well (Glenn & Mumby, 1998). Working memory generally refers to the acquisition and retention of information for a short period of time, and this information is only applicable to an individual trial and thus changes from trial to trial.

In DNMS, the animal must remember the sample object over the retention delay to make an accurate choice during the test portion of the trial. In the water maze, working memory is usually assessed by administering paired swims in which the platform moves on each trial. On the first swim the animal acquires information about where the platform is located on that trial. A delay is interposed between the two swims during which the animal must retain the information about the platform's location. Thus, on the second swim the animal demonstrates that it retained that information by locating the platform in a timely manner. Both allocentric reference and working spatial memory in the water maze are impaired following lesions of the HPC (Morris et al., 1982; Glenn & Mumby, 1998).

The radial-arm maze is a dryland, elevated apparatus that is also designed to tax allocentric place memory in rats (Olton, Walker, & Wolf, 1982). Many variations of this maze have been used and in most versions both reference and working memory abilities can be evaluated. The water and radial-arm mazes are two tasks that have figured prominently in the accumulation of empirical support for the specialized role of the HPC in the processing of place information. Consequently, both tasks have also been used to investigate the contribution of other brain regions to place memory.

1.3.4.3 Place version of NPT

In Section 1.3.3, NPT was described as a test of object-recognition memory. But as briefly mentioned in Section 1.3.3.1, it has been modified to test other aspects of memory (see Dix & Aggleton, 1999). In the place version of NPT, the sample session and retention delay proceeds exactly as in the object version; rats are placed in an open-field arena with two identical sample objects, followed by the retention delay, which can be of variable duration. During the test session, however, the same two sample objects are present but one object is moved to a new location and the other remains in the same location as during the

sample session. Normal rats will spend more time exploring the object that was moved to a new location (Dix & Aggleton, 1999; Mumby et al., 2002).

The main advantage of the NPT task is that affords a unique opportunity to examine two different aspects of memory for the same learning event. The sample session is exactly the same for the place and object versions, but the object test assesses the ability of rats to detect that a novel object is present, whereas the place test assesses the ability of rats to detect that a familiar object has moved to a new location. A second advantage to the place version of NPT, as with the object version, is that it can be easily modified for retrograde memory testing.

1.3.4.4 Lesions studies with place memory tasks

There is ample evidence of the devastating effects of HPC lesions on place memory. Rats with lesions of the HPC invariably fail on the standard reference (e.g. Morris et al., 1982) and working (e.g. Glenn & Mumby, 1996; 1998) memory versions of the water maze. On the radial-arm maze they make substantially more reference and working memory errors than normal rats (e.g. Jarrad, 1993; Olton & Papas, 1979; Olton et al., 1982). Finally, Mumby et al. (2002) recently found that rats with HPC lesions did not show a preference for the object that was moved to a novel location on the place version of NPT.

The contribution of the PRh to place memory is not clear. The findings from a number of studies that examined the performance of rats with PRh lesions on all versions of the water and radial-arm mazes are mixed. Lesions restricted to the PRh in rats produced deficits on the reference (Wiig & Bilkey, 1994; Liu & Bilkey, 1998a; 2001) and working (Liu & Bilkey, 1998b; also see Nagahara, Otto, & Gallagher, 1995) memory versions of the water maze. Lesions restricted to the PRh were also reported to increase reference and working memory errors on the radial-arm maze (Liu & Bilkey, 1998c; 1999).

It should be noted, however, that the magnitude of the deficits following PRh lesions was much less than is typically observed following lesions of the HPC. Additionally, there are a number of reports in which PRh lesions or combined PRh and postrhinal cortex lesions did not impair place memory. Glenn & Mumby (1996; 1998) found that lesions of the PRh did not produce deficits on the spatial working memory version of the water maze. Machin, Vann, Muir, and Aggleton (2002) found that PRh lesions did not produce deficits in reference or working memory using a radial-arm maze. Bussey, Duck, Muir, and Aggleton (2000) found that combined PRh and postrhinal cortex lesions did not impair spatial delayed alternation on a t-maze.

1.4 Proposed Functions of the PRh

In general, the preponderance of findings from lesion studies in rats and monkeys, as discussed in the previous section, suggest that the PRh (Baxter & Murray, 2001; Buckley & Gaffan, 1998b; Buckley, Gaffan, & Murray, 1997; Bussey et al., 2000; Gaffan, 1994; Gaffan & Murray, 1992; Eacott, Gaffan, & Murray, 1994; Glenn & Mumby, 1996; Meunier et al., 1993; 1996; Mumby et al., 1994), but not the HPC (Baxter & Murray, 2001; Bussey et al., 2000; Gaffan, 1994; Glenn & Mumby, 1996; Meunier et al., 1996; Mumby et al., 1992; Zola-Morgan et al., 1987), is important for normal object-recognition memory. Furthermore, the HPC is clearly important for normal place memory (Bouffard & Jarrard, 1988; DiMattia & Kesner, 1988; Devan, Goad, & Petri, 1996; Douglas, 1967; Glenn & Mumby, 1996; 1998; Jarrard, 1993; O'Keefe & Nadel, 1978; Olton & Papas, 1979; Olton, et al. 1982; Morris et al., 1982; Sherry, Jacobs, & Gaulin, 1992), while the role of the PRh in this type of memory is not yet well understood (Glenn & Mumby, 1998; Machin et al., 2002; but see Liu & Bilkey, 1998a; 1998b; 2001).

Evidence from electrophysiological studies is consistent with the findings from lesion studies. Neurons in the PRh appear to fire preferentially in response to novel stimuli, and their firing rate decreases as stimuli become more familiar (Brown & Xiang, 1998; Zhu, Brown, & Aggleton, 1995). Additionally, PRh neurons also show particular firing patterns during the delay period on object recognition tasks like DNMS (Erickson & Desimone, 1999). Neurons in the HPC are not observed to display these response patterns (Zhu et al., 1995). HPC neurons do fire preferentially to particular locations in familiar environments (O'Keefe, 1976; O'Keefe & Nadel, 1978). These neurons are specifically referred to as 'place cells'. Furthermore, there is neuronal activation in the PRh following the presentation of objects, but not places (Wan, Aggleton, & Brown, 1999; Zhu, Brown, McCabe, & Aggleton, 1995; Zhu, McCabe, Aggleton, & Brown, 1996). The converse was observed in the HPC (Wan et al., 1999; Zhu, McCabe, Aggleton, & Brown, 1997).

Several models of MTL function have been proposed to account for these findings as well as those from human amnesic patients. In the following sections those models relevant to the present thesis will be described. In each section, the functions attributed to the PRh will be emphasized.

1.4.1 The PRh as a component in a MTL memory system

A prevalent view is that MTL structures comprise a single functional system (Squire, 1986; Squire & Zola-Morgan, 1988; 1991; also see Eichenbaum, Otto, & Cohen, 1994). The primary role of this system is believed to be the formation of long-term explicit memory (Alvarez & Squire, 1994; Zola-Morgan & Squire; 1990). The process by which long-term memories are formed is conventionally referred to as *consolidation*. This process is viewed as time-limited, thus making the role of the MTL temporary. Accordingly, MTL structures must be intact at the time of learning and for a period of time after learning is complete. As

consolidation proceeds, the MTL is also required for retrieval of the newly learned information. Once consolidation is complete and the information is permanently established in long-term memory, presumably in neocortex, the MTL is no longer needed for retrieval.

The primary role of the PRh as a component in this system is to provide the HPC with highly processed uni- and polymodal information arising from neocortex. Proponents of this view regard HPC function as critical for rapid encoding of new information, binding of elemental features of a whole representation, the formation of flexible relations between individual items of information, and/or temporal and spatial indexing of representations. In order for the HPC to successfully perform any or all of these functions it requires access to sensory details of a learning event encoded by disparate regions of the neocortex. The PRh and the postrhinal cortex are sites of convergence of such information from neocortex, and a substantial portion of their output is directed to the entorhinal cortex and the HPC. Thus, the perirhinal cortex is viewed as the necessary route through which sensory information reaches the HPC.

Support for the view that the HPC and rhinal cortex of the MTL comprise a single functional system is mixed. The proximate anatomical location and intimate connectivity of these structures is compelling evidence that they may function interdependently. However, the findings that the functions of the PRh and HPC can be doubly dissociated are not consistent with this model. If the PRh and HPC are components in a unitary memory system, it may be possible to observe single dissociations in function, but it should not possible to observe that PRh, and not HPC, lesions impair object memory whereas HPC, and not PRh, lesions impair place memory. However, as described in Section 1.3.4.5, the effects of PRh lesions on place memory are mixed.

The findings that PRh lesions produce deficits in the acquisition of place information provide some support for the notion that some HPC functions may be dependent on the integrity of the rhinal cortex region, but there are as many findings that PRh lesions do not produce place memory deficits. Therefore, further research is required to establish the contribution of the PRh to place memory and this was addressed in the present thesis. There is also little evidence regarding the contribution of the PRh to the consolidation process proposed to rely on the MTL. For this reason, one aim of the experiments in this thesis was to examine the effects of PRh lesions on the ability of rats to recall information learned before surgery.

1.4.2 The PRh as a component in an object-processing system

The view that the rhinal cortex, particularly the PRh, is the neural region critical for the processing of objects was initially outlined by Mishkin and Murray (1994) and was further described by Murray (1996), Buckley and Gaffan (2000), and Murray and Richmond (2001). The notion is based largely on empirical evidence from ablation studies using monkeys, and, to a lesser extent, rats. As noted in the introduction to Section 1.4, PRh lesions impair DNMS performance, while lesions of other MTL structures have little or no effect. The stability of these findings over time and setting provided a compelling impetus to reevaluate the ways in which MTL function is viewed.

The close anatomical relation between the PRh and unimodal, visual processing areas make it an ideal candidate for the representation and association of sensory features necessary for identifying objects. Stimulus identification refers to the ability of an animal to know that an object is the same across different exposures to it, even if some information regarding its sensory features is unavailable (Murray & Richmond, 2001).

It has also been argued that the PRh may be part of a perceptual system, rather than a mnemonic one (Buckley, Booth, Rolls, & Gaffan, 2001). Evidence for this notion has been primarily derived from studies in which PRh lesions produce a more devastating impairment as the complexity of the discriminanda increases. Examples include varying object views (Buckley & Gaffan, 1998a), or using very large stimulus sets (Eacott et al., 1994). Buckley et al. (2001) reported that PRh lesions in monkeys specifically led to an inability to process information that required a complete representation of the object, rather than just the identification and utilization of simple or even moderately complex features of the object. They, therefore, contend that the PRh is needed to assemble a representation of an entire object and PRh lesions will only disturb object perception and recognition when there is a demand to recall the object as a whole. A broader view is that the PRh serves both perceptual and mnemonic functions, and collectively the evidence supports this position (Buckley & Gaffan, 1997; 1998a; Bussey, Saksida, & Murray, 2002; Hampton & Murray, 2002; Murray & Richmond, 2001; but see Buffalo, Ramus, Squire, & Zola, 2000; Buffalo, Reber, & Squire, 1998; Buffalo et al., 1999).

The object processing system described by its proponents appears to be critical for both the short-term and long-term representation of objects (e.g. Bussey & Saksida, 2002; Buckley & Gaffan, 1998b). The PRh may be the site at which complex features of stimuli are assembled and/or associated, the individual features being stored in uni- and polymodal sensory processing areas. If this is the case, then the PRh would always be required for retrieval of the representation. Accordingly, both anterograde and retrograde memory for objects would be compromised following PRh lesions. This was examined in the present thesis.

The electrophysiological evidence that was cited previously is also consistent with a specialized object-recognition system. That neurons in the PRh respond according to whether an object is novel or familiar is consistent with a system designed to make judgements regarding whether an object has been previously encountered. However, the view that the PRh is an essential component in a system specialized for processing object information does not preclude the likelihood that the PRh also functions interdependently with the HPC under certain circumstances. Gaffan and Parker (1996), for example, found that both PRh and HPC lesions impaired performance on an 'object-in-place' task, in which both place and object information was necessary for success. It is also conceivable that the PRh may participate in the formation of a cognitive map to process information about environmental cues, or landmarks.

1.4.3 The PRh as a component in a recognition system

Aggleton and Brown (1999) proposed a model of memory function that, unlike preceding views, attempts to separate individual MTL structures on the basis of their connectivity with regions of the diencephalon. Aggleton and Brown specifically target two mnemonic processes: recognition and recollection. Recognition memory is described as a process of *knowing* and occurs through judgments of relative familiarity, whereas recollection is described as a process of *remembering* and occurs through the recall of the learning event, akin to episodic memory. According to their model, recognition memory relies on a PRh-medial dorsal thalamus circuit and it is functionally independent of recollection, which relies on a HPC-anterior thalamus circuit.

Aggleton and Brown argue that the tendency of researchers to primarily use tests of recognition memory as the hallmark assessment of amnesia has incorrectly distanced the contribution of thalamic structures and their MTL connections. Findings from lesion-based

studies with animals provide support for their dissociation: lesions of the PRh or the mediodorsal nucleus produce object recognition deficits, while lesions of the HPC, mammillary bodies, or anterior thalamic nuclei produce deficits in place memory. The process of recollection or episodic memory in general is difficult to evaluate in nonhuman animals. However, Gaffan (1991) notes that episodic memories in humans are characterized by their ability to recall the spatial and temporal features of a learning event. He suggests that place memory in animals may be analogous to episodic memory in humans. In the present thesis, object-recognition and place-memory tasks are utilized. The effects of PRh lesions on these tasks will aid in evaluating the validity of the model proposed by Aggleton and Brown.

1.5 Summary and Intent

If a focus is placed mainly on the findings from animal research, there is a convergence of evidence from different types of investigations that strongly indicates a specialized role for the PRh in object recognition and its functions seem to be distinct from those of other MTL structures. Rats and monkeys with PRh lesions show an inability to hold object information in working memory, as assessed by DNMS and NPT. Neurons in the PRh of both rats and monkeys are found to fire in response to novel stimuli, and the firing rate is observed to decrease with repeated presentations of the same stimuli, suggesting that neurons in the PRh encode for familiarity. Finally, increases in neuronal activation are observed in the PRh following the presentation novel objects.

The findings from studies that examine the contribution of the PRh to the processing of other types of information are more difficult to interpret. It is possible that in addition to being a central component in a system that is devoted to object recognition the PRh is also part of other systems that process different types of information. That PRh lesions, under some circumstances, impair the acquisition of place information may reflect its contribution

to a HPC system. Given that this type of deficit is not always observed, further research is necessary to identify the conditions under which PRh lesions may lead to place memory deficits. In addition, there are few studies that examine the role of individual MTL structures in retrograde memory. Therefore, to obtain a more complete view of the mnemonic processes that may rely on the integrity of the PRh, anterograde and retrograde memory for objects and places were examined in the present thesis.

1.5.1 Main Questions

The PRh is a site of convergence of highly processed sensory information arising from neocortex. It also shares reciprocal connections with many other brain regions, including the HPC, amygdala, and thalamus, and it can be dissociated from other, adjacent, cortical areas based on its input and output. There is ample evidence that PRh functions are dissociable from those of nearby regions, but there exists inconsistencies that limit the extent to which a broad view of PRh function can be formulated. Therefore, the overall purpose of the present thesis was to conduct a comprehensive evaluation of the effects of PRh lesions on learning and memory in rats. A multifaceted approach was used; the ability of rats with PRh lesions to learn and remember different types of information was assessed using a variety of behavioural tasks. In addition, the contributions of different methodologies to the inconsistencies in the effects of PRh lesions were investigated. Thus, the findings from this work will afford the opportunity to evaluate the validity of different views of PRh function and, to some extent, MTL function.

The first major aim of the present thesis is to examine the effects of PRh damage on the ability of rats to learn and remember the location of a stationary, hidden platform in a water maze. In Chapter 2, a series of experiments are described in which anterograde and retrograde place memory is assessed using the water maze task. The second major aim of the

present thesis is to examine the effects of PRh damage on the ability of rats to learn and remember information about objects. In Chapter 3, a series of experiments are described in which anterograde and retrograde object memory is assessed using the NPT task. The experiments described in Chapter 4 were conducted to more fully characterize the contribution of the PRh to place memory. To this end, the place version of NPT was used to assess anterograde and retrograde place memory in rats with PRh damage.

Another aim of this thesis was to investigate the types of procedural aspects that may contribute to and/or confound results in behaviorally-based lesion studies. In Chapters 2 and 3, lesion method and experimental design were varied to determine whether these methodological features alter the pattern or magnitude of memory loss. In Chapter 5, the expression of the protein products of the immediate-early gene, *c-fos*, was used as a marker for neuronal activation to explore the possibility that different lesion techniques may differentially activate structures outside of the PRh.

Chapter 2

Memory for Places

The main purpose of the experiments described in this chapter was to examine the effects of PRh lesions on anterograde and retrograde place memory, using the stationary platform, reference memory version of the water maze task. Anterograde place learning was examined in the first experiment. We previously found that PRh lesions did not impair working memory in the water maze (Glenn & Mumby, 1996; 1998), thus, it was important to determine whether our PRh-lesioned rats would be able to solve the reference memory version using our normal procedures and testing room. The effects of PRh damage on long-term retention of anterograde learning is not known, thus we also examined retention of the place problem 3 weeks after learning.

It is also not known whether the PRh participates in the retention of presurgically acquired place information. Therefore, retrograde place memory was examined in the remaining experiments. In Experiment 2, we assessed the ability of rats with electrolytic lesions of the PRh to remember place problems learned 4 weeks and 2 days before surgery. In Experiment 3, the training and testing procedures were the same as in Experiment 2, but we made aspiration lesions of the PRh. In Experiment 4, we assessed the ability of rats with aspiration PRh lesions to remember a single place problem learned either 4 weeks or 2 days before surgery. These three experiments offer new information about the contribution of the PRh to the retention of water-maze place problems, and also provide important knowledge regarding the influence of both lesion method (Experiments 2 and 3) and experimental design (Experiments 3 and 4) on patterns of memory loss in animal models of retrograde amnesia.

2.1 General Method

The following methodological information was common to all the experiments described in this chapter.

2.1.1 Subjects

Male, Long-Evans rats (Charles Rivers, St. Constant, Quebec), weighing between 300 and 350 g at the start of the experiments, served as subjects. Rats were singly housed in opaque cages and had free access to food and water throughout the experiment. The colony was maintained at 21 degrees Celsius with a 12:12 light-dark cycle (lights on at 8 am). All procedures were conducted during the light phase of the cycle.

Upon arrival to the colony, all rats were permitted to acclimate for 2-3 days. After this they were handled for a minimum of 1 week prior to the onset of the experiment. Throughout training and testing rats were handled at least once per week for the duration of the experiment.

2.1.2 Apparatus and Materials

The water maze was a circular pool (137 cm diameter, 46 cm deep). A Plexiglas platform (10 x 10 x 28 cm) was submerged approximately 2 cm below the surface of the water, which was made opaque through the addition of skim milk powder. Water temperature was 23 $\pm$1 ° C. Distal, extramaze cues (including laboratory equipment, posters on the wall, and sounds from a radio) were available. Rats could use these cues to locate the hidden platform in the maze. A VP118 Super Tracker (HVS Image, Hampton, UK), and a Panasonic video camera (WV-BP120), configured to an IBM-compatible computer with HVSWater software were used to record the position of the rat in the maze 10 times per second and the swim path and other dependent measures were derived from these data.

2.1.3 Histology

After the completion of behavioural testing, rats were administered an overdose of pentobarbital and were transcardially perfused with 0.09 % saline followed by 10 % formalin. Brains were extracted and stored in a 30 % sucrose-formalin solution for a minimum of 72 hours prior to sectioning. Using a cryostat, brains were sectioned at a width of 30 μm and every fifth section through the lesion was retained and mounted on gel-coated slides for analysis. The slides were Nissl stained and examined microscopically to determine the extent of perirhinal and extra-perirhinal damage.

We quantified the lesions by estimating the percentage of tissue loss to the PRh in the rats with the largest and smallest lesions; all other lesions fell within the reported ranges. Percentages of tissue damage were made based on calculations of the approximate total area of the target region and the area of the damage within that region. The boundaries of the PRh were based on those described by Burwell (2001); the rostral border was placed at -2.80 mm relative to Bregma, and the caudal border was placed at -7.8 mm relative to Bregma.

Estimates of PRh damage were made by examining the lateral reconstruction of the lesion and the coronal reconstruction at each of the three planes shown in Figure 2 (-3.8, -5.3, and -6.8 mm relative to Bregma). In each of these four views, an estimate of the percentage of PRh damaged was made for both hemispheres, which were then averaged. Damage to the lateral entorhinal cortex was evaluated from both the lateral and coronal reconstructions of the lesions, whereas damage to the postrhinal cortex was evaluated from the lateral reconstruction only. All other extra-PRh damage was evaluated by examining the coronal reconstruction of the lesion.

2.1.4 Statistical Analysis

The main dependent measure in each of the experiments was the latency, in seconds, of rats to locate and escape onto the hidden platform. We also examined swim speed (cm/s) on all trials, summarized in Appendix A, to ensure that between-group differences in latency were not a result of increased or decreased swimming speed. At various points in the experiments probe trials were conducted in which the platform was removed from the pool. In Experiment 1, probe trials were either 30 or 60 seconds in duration. The 60-second probe trials failed to provide any meaningful differences between Sham and PRh rats, therefore only the first 30 seconds of these trials was used in the analyses. In Experiment 2, probe trials were 25 seconds in duration and all other probe trials were 30 seconds long. The primary dependent measure on probe trials was the percent of the total swim time rats spent in the target quadrant (the quadrant that contained the platform on normal trials). Additional measures from probe trials were the latency to the first platform crossing and the number of platform crossings; these data are also summarized in Appendix A. Means and standard error of means (S.E.M.) are displayed in Figures.

Statistical analyses consisted primarily of Analyses of Variance (ANOVA). Factors used in analyses included Lesion (between-subjects factor: PRh versus Sham), Trials (within-subjects factor), Quadrant (within-subjects factor: NE, SE, SW, and NW), Probe (within-subjects factor: early versus late), and Time of Learning (within-subjects factor in Experiments 2 and 3, between-subjects factor in Experiment 4: REMOTE versus RECENT). One-sample t-tests were used to compare the percent time Sham and PRh rats spent in each quadrant with chance performance (25 percent) for each probe trial in all experiments. Where applicable, independent sample t-tests were used to compare Sham and PRh rats. Significance level was set at .05 for all statistical tests, but statistical trends ($p = .05$ to .10) were noted.

2.2 Experiment 1: Anterograde reference memory in the water maze following aspiration lesions of the PRh

A number of reports indicate that lesions of the PRh lead to an impairment in the ability of rats to learn the location of a hidden, stationary platform in a water maze (Liu & Bilkey, 1998a; 2001; Wiig & Bilkey, 1994). In these studies, PRh rats typically displayed a mild deficit in acquisition. On initial trials, there were no significant differences between PRh and sham rats; neither group had learned the platform's location. After approximately 10-15 trials PRh rats displayed significantly longer latencies to reach the platform's location relative to Sham rats. However, by the end of training (approximately 24 training trials), there were no significant differences between PRh and Sham rats. This acquisition deficit in the PRh rats differs from the pattern of deficits following hippocampal lesions; rats with lesions of this type still take longer to locate the hidden platform after 24 training trials (Morris, Garrud, Rawlins, & O'Keefe, 1982; Glenn and Mumby, unpublished data). Nevertheless, the acquisition deficits in PRh rats have been interpreted as an inability of the HPC to obtain sensory information from neocortex due to the loss of PRh tissue (Liu & Bilkey, 1998a; 1998b; 2001).

The above findings can be contrasted with the results from other studies in which PRh lesions do not impair spatial memory. We previously found that PRh rats were unimpaired on a working memory version of the water maze task (Glenn & Mumby, 1996; 1998, but see Liu & Bilkey, 1998c). Additionally, Bussey et al. (1999) and Ennaceur & Aggleton (1997) failed to detect an impairment in spatial working or reference memory in PRh rats using the radial-arm maze task (but see Liu & Bilkey, 1999), and Bussey et al. (2000) and Ennaceur et

al. (1996) failed to detect an impairment on the acquisition of and performance on a T-maze task in PRh rats.

Wiig and Bilkey (1994) and Liu and Bilkey (1998a; 1998b; 1999) made electrolytic lesions of the PRh, whereas we used aspiration lesions of the PRh (Glenn & Mumby, 1996; 1998). Since we had not purposefully tested the ability of PRh rats to learn and remember the location of a stationary, hidden platform, the main goal of this first experiment was to do this using a training regime of 8 trials per day for 3 days. To keep our preparation similar to our previous study, aspiration lesions of the PRh were used in this experiment. In this way, we retained the ability to make some comparison between working (Glenn & Mumby) and reference (present experiment) place memory, as well as between electrolytic (Wiig & Bilkey; Liu & Bilkey) and aspiration (present experiment) lesions. A second goal of this experiment was to assess the ability of rats with PRh lesions to successfully retain place information acquired after surgery by conducting a retention test 3 weeks after learning. To our knowledge, the long-term retention of place information acquired after PRh lesions has not been assessed.

2.2.1 Method

2.2.1.1 Subjects

Eleven experimentally naïve rats served as subjects in this experiment.

2.2.1.2 Procedure

2.2.1.2.1 Surgery. Rats received either bilateral PRh lesions (n = 6) or sham surgery (n = 5). Rats were anaesthetized with pentobarbital (65 mg/kg). For PRh rats, 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. Tissue was aspirated using a glass pipette attached to a vacuum pump. Sterile gelfoam (Upjohn Company, Don Mills, Ontario,

Canada) was placed in the cavity, the muscle was replaced, and the incision was sutured. For Sham rats, a scalp incision was made and sutured. Scalp wounds of all rats were treated with a topical antibiotic. Rats were permitted to recover for two weeks before water-maze training commenced.

2.2.1.2.2 Water-maze training. Rats were transported to the water-maze room in groups of 5 or 6, and each lesion condition (PRh and sham) was represented in each group. Rats received a total of 24 training trials and 4 probe trials on the water maze task, conducted over a 3-day period. Each trial involved placing the animal in the maze at one of four release positions (arbitrarily designated N, S, W, and E). The order of release positions used across trials was randomly determined in blocks of 4 trials, such that each position was used once before any were repeated. Rats were permitted to search the maze for the hidden platform for 60 seconds. If they did not find the platform in 60 seconds the experimenter guided them to it. All rats spent 10 seconds on the platform before being removed from the maze. The intertrial interval was approximately 5 minutes.

On probe trials the platform was removed from the pool, rats were placed in the maze as usual, and their swim paths were recorded for durations of 30 or 60 seconds. On Day 1 rats received 8 normal trials. On Days 2 and 3 rats received 10 trials: 8 normal trials and 2 probe trials. On Day 2 the first probe trial occurred on Trial 1 (early probe) and the second occurred on Trial 10 (late probe). On Day 3 the first probe trial occurred on Trial 1 (early probe) and the second occurred on Trial 8 (late probe).

2.2.1.2.3 Retention testing. Three weeks after training rats received a single retention test consisting of 10 trials. Trials 2 and 10 were probe trials (early and late probe, respectively). All other procedures were the same as during training.

2.2.2 Results

2.2.2.1 Histological results

Figure 4 shows the location and extent of the largest and smallest lesions. There was substantial and nearly complete, bilateral damage to the PRh in each lesioned rat. The PRh was 100 percent destroyed in the rat with the largest lesion, and 85 percent destroyed in the rat with the smallest lesion. All PRh rats also had bilateral damage to the lateral entorhinal cortex; this damage was primarily in the posterior extent of the lesions, with less, and frequently unilateral, damage evident in the anterior extent of the lesions. The rat with the largest lesion had approximately 50 percent damage to the lateral entorhinal cortex, whereas the rat with the smallest lesion sustained about 30 percent damage to this region. Damage to the anterior portion of the postrhinal cortex occurred in all PRh rats. In four of these rats this damage was bilateral and was estimated to include between 5 and 15 percent of the postrhinal cortex. In the other two rats, the postrhinal damage was unilateral and was estimated to include approximately 10-15 percent of the postrhinal cortex.

Bilateral, though minor, damage to temporal association cortex, Te2, was evident in all rats. Three of the six rats sustained unilateral damage to the CA1 and subiculum, and in one rat these areas sustained bilateral damage in the ventral portions.

2.2.2.2 Behavioural Results

2.2.2.2.1 Water-maze training. Figure 5 shows the average latency of each group of rats to find and escape onto the hidden platform on the 28 trials of water-maze training administered over three days. The average latencies to the first platform crossing were used in the Figure, but not in this analysis. A 2 x 3 x 8 (Lesion x Day x Trial) mixed-factorial ANOVA revealed significant main effects of Day ($F[2,18] = 26.975$, $p = .001$) and Trial

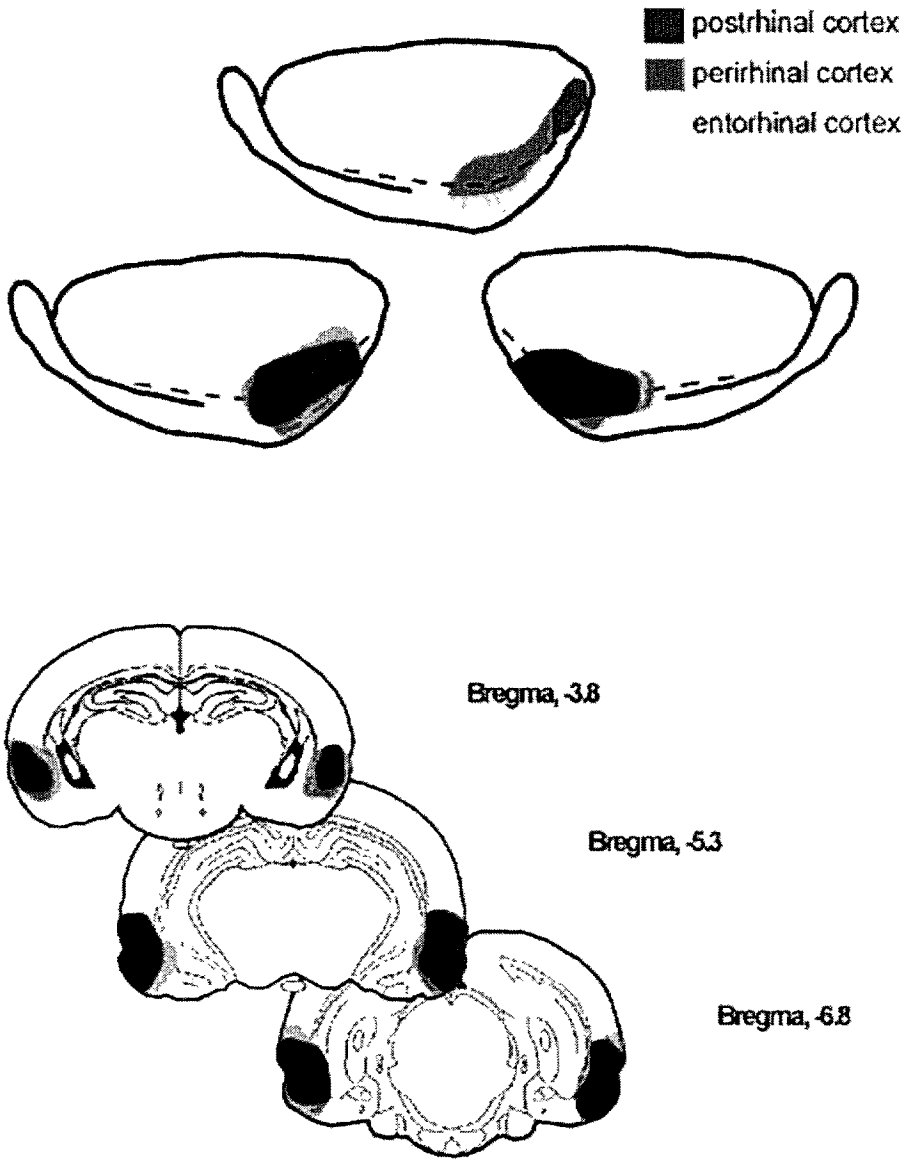


Figure 4. The location and extent of the PRh lesions are shown on the lateral surface of the rat brain in the top panel and in three coronal views in the bottom panel. In each view, the largest lesion is shown in grey and the smallest lesion is shown in black.

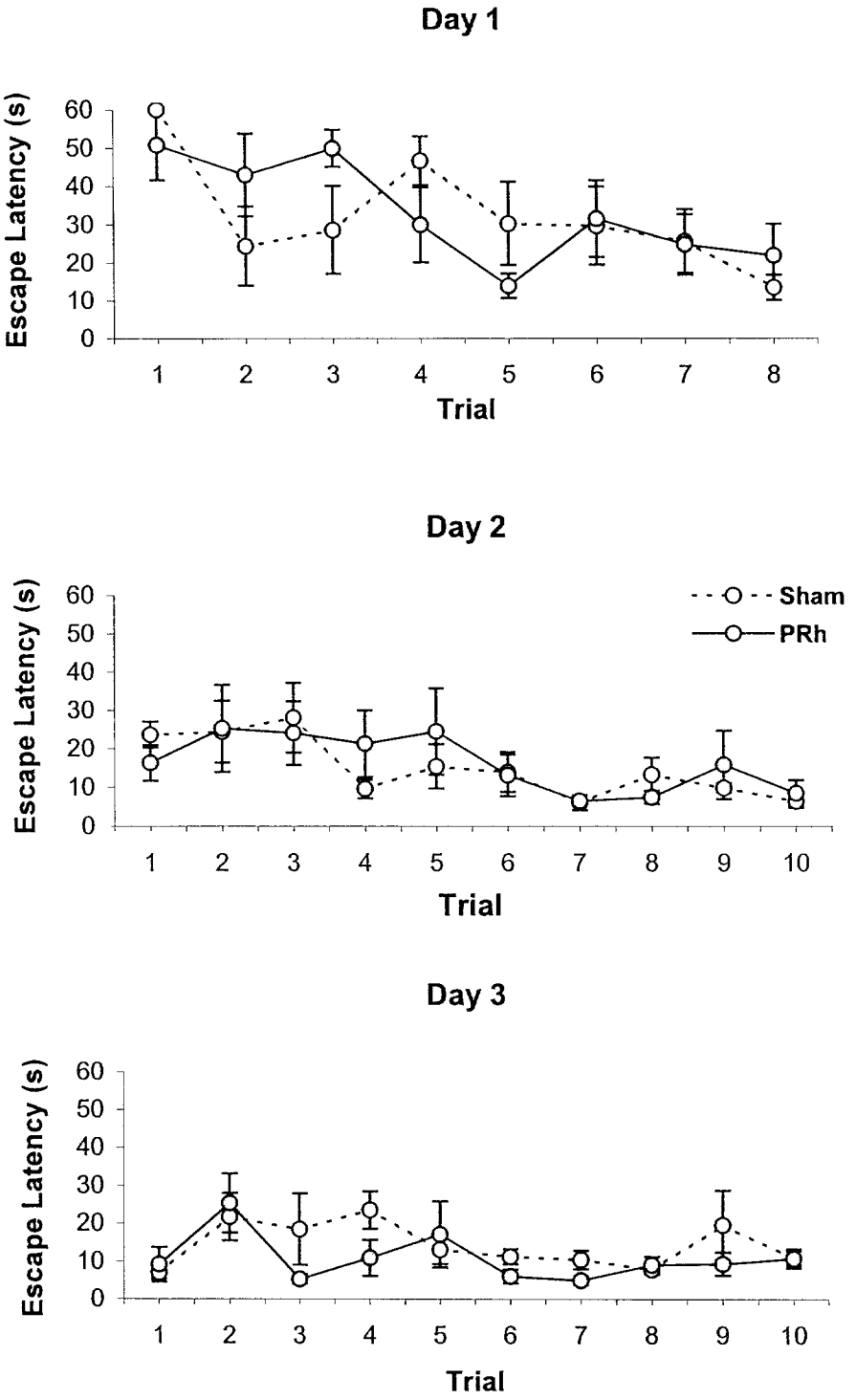


Figure 5. Mean escape latencies on the 28 water-maze acquisition trials, shown by day. The error bars represent S.E.M

($F[7,63] = 6.427$, $p = .001$). Overall, rats had longer latencies on Day 1 and on initial trials. No statistically significant differences between PRh and Sham rats were detected; the main effect of Lesion and all interactions were not statistically significant (all $ps > .10$).

The percent time Sham and PRh rats spent in the target quadrant of the maze during the early and late probe trials conducted on Days 2 and 3 of acquisition is shown in Figure 6. A 2 x 4 (Lesion x Probe) mixed factorial ANOVA revealed a significant main effect of Probe ($F[3,27] = 16.531$, $p = .001$); overall, rats spent more time in the target quadrant on the late probe trial of Day 3. There was also a significant interaction between Lesion and Probe ($F[3,27] = 4.908$, $p = .008$). The main effect of Lesion was not statistically significant ($p > .10$).

One sample t-tests comparing percent time in the target quadrant by Sham and PRh rats on each of the four probe trials revealed that neither group displayed a preference for the target quadrant on either probe conducted on Day 2 ($ps > .10$), whereas on Day 3 PRh rats showed a preference for the target quadrant on both the early and late probe trials ($t[5] = 4.569$, $p = .003$ and $t[5] = 4.561$, $p = .003$, respectively) and Sham rats showed a preference for the target quadrant on the late probe trial only ($t[4] = 4.743$, $p = .005$).

2.2.2.2.2 Retention testing. The mean escape latency of Sham and PRh rats on each trial of the retention test conducted three weeks after training are shown in Figure 7. A 2 x 10 (Lesion x Trial) mixed-factorial ANOVA conducted on escape latency revealed a significant main effect of Trial ($F[9,81] = 2.12$, $p = .037$). Overall, rats tended to have longer escape latencies on initial trials. The main effect of Lesion and the interaction between Lesion and Trial were not statistically significant ($ps > .10$).

Figure 8 shows the percentage of time each group spent in the maze quadrants on the early (Trial 2) and late (Trial 10) probe trials of the retention test. A 2 x 2 x 4 (Lesion x Probe

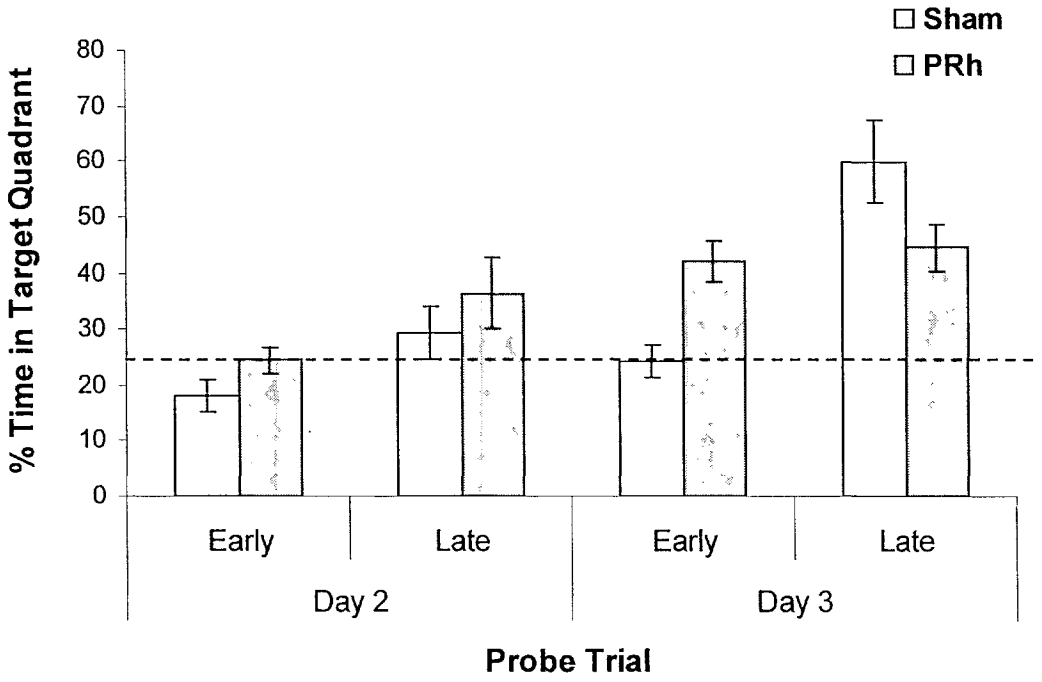


Figure 6. The percent time Sham and PRh rats spent in the target quadrant of the maze during the early and late probe trials conducted on Trials 1 and 10 of Day 2 and Trials 1 and 8 of Day 3. The error bars represent S.E.M. The dashed line indicates chance performance (25%).

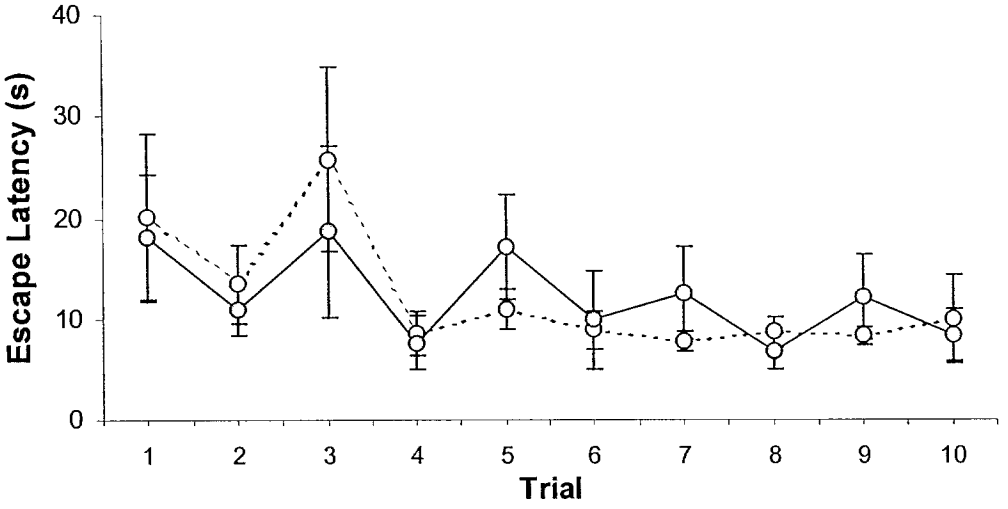


Figure 7. Mean escape of Sham and PRh rats on the 10 trials of the retention test. The error bars represent S.E.M.

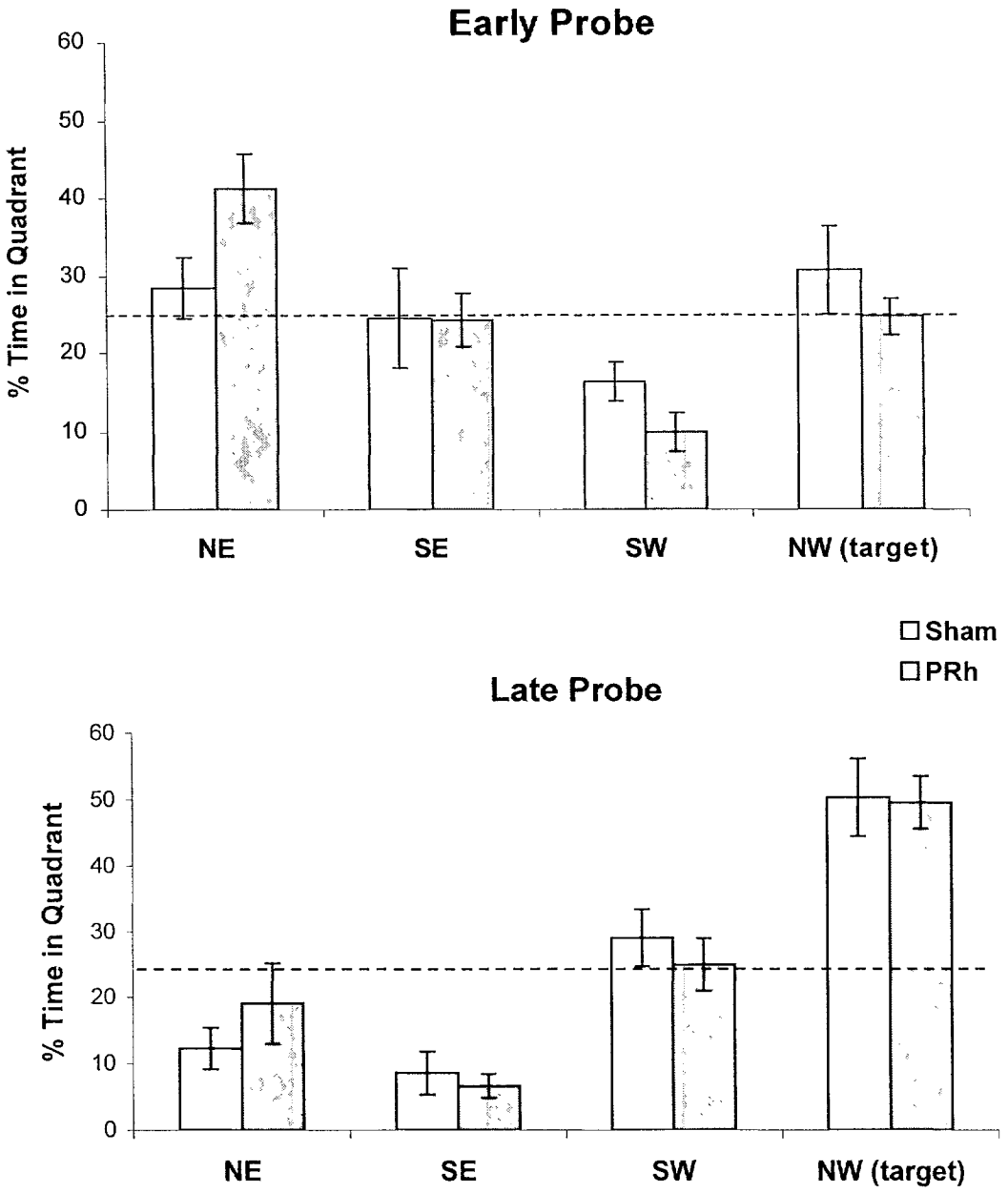


Figure 8. The percent time Sham and PRh rats spent in each quadrant of the maze during the early probe trial that was conducted on Trial 2 and the late probe trial that was conducted on Trial 10 of the retention test.. The error bars represent S.E.M. The dashed line indicates chance performance (25%).

x Quadrant) mixed-factorial ANOVA revealed a significant main effect of Quadrant ($F[3,27] = 15.132$, $p = .001$) and a significant interaction between Probe and Quadrant ($F[3,27] = 21.782$, $p = .001$). Overall, rats displayed a preference for the target quadrant; however, this effect was due to rats showing a preference for the target quadrant on the late probe trial, but not on the early probe trial. No other main effects or interactions were statistically significant ($ps > .10$).

One sample t-tests revealed that neither the PRh or sham rats spent a significant portion of their swim time in the target quadrant on the early probe trial ($t[5] = -.149$, $p = .444$ and $t[4] = 1.00$, $p = .187$, respectively). However, PRh rats, but not Sham rats, displayed a significant preference for the NE quadrant on the early probe trial ($t[5] = 3.61$, $p = .008$), and both PRh and Sham rats spent significantly less time in the SW quadrant on the early probe trial than would be expected by chance ($t[5] = -6.052$, $p = .001$ and $t[4] = -3.449$, $p = .013$, respectively). On the late probe trial, both PRh and Sham rats displayed a significant preference for the target quadrant ($t[5] = 6.20$, $p = .001$ and $t[4] = 4.32$, $p = .006$, respectively), and spent significantly less time in the SE quadrant than would be expected by chance ($t[5] = -10.247$, $p = .001$ and $t[4] = -5.10$, $p = .004$, respectively). Sham rats, but not PRh rats, also spent significantly less time than expected by chance in the NE quadrant on this trial ($t[4] = -4.106$, $p = .008$).

2.2.3 Summary

The main finding of this experiment was that aspiration lesions of the PRh did not impair the ability of rats to learn the location of a stationary and hidden platform in a water maze. PRh and Sham displayed typical learning curves over the 24 training trials, and similar degrees of preference for the target quadrant on most probe trials conducted. All rats displayed long latencies on the initial day of training and only a mild preference for the target

quadrant on early probe trials. On the final day of training, all rats showed short latencies to reach the platform on normal trials and a preference for the target quadrant on the last probe trial.

The retention test conducted 3 weeks after training revealed that both PRh and Sham rats displayed good retention of the platform's location, mostly evidenced by short latencies to find the hidden platform. Neither lesion group showed a substantial preference for the target quadrant on the early probe trial of the retention test, but both groups spent a significant portion of their swim time in this quadrant on the late probe trial. As during training, the groups did not show any marked differences in swim speed during the retention test, nor were their latencies to the first platform crossing and the numbers of platform crossings on the early and late probe trials significantly different.

The intact anterograde memory for places in rats with PRh lesions observed in the present experiment is consistent with our previous findings that PRh lesions do not impair spatial working memory (Glenn & Mumby, 1996; 1998). It is also consistent with the findings of Bussey et al. (1999; 2000) and Ennaceur et al. (1996; 1997) that PRh rats perform normally on the radial-arm and t-mazes. The findings are, however, inconsistent with those of Wiig and Bilkey (1994) and Liu and Bilkey (1998a; 1998b; 1998c; 1999; 2000). Several differences in methodology may have contributed to the differences between the latter findings and those of the present experiment. Of note, they used electrolytic or excitotoxic lesions of the PRh, whereas we used aspiration lesions. However, our lesions were larger and more complete, yet we did not detect a deficit in our PRh rats. Also, both lesion techniques produce gross tissue damage and destroy both cell bodies and fibers in the target area. Other differences in methodology include their use of a non-pigmented rat strain and a large testing room (Bilkey, personal communication). The combined effect of the lesion method, the

poor visual acuity in their rats, and the large room, in which distal cues may be more difficult to detect, may have led to the mild deficits they observed.

The combined findings from the present experiment, our past research, and the work of others provide compelling evidence that the PRh does not make an essential contribution to anterograde place memory. We further demonstrated in the present study that the PRh is also not critical for the long-term retention of place information. It is therefore possible that the PRh is not participating in spatial learning and memory functions, but instead may be contributing to some other aspect of performance that is revealed only under specific circumstances.

2.3 Experiment 2: Retrograde place memory following electrolytic lesions of the PRh: Within-subjects design

Several reports indicate that, in addition to anterograde amnesia, HPC lesions also produce a complete retrograde amnesia for place information acquired prior to surgery (Francis, 1997; Mumby et al., 1999). It is not known whether other medial temporal lobe structures make significant contributions to the retention of place information; few experiments have examined the effects of lesions to other regions on retrograde place memory. Francis (1997) assessed retrograde memory in rats with PRh lesions that had learned a place problem (stationary platform task in a water maze) either 72 or 1 hours prior to surgery. During retention testing after surgery, PRh rats did not differ from Sham rats; however neither group showed good retention of the problems learned before surgery. The poor retention displayed by rats in this experiment may have been due to the limited amount of learning that had occurred prior to surgery; rats were taught the place problem in a single training session consisting of only 10 trials.

The present experiment sought to determine whether the PRh participates in the retention of place information, an ability demonstrated to be dependent on the integrity of the hippocampus. In this experiment, rats learned two place problems prior to surgery. The first problem was learned 4 weeks before surgery (REMOTE problem) and the second problem was learned during the week before surgery (RECENT problem). A more extensive training regime was utilized to increase retention in Sham rats: 3 training sessions of 8 trials each for a total of 24 training trials on each problem.

2.3.1 Method

2.3.1.1 Subjects

Sixteen, experimentally naive rats served as subjects in this experiment.

2.3.1.2 Procedure

2.3.1.2.1 Presurgery water-maze training. All rats learned two place memory problems prior to surgery. The first problem (REMOTE) was learned over a 3-day period, 4 weeks before surgery. The second problem (RECENT) was learned during the week of surgery. Two distinct testing rooms were used to distinguish the REMOTE and RECENT problems. The use of the rooms was counterbalanced across the two time points.

Training on both problems consisted of 3 sessions of 8 trials conducted on 3 consecutive days, for a total of 24 training trials. Trials were conducted as in Experiment 1: Rats were placed in the maze at one of the four release positions and received 60 seconds to search the pool for the stationary, hidden platform. Each trial ended with rats spending 10 seconds on the platform before being removed from the pool. No probe trials were conducted during training on either of the problems.

2.3.1.2.2 Surgery. Between 24 and 48 hours after the last training day of the RECENT problem rats received either bilateral, electrolytic lesions of the PRh (n=7) or Sham surgery (n=9). As in Experiment 1, rats were anaesthetized using sodium pentobarbital (65 mg/kg). For the PRh surgery, a scalp incision was made and the muscle overlying the temporal skull was displaced. A portion of the skull overlying the PRh was removed using a hand-held dental drill. The lesion was made with a bipolar, stainless steel electrode, insulated with Teflon except for approximately 1 mm at the tip angled at 10° to the vertical plane. An electric current (1.5 mA for 10 seconds) was delivered to five sites per hemisphere using a current generator. The coordinates for each current site is shown in Table 1. Sham rats

Table 1

Coordinates in mm relative to Bregma for each of the 5 sites at which current was delivered during the electrolytic lesions of the PRh

Anterior-Posterior	*Medial-Lateral*	*Dorsal-Ventral*
7.5	8.5	8.4
6.5	8.5	9.2
5.5	8.5	9.2
4.5	8.5	9.2
3.5	8.5	9.2

received a scalp incision only. All incisions were sutured and treated with topical antibiotic. Rats were permitted to recover from surgery for two weeks prior to the commencement of retention testing.

2.3.1.2.3 Postsurgery retention testing. Retention testing consisted of two 16-trial sessions conducted one per day for two consecutive days: one session per testing room. Thus, half of the rats from each group received a retention test for the REMOTE problem first, and the other half was tested on the RECENT problem first. Procedures were the same as before surgery. However, trials 2 and 14 of the retention test were 25-second probe trials in which the platform was removed from the pool (early and late probe, respectively).

2.3.2 Results

2.3.2.1 Histological results

The location and extent of the largest and smallest PRh lesion are shown in Figure 9. Each lesion resulted in nearly complete, bilateral damage to the PRh. The largest lesion included approximately 95 percent and the smallest lesion included approximately 75 percent of the PRh. All rats sustained bilateral damage to the lateral entorhinal cortex: about 35 percent in the largest lesion and 10 percent in the smallest lesion. The anterior portion of the postrhinal cortex was also damaged in all PRh rats, but this damage was minimal— between 5 and 10 percent. The temporal association cortices sustained bilateral damage in all rats, though it was minor in most rats. There was slight, unilateral damage to the subiculum and CA1 region of the HPC in three rats.

2.3.2.2 Behavioural results

The groups were matched prior to surgery based on the escape latencies on the final two learning trials on the last day of testing for the REMOTE (PRh: M = 5.40 s, SEM =

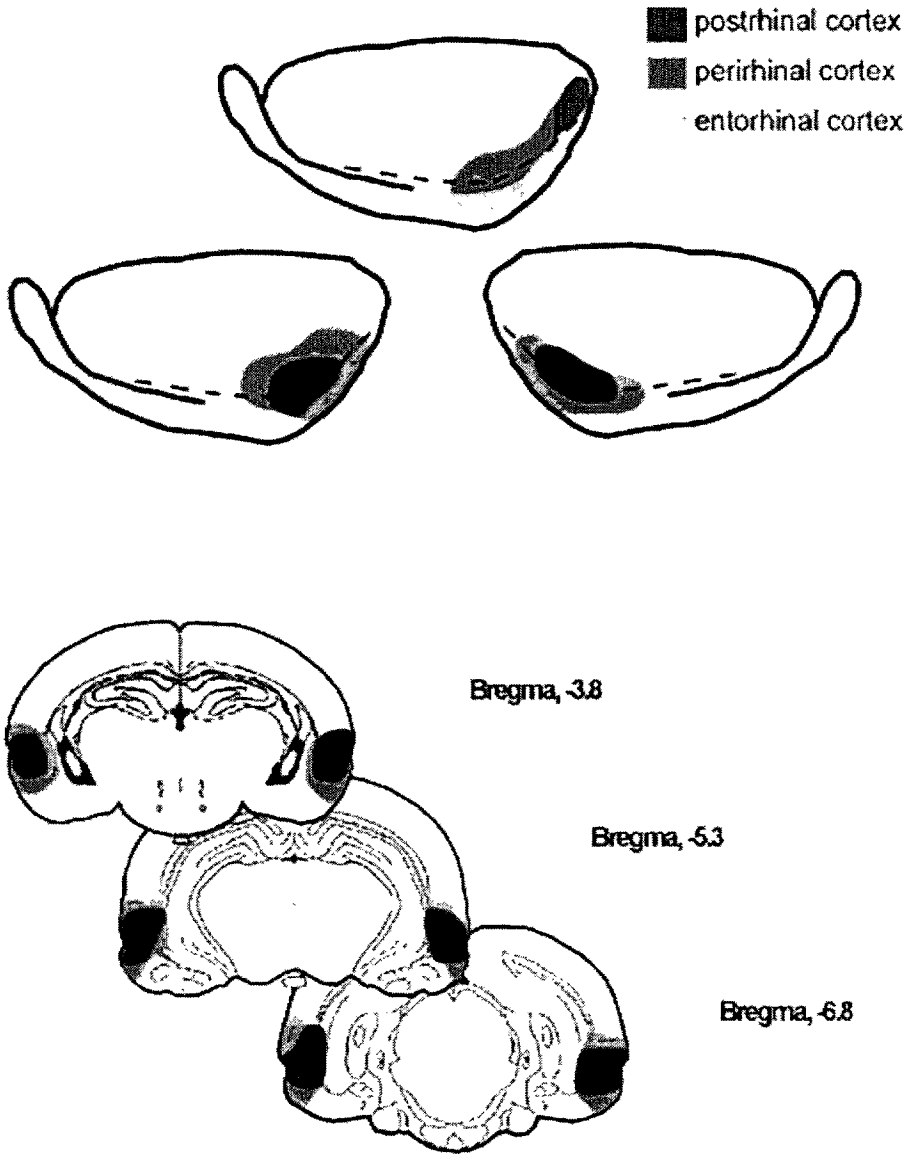


Figure 9. The location and extent of the PRh lesions are shown on the lateral surface of the rat brain in the top panel and in three coronal views in the bottom panel. In each panel, the largest lesion is shown in grey and the smallest lesion is shown in black.

0.89; Sham: M = 4.73 s, SEM = 0.35) and RECENT problems (PRh: M = 4.53 s, SEM = 0.80; Sham: M = 4.63 s, SEM = 0.73).

The average escape latencies of both groups during the retention tests for the REMOTE and RECENT problems are shown in Figure 10. A 2 x 2 x 16 (Lesion x Time of Learning x Trial) mixed-factorial ANOVA revealed a significant main effect of Trial ($F[15,210] = 14.088$, $p = .001$) and the interaction between Time of Learning and Trial approached statistical significance ($F[15,210] = 1.663$, $p = .060$). Overall, rats had longer latencies at the beginning of the session than at the end, and this was more so true during the REMOTE retention test compared to the RECENT retention test. No other main effects or interactions were statistically significant (ps $> .10$).

Figure 11 shows the percentage of time Sham and PRh rats spent in each of the quadrants on the early probe trials conducted during the REMOTE and RECENT retention tests. A 2 x 2 x 4 (Lesion x Time of Learning x Quadrant) mixed-factorial ANOVA revealed a significant main effect of Quadrant ($F[3,42] = 12.381$, $p = .001$) and a significant interaction between Lesion and Quadrant ($F[3,42] = 12.997$, $p = .001$). Overall, rats spent more time in the target quadrant. This effect was due to Sham rats, but not PRh rats, spending more time in the target quadrant. No other main effects or interactions were statistically significant (ps $> .10$).

One-sample t-tests confirmed that Sham rats displayed a significant preference for the target quadrant during the early probe trial of the REMOTE retention test ($t[8] = 3.12$, $p = .008$), and spent significantly less time in the SE and SW quadrants than would be expected by chance ($t[8] = -3.31$, $p = .006$ and $t[8] = -3.30$, $p = .006$, respectively). By contrast, PRh rats spent only as much time in each quadrant as would be expected by chance (all ps $> .10$).

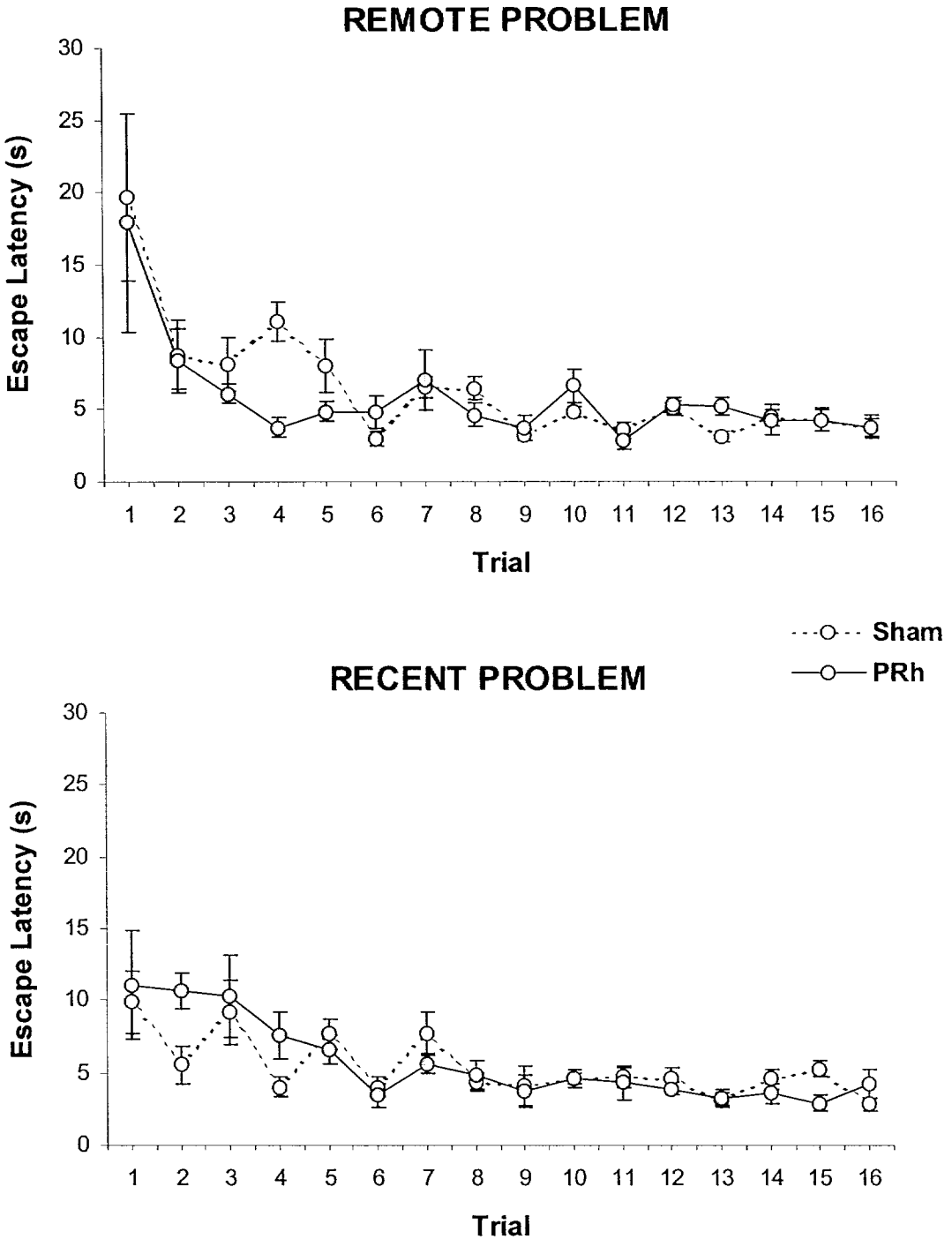


Figure 10. Mean escape latencies of Sham and PRh rats during retention and reacquisition testing on the REMOTE and RECENT place problems. The error bars represent S.E.M.

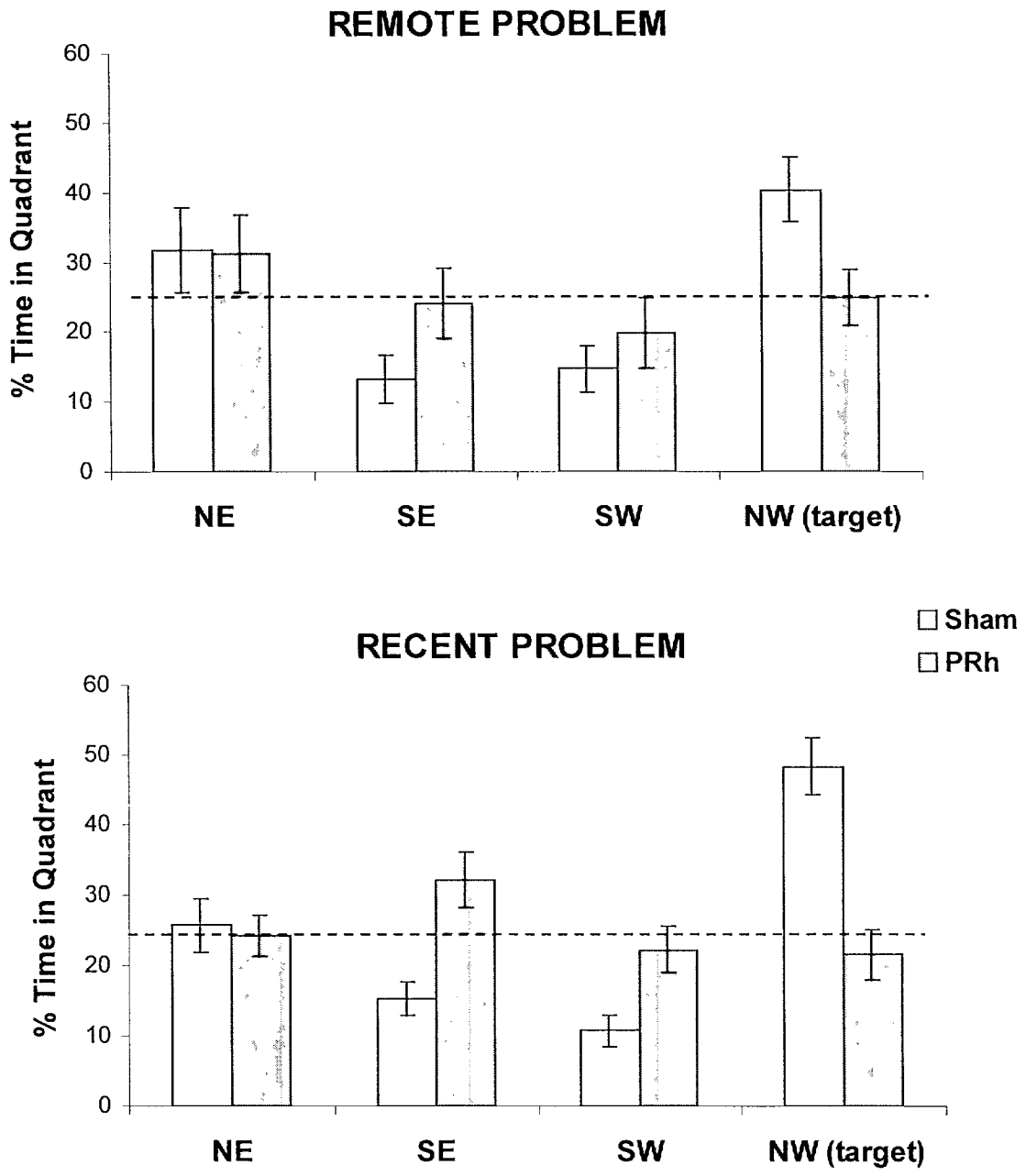


Figure 11. Mean percent time Sham and PRh rats spent in each quadrant of the pool on the early probe trials of the REMOTE and RECENT retention test. The error bars represent S.E.M.

Additionally, Sham rats spent significantly more time in the target quadrant than PRh rats ($t[14] = 2.426$, $p = .015$).

A similar pattern of results was observed for the percent time spent in quadrants on the early probe trial of the RECENT retention test. One-sample t-tests showed that Sham rats significantly preferred the target quadrant ($t[8] = -5.72$, $p = .001$) and spent significantly less time in the SE and SW quadrants than would be expected by chance ($t[8] = -3.27$, $p = .006$ and $t[8] = -6.20$, $p = .001$, respectively). PRh rats, again, showed no preference for any quadrant, and spent only as much time in each as would be expected by chance (all $ps > .10$). Additionally, Sham rats spent significantly more time in the target quadrant than PRh rats ($t[14] = 5.31$, $p = .001$), and significantly less time than PRh rats in the SE ($t[14] = -3.65$, $p = .002$) and SW ($t[14] = -2.62$, $p = .010$) quadrants.

Figure 12 shows the percentage of time Sham and PRh rats spent in each of the quadrants on the late probe trials conducted during the REMOTE and RECENT retention tests. A 2 x 2 x 4 (Lesion x Time of Learning x Quadrant) mixed-factorial ANOVA revealed a significant main effect of Quadrant ($F[3,42] = 70.967$, $p = .001$). Overall, rats spent a larger proportion of their time in the target quadrant. There were also significant interactions between Lesion and Time of Learning ($F[1,14] = 4.430$, $p = .054$) and Time of Learning and Quadrant ($F[3,42] = 2.851$, $p = .049$). The former effect was not meaningful due to the averaging of percent times spent in quadrants. The latter effect was also not particularly informative, but seemed to indicate that, overall, rats spent more time in the NE quadrant during the late probe trial of the RECENT retention test than on the REMOTE retention test. The opposite was true for the target quadrant; rats spent more time in the target quadrant during the late probe trial of the REMOTE than on the late probe trial of the

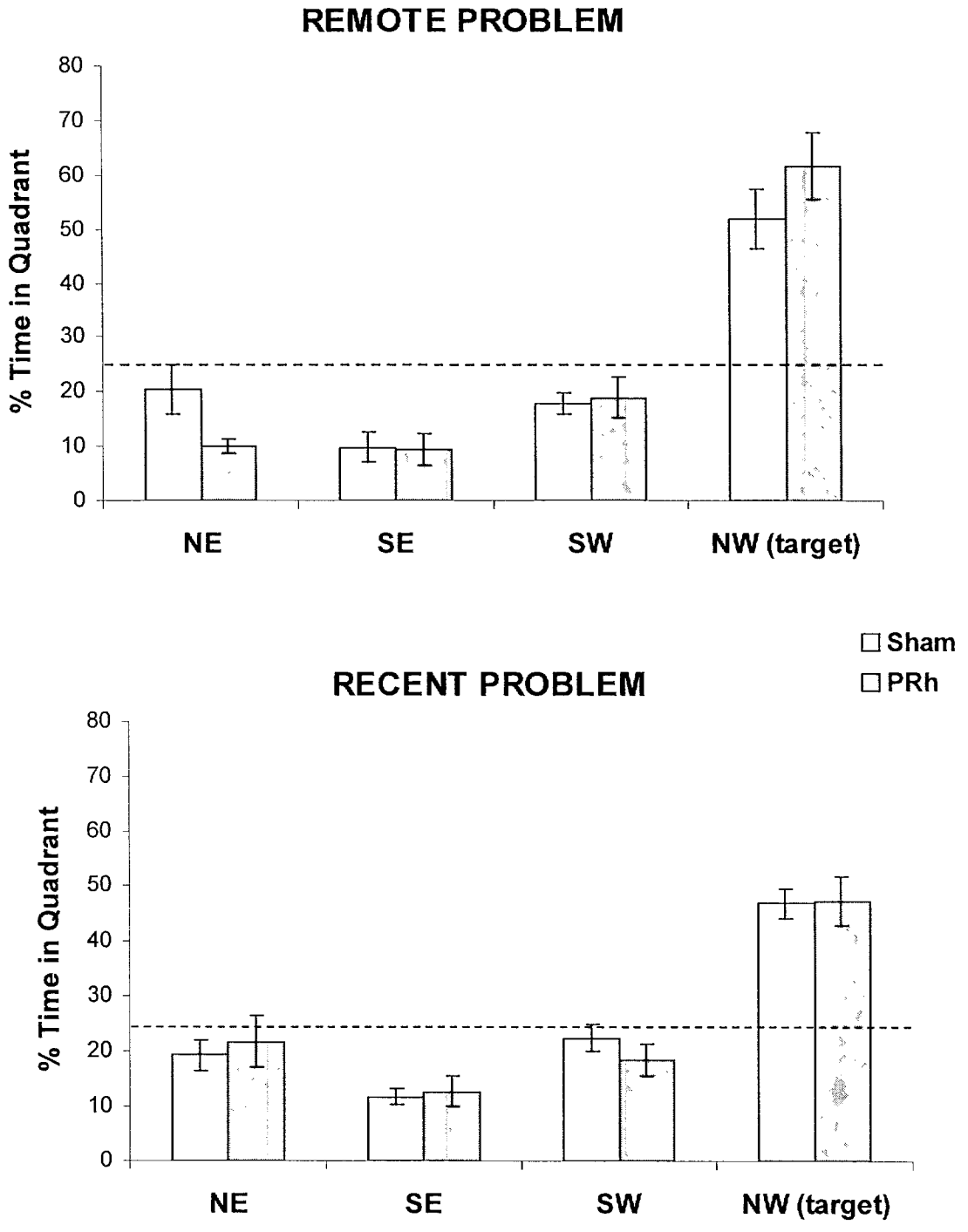


Figure 12. Mean percent time Sham and PRh rats spent in each quadrant of the pool on the late probe trials of the REMOTE and RECENT retention test. The error bars represent S.E.M.

RECENT retention test. No other main effects or interactions were statistically significant (ps >.10).

One sample t-tests showed that both Sham and PRh rats spent more time in the target quadrant during the late probe trial of the REMOTE retention test, relative to chance ($t[8] = 4.80$, $p = .001$ and $t[6] = 5.82$, $p = .001$, respectively). Additionally, Sham rats spent significantly less time in the SE and SW quadrants, relative to chance ($t[8] = -5.68$, $p = .001$ and $t[8] = -3.54$, $p = .004$, respectively) and PRh rats spent significantly less time in the NE and SE quadrants, relative to chance ($t[6] = -11.51$, $p = .001$ and $t[6] = -5.17$, $p = .001$, respectively). There was no significant difference between Sham and PRh rats in the percent time they spent in the target quadrant ($p > .10$).

During the late probe trial of the RECENT retention test both Sham and PRh rats again showed a significant preference for the target quadrant ($t[8] = 7.85$, $p = .001$ and $t[6] = 4.96$, $p = .002$, respectively). As well, Sham rats spent significantly less time in the NE and SE quadrants, relative to chance ($t[8] = -2.10$, $p = .035$ and $t[8] = -9.18$, $p = .001$, respectively), and PRh rats spent significantly less time in the SE and SW quadrants, relative to chance ($t[6] = -4.65$, $p = .002$ and $t[6] = -2.24$, $p = .033$, respectively). As with the REMOTE problem, there was no significant difference between Sham and PRh rats in the percent time they spent in the target quadrant ($p > .05$).

2.3.3 Summary

The main finding of this experiment was that rats with electrolytic lesions of the PRh displayed impaired retention of place information acquired before surgery. Retrograde amnesia was primarily evidenced by the poor performance of PRh rats on the early probe trials, in which the platform was absent from the pool. On these trials, PRh rats did not show a significant preference for the target quadrant, which previously contained the

platform, during either the REMOTE or RECENT retention test; they spent only as much time in this quadrant as would be expected by chance. Unlike PRh rats, Sham rats spent a significant portion of their swim time in the target quadrant on the early probe trials during both retention tests. PRh rats also took significantly longer than Sham rats to locate the platform on the initial trials of the retention test for the RECENT problem, and displayed fewer platform crossings than Sham rats on the early probe trial of that test (see Appendix B). These differences were not evident on the retention test for the REMOTE problem, and provide some evidence that the retrograde amnesia may have a temporal gradient.

Despite the clear lack of a preference for the target quadrant by the PRh rats on the early probe trials, there were no significant differences between the groups on the late probe trials; both PRh and Sham rats spent a significant portion of their swim time in the target quadrant. As well, the latencies of PRh rats on trials following the early probe trials did not differ from those of Sham rats during the REMOTE and RECENT retention test. Thus, despite showing deficient retention for the platform's location on initial trials, the PRh rats showed normal performance on subsequent trials, including the late probe trials, indicating rapid reacquisition of the problems. These findings also indicate that it is unlikely that the PRh rats displayed deficits on the early probe trial due to a lesion-induced change in motivation or swim strategy. It is expected that these types of changes would be most evident on the first retention test, not both. The retention tests were conducted on consecutive days with half of each group tested for the REMOTE problem first and RECENT problem second; the remaining rats received the reverse order. That we observed the same pattern of impaired retention on initial trials and excellent performance on later trials on both days is not consistent with non-specific and transient lesion effects masking normal retention.

The present, novel result, that PRh lesions produced retrograde amnesia for places, is consistent with reports that rats with PRh lesions display anterograde deficits in working and reference memory on the water maze and radial-arm maze tasks (Wiig & Bilkey, 1994; Liu & Bilkey, 1998a; 1998b; 1998c; 1999; 2000). However, it is inconsistent with other evidence that PRh lesions do not produce anterograde amnesia for places on the same tasks (Bussey et al., 1999; 2000; Ennaceur et al., 1996; 1997; Glenn & Mumby, 1996; 1998). The combined findings from these experiments, that PRh lesions produce acquisition deficits in some experiments, no deficits in others, and a transient memory loss for place information acquired prior to surgery (present experiment), suggests that the PRh plays a minor role in normal anterograde and retrograde place memory.

Though the contribution of the PRh to normal place memory appears to be slight, the evidence that it may play some role in the acquisition and/or retention of place information is intriguing. One question that arises is whether the PRh is important for spatial processing or navigation, functions typically attributed to the HPC. That is, are the deficits, when they occur, due to an inability of the HPC to access sensory information about some features of the environment that normally arise from PRh? A related question is whether the PRh is actually contributing to the processing of information about the environment that is a nonessential, but useful, aspect of the entire learning event. Identifying the nature of the contribution of the PRh to place memory may yield important clues about PRh function specifically, and the organization of memory in the MTL generally.

2.4 Experiment 3: Retrograde place memory following aspiration lesions of the PRh: Within-subjects design

In Experiment 2, we found that PRh lesions produced retrograde amnesia, without a temporal gradient, for place information acquired 4 weeks and 2 days before surgery. This finding was remarkable, as we had not previously observed anterograde place memory deficits in rats with PRh lesions (Glenn & Mumby, 1996; 1998; and Experiment 1, present thesis). It also provided further support for the notion that under specific circumstances the PRh is recruited to support some, as yet unidentified, aspect of place memory. Since there are little data available on the effects of PRh lesions on retrograde place memory, we sought to further investigate this finding.

One methodological difference between Experiment 2 and our previous reports of intact anterograde memory for water-maze problems (Glenn & Mumby, 1996; 1998; Experiment 1, present thesis) is the lesion technique used to ablate PRh tissue. In Experiment 1, and in our previous work, PRh lesions were made using aspiration, whereas in Experiment 2 we made the PRh lesions electrolytically. Also, some key studies discussed above, which reported anterograde place memory deficits, made electrolytic PRh lesions (Liu & Bilkey, 1998a; 1998b; Wiig & Bilkey, 1994). Thus, it was of interest to us to determine whether the lesion method could be a critical factor in the retrograde amnesia that we observed. While both techniques produce widespread damage to both cell bodies and fibres in the target region, it is possible that electrolytic, but not aspiration lesions, disrupt processes elsewhere in the MTL, perhaps in the entorhinal cortex or the HPC. This might occur because the strong current used during the electrolytic surgery (1.5 mA for 10 s at 5

sites per hemisphere) produces an abnormal cascade of activation that may disturb normal function in structures efferent to the PRh.

The main purpose of the present experiment was to reproduce the findings from Experiment 2 in rats with aspiration lesions of the PRh. Several recent studies have attempted to determine whether certain aspects of the training and testing procedures can account for the inconsistent results discussed above, but no critical procedural features have been identified so far (Glenn & Mumby, unpublished data, also see Liu & Bilkey, 1998c; 2001). To our knowledge, only electrolytic lesions of the PRh produce retrograde amnesia for places. Thus, it was important to determine whether the retrograde amnesia we observed in Experiment 2 occurs following PRh lesions that are made in other ways. We also decided to make aspiration lesions of the PRh in the present experiment because deficits in the acquisition of place information were not observed when this technique was used (Glenn & Mumby, 1996; 1998; Experiment 1, present thesis). All other features of Experiment 2 were replicated in the present experiment.

2.4.1 Method

2.4.1.1 Subjects

Seventeen experimentally naïve rats served as subjects.

2.4.1.2 Procedure

2.4.1.2.1 Presurgery water-maze training. Presurgery training on each problem was the same as in Experiment 2: rats learned one problem 4 weeks before surgery (REMOTE) and the other problem was learned during the week of surgery (RECENT). Rats received a total of 24 training trials administered over 3 days on each problem.

2.4.1.2.2 Surgery. Approximately 48 hours after the final day of training on the RECENT problem, rats received either bilateral PRh lesions (n = 9) or sham surgery (n = 8).

Surgical procedures were as in Experiment 1. Following surgery rats were permitted to recover for 2 weeks prior to the commencement of retention testing.

2.4.1.2.3 Postsurgery retention testing. As in Experiment 2, retention tests were conducted on 2 consecutive days, 1 day for each problem, with the sequence counterbalanced across groups. Each test session consisted of 16 trials. Trials 2 and 14 were 30-second probe trials (EARLY and LATE probe respectively) in which the platform was removed from the pool and the search patterns of rats were recorded. The latency to the first platform crossing was used as a measure of escape latency on probe trials.

2.4.2 Results

2.4.2.1 Histological results

Figure 13 shows the location and extent of the PRh lesions. There was substantial and nearly complete, bilateral damage to the PRh in each lesioned rat. The PRh was 90 percent destroyed in the rat with the largest lesion, and 70 percent destroyed in the rat with the smallest lesion. All PRh rats also had bilateral damage to the lateral entorhinal cortex; this damage was primarily in the posterior extent of the lesions, with less, and frequently unilateral, damage evident in the anterior extent of the lesions. The rat with the largest lesion had approximately 40 percent damage to the lateral entorhinal cortex, whereas the rat with the smallest lesion sustained about 25 percent damage to this region.

Damage to the anterior portion of the postrhinal cortex occurred in 7 of the 9 PRh rats. In four of these rats this damage was bilateral and was estimated to include approximately 10 percent of the postrhinal cortex. In the other three rats, the postrhinal damage was unilateral and was estimated to include approximately 10-15 percent of the postrhinal cortex.

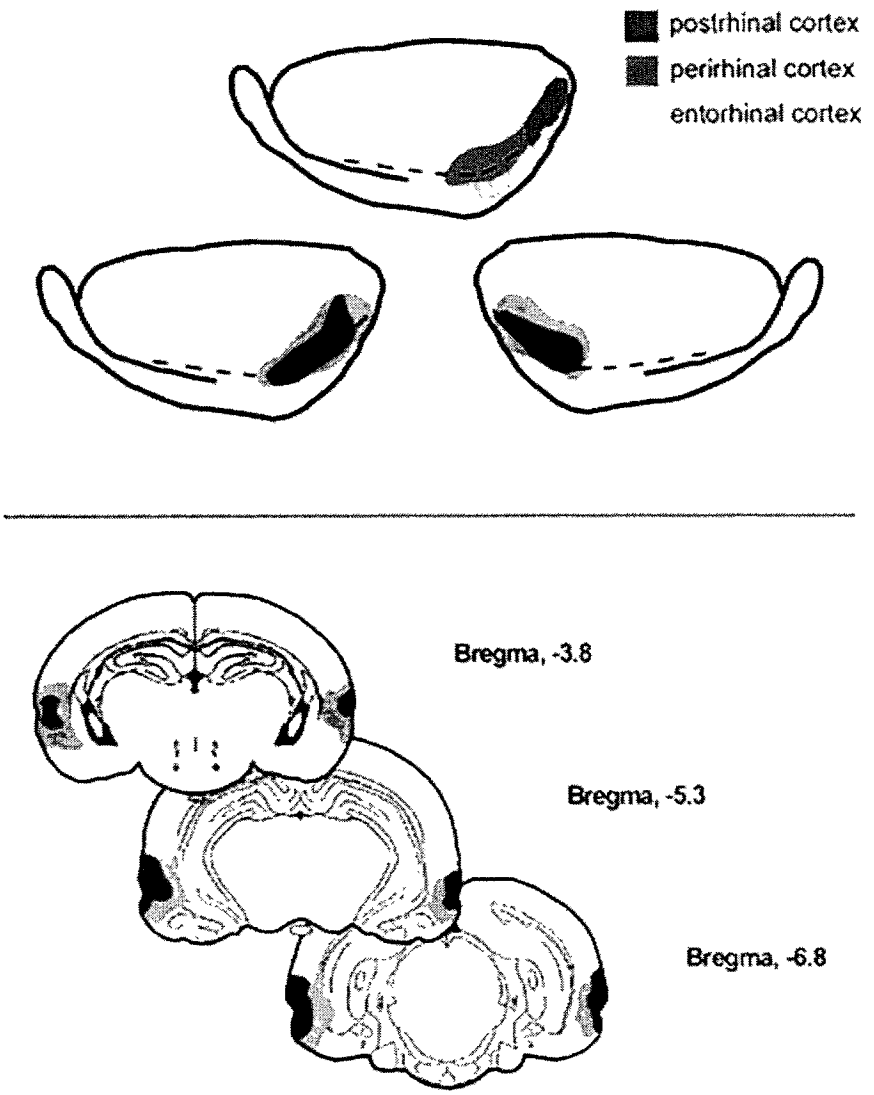


Figure 13. The location and extent of the PRh lesions are shown on the lateral surface of the rat brain in the top panel and in three coronal views in the bottom panel. In each panel, the largest lesion is shown in grey and the smallest lesion is shown in black.

Four of the 9 PRh rats also had unilateral damage to the ventral portions of the temporal association cortex. This region was estimated to be about 10 percent damaged in both the rat with the largest lesion and the rat with the smallest lesion. The CA1 subfield of the hippocampus sustained minor damage, unilaterally, in three rats. One rat had unilateral damage to the piriform cortex, and another rat had unilateral damage to the dorsolateral amygdala.

2.4.3.2 Behavioural results

PRh and Sham groups were matched on their latencies to escape onto the platform on the final 2 trials of training for both the REMOTE (PRh: $\underline{M}$ = 6.91 s, $\underline{SEM}$ = 0.55; Sham: $\underline{M}$ = 7.90 s, $\underline{SEM}$ = 0.86) and RECENT (PRh: $\underline{M}$ = 5.74 s, $\underline{SEM}$ = 0.32; Sham: $\underline{M}$ = 5.38 s, $\underline{SEM}$ = 0.37) problems.

Figure 14 shows the average escape latencies of both groups on each trial of the REMOTE and RECENT retention tests. As in Experiment 2, trials 2 and 14 were probe trials in which the platform was removed from the pool. The latencies to the first platform crossing are shown in the Figure and were used in the analysis. A 2 x 2 x 16 (Lesion x Time of Learning x Trial) mixed factorial ANOVA revealed significant main effects of Time of Learning ($\underline{F}[1, 15] = 6.145$, $\underline{p} = .026$) and Trial ($\underline{F}[15,225] = 5.795$, $\underline{p} = .001$). Overall, escape latencies were higher on the REMOTE retention test than on the RECENT retention test and, on both tests, latencies tended to be longer on initial trials compared to later trials. The main effect of Lesion and the interactions were not statistically significant ($\underline{p}$s $>.10$).

An interesting finding was that PRh-lesioned rats tended to have longer escape latencies on trials that followed probes (Figure 14, top panel see trial 3, and bottom panel see trials 3 and 15). However, the large variance on those trials obscured any statistically significant group effects, even when analyzed separately ($\underline{p}$s $>.10$).

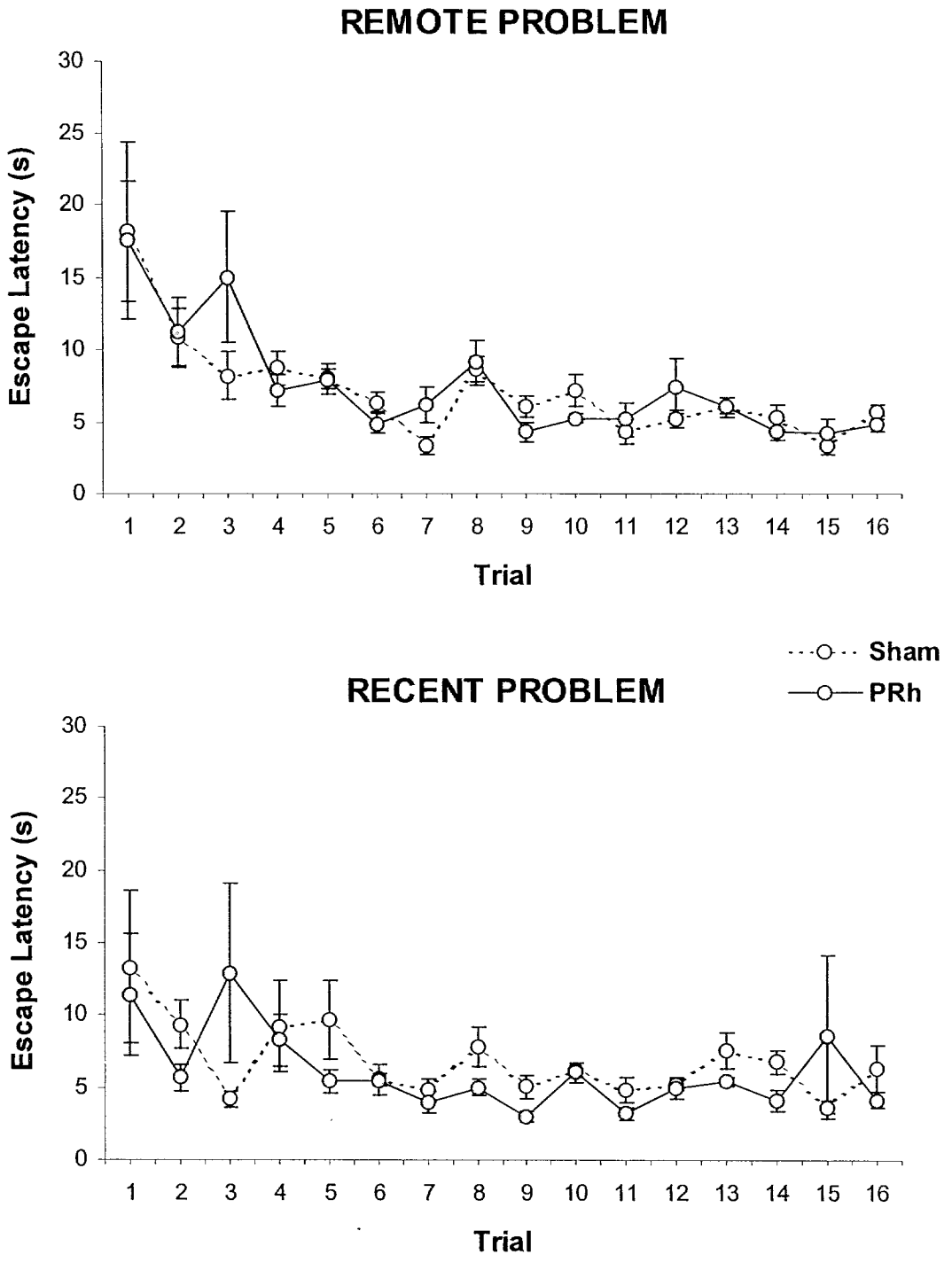


Figure 14. Mean escape latencies of Sham and PRh rats during retention and reacquisition testing on the REMOTE and RECENT place problems. The error bars represent S.E.M.

As in Experiment 2, the performance of rats on the early probe trial was considered an important indicator of how well the rats recalled the problems learned before surgery. The percentage of time both groups spent in each of the quadrants on the early probe trials of the REMOTE and RECENT retention tests is depicted in Figure 15. A 2 x 2 x 4 (Lesion x Time of Learning x Quadrant) mixed factorial ANOVA revealed a significant main effect of Quadrant ($F[3,45] = 15.950$, $p = .001$) and the interaction between Time of Learning and Quadrant approached statistical significance ($F[15,225] = 2.458$, $p = .075$). Overall, rats tended to spend most of their time in the NE and NW (target) quadrants. However, this was more so true of the REMOTE problem, whereas on the RECENT problem rats spent more time in the target quadrant than in the other three quadrants. The other main effects and interactions were either not statistically significant or were not interpretable ($ps > .10$).

One-sample t-tests comparing the percent time each group spent in each quadrant, relative to chance performance, on the early probe trials provided further evidence that rats did not remember the REMOTE problem as well as the RECENT problem. PRh rats displayed a significant preference for the target quadrant on the REMOTE problem ($t[8] = 1.865$, $p = .050$), whereas this was only marginally the case for Sham rats ($t[7] = 1.671$, $p = .070$). Sham rats did show a significant preference for the NE quadrant ($t[7] = 1.849$, $p = .054$), and spent significantly less time in the SW quadrant than would be predicted by chance ($t[7] = -3.853$, $p = .003$). PRh rats spent significantly less time than chance in the SE quadrant ($t[8] = -2.633$, $p = .015$). Time spent in all other quadrants by Sham and PRh rats was not significantly different from chance ($ps > .10$).

For the RECENT problem, both PRh and Sham rats displayed a significant preference for the target quadrant ($t[8] = 3.688$, $p = .003$ and $t[7] = 5.140$, $p = .001$, respectively). Additionally, both PRh and Sham rats spent significantly less time, relative to chance, in the

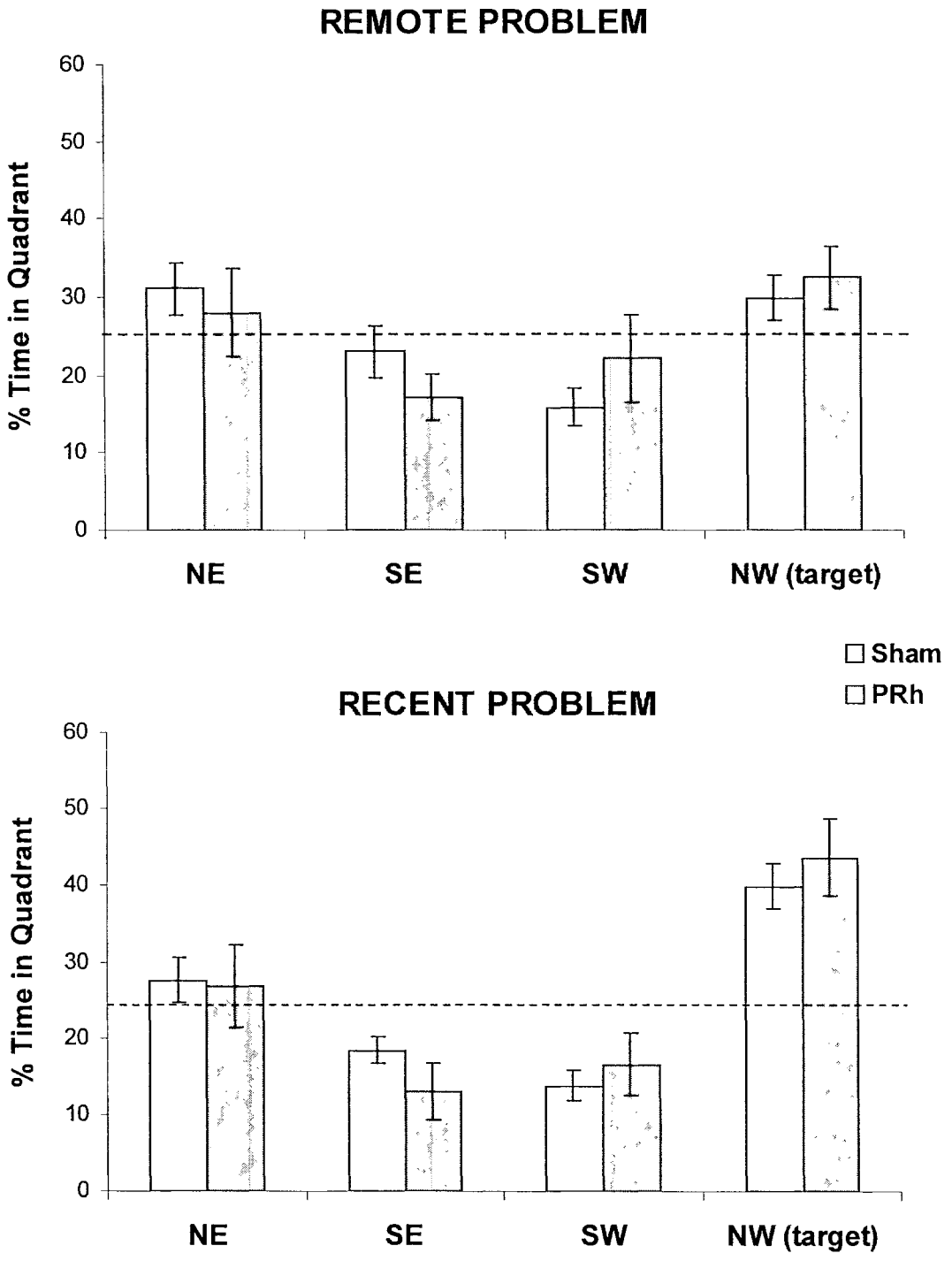


Figure 15. Mean percent time Sham and PRh rats spent in each quadrant of the pool on the early probe trials of the REMOTE and RECENT retention test. The error bars represent S.E.M.

SE ($t[8] = -3.251$, $p = .006$ and $t[7] = -3.772$, $p = .004$, respectively) and SW ($t[8] = -2.007$, $p = .040$ and $t[7] = -5.516$, $p = .001$, respectively) quadrants. Neither group spent significantly more or less time in the NE quadrant, relative to chance.

Figure 16 shows the percentage of time both groups spent in each of the quadrants on the late probe trials of the REMOTE and RECENT retention tests. A 2 x 2 x 4 (Lesion x Time of Learning x Quadrant) mixed factorial ANOVA revealed a significant main effect of Quadrant ($F[3,45] = 89.307$, $p = .001$) and a significant interaction between Lesion and Quadrant ($F[3,45] = 4.795$, $p = .006$). Overall, rats spent the largest proportion of their swim time in the target quadrant, and PRh rats spent significantly less time than Sham rats in the NE quadrant and significantly more time in the target quadrant ($ps < .05$) during the REMOTE retention test, whereas the groups spent similar proportions of time in each quadrant during the RECENT retention test.

One sample t-tests conducted on the percent time in quadrants on the late probe trials also showed that both PRh and Sham rats spent significant proportions of their time in the target quadrant, relative to chance, on the REMOTE ($t[8] = 7.883$, $p = .001$ and $t[7] = 5.160$, $p = .001$, respectively) and RECENT ($t[8] = 3.553$, $p = .005$ and $t[7] = 5.618$, $p = .001$, respectively) problems. Additionally, for the REMOTE problem, PRh and Sham rats spent significantly less time than would be expected by chance in the SE ($t[8] = -7.458$, $p = .001$ and $t[7] = -9.732$, $p = .001$, respectively) and SW ($t[8] = -1.955$, $p = .043$ and $t[7] = -2.046$, $p = .040$, respectively) quadrants. PRh also spent significantly less time in the NE quadrant ($t[8] = -4.338$, $p = .001$), but Sham rats did not differ from chance in the proportion of time they spent in this quadrant. For the RECENT problem, PRh and Sham rats spent significantly less time in the SE quadrant than chance would predict ($t[8] = -5.501$,

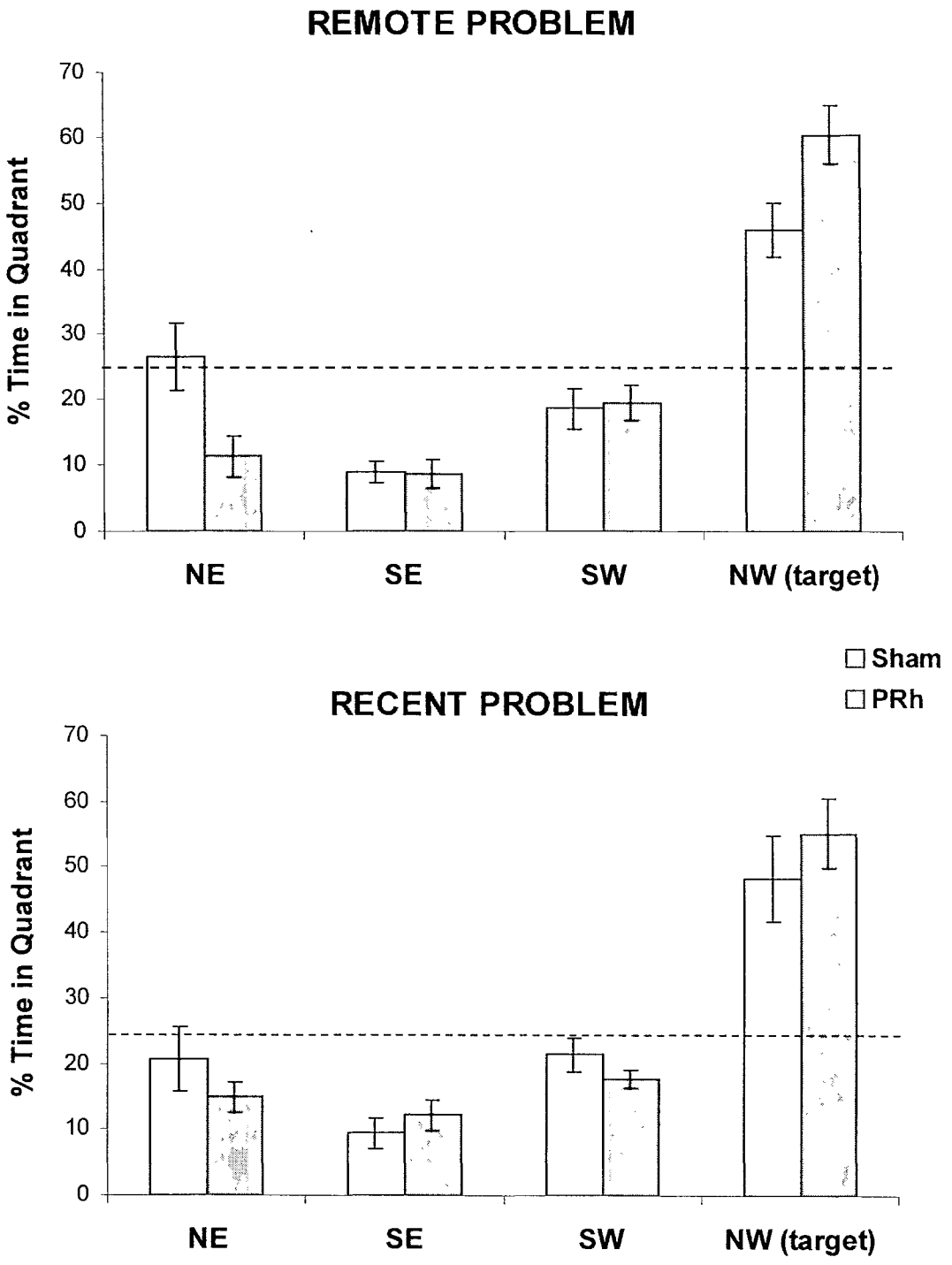


Figure 16. Mean percent time Sham and PRh rats spent in each quadrant of the pool on the late probe trials of the REMOTE and RECENT retention test. The error bars represent S.E.M.

$p = .001$ and $t[7] = -6.446$, $p = .001$, respectively), but only PRh rats spent significantly less time in the NE ($t[8] = -4.286$, $p = .002$) and SW ($t[8] = -5.340$, $p = .001$); Sham rats were not significantly different from chance.

2.4.3 Summary

The PRh lesions in this experiment did not produce retrograde amnesia for the water-maze problems learned 4 weeks and 2 days prior to surgery. PRh rats spent as much time as Sham rats searching for the platform in the target quadrant on the early probe trials for both the REMOTE and RECENT problems. This finding is inconsistent with our findings from Experiment 2, that PRh rats did not show a preference for the target quadrant on early probe trials for either the REMOTE or RECENT problem. We also observed that in Experiment 2 PRh rats had significantly longer latencies to escape onto the hidden platform on the first trial compared to Sham rats during the retention test for the RECENT problem, but not the REMOTE problem. In the present experiment, PRh and Sham rats' latencies did not differ significantly at any point during either retention tests.

Though this experiment utilized the same design and training/testing procedures as in Experiment 2, subtle differences resulting from conducting the experiments several months apart may have contributed to the disparate findings. However, in our previous study, PRh lesions were made electrolytically, whereas in the current experiment they were made by aspiration, and this difference may have contributed substantially to the varied effects. Both techniques produced widespread damage to the PRh, however the aspiration lesions in the present experiment tended to include more tissue in adjacent regions. The electrolytic lesion itself may have consequences for normal learning and memory, perhaps by disrupting function in the entorhinal cortex or HPC— regions efferent to the PRh. It seems unlikely

that the larger, less specific lesions of this experiment would not produce deficits, and the more discrete lesions in our earlier experiment would.

Despite the lack of retrograde amnesia in the PRh rats in the current study, there was other evidence that they were not performing the task in the same way as the Sham rats. We observed that PRh rats took longer to locate the hidden platform on normal trials (in which the platform was present) that immediately followed probe trials (in which the platform was absent). While the differences were not statistically significant, they were observed following 3 of the 4 probe trials administered during the retention tests. This finding suggests that PRh damage may disturb some aspect of task performance that may be of use in solving the task, but is not critical to successful retention. Thus, this transient contribution may be subtle and difficult to detect.

2.5 Experiment 4: Retrograde place memory following aspiration lesions of the PRh: Between-subjects

In Experiment 3, we found that aspiration lesions of the PRh did not produce retrograde amnesia for the platform locations learned prior to surgery. Combined with the findings from Experiment 2, this suggests that the lesion method may be a critical factor in the expression of retrograde amnesia for place information. In this experiment, we addressed the possibility that the experimental design may also play an important role in whether retrograde amnesia is observed.

This experiment focused on our previous use of a mixed design (Experiments 2 and 3) in which the Time of Learning variable (REMOTE versus RECENT) was a within-subjects factor. This type of design is typically favoured in studies assessing retrograde amnesia as it is thought to more closely reflect the syndrome as it occurs in human patients. There are, however, disadvantages to using this type of design in animal studies (for a comprehensive discussion see Murray & Bussey, 2001). For example, animals may acquire a learning set that will affect the rate of learning or the manner in which they learn subsequent problems. Therefore, it is difficult to ensure that the same amount and type of learning is occurring when subjects are trained on ostensibly equivalent problems. In this experiment, we sought to explore the possible impact of this by conducting the same study as in Experiment 3 using a completely between-groups design. Thus, rats learned only a single place problem either 4 weeks before or during the week of surgery.

2.5.1 Method

2.5.1.1 Subjects

Twenty-two experimentally naïve rats served as subjects.

2.5.1.2 Procedure

2.5.1.2.1 Presurgery water-maze training. Four weeks prior to surgery (REMOTE), half the rats were taught the location of a stationary, hidden platform in a water maze. During the week before surgery (RECENT) the other half of the rats were taught the problem. The same two, distinct testing rooms used in Experiment 1 were used in this experiment. The rooms were counterbalanced at each time point. Each rat received the same training as in Experiment 2 and 3; three 8-trial sessions on consecutive days.

2.5.1.2.2 Surgery. Approximately 48 hours after the final day of training on the RECENT problems, all rats underwent either bilateral aspiration lesions of the PRh (REMOTE, n = 6; RECENT, n=6) or sham surgery (REMOTE, n=5; RECENT, n = 5). Surgical procedures were the same as in Experiment 1. Rats recovered for 2 weeks prior to the commencement of retention testing. One rat died during recovery, leaving 4 sham rats in the REMOTE condition.

2.5.1.2.3 Postsurgery retention testing. The retention test consisted of a single, 15-trial session, and trials 2 and 13 were probe trials (EARLY and LATE probe respectively) in which the platform was removed from the pool and the search patterns of rats were collected for a 30-second period.

2.5.2 Results

2.5.2.1 Histological results

Figure 17 shows the location and extent of the PRh lesions. As in Experiment 1, there was nearly complete, bilateral destruction of the PRh in each lesioned rat. The PRh was 100 percent damaged in the rat with the largest lesion, and approximately 80 percent damaged in the rat with the smallest lesion. All PRh rats in this experiment also had bilateral damage to

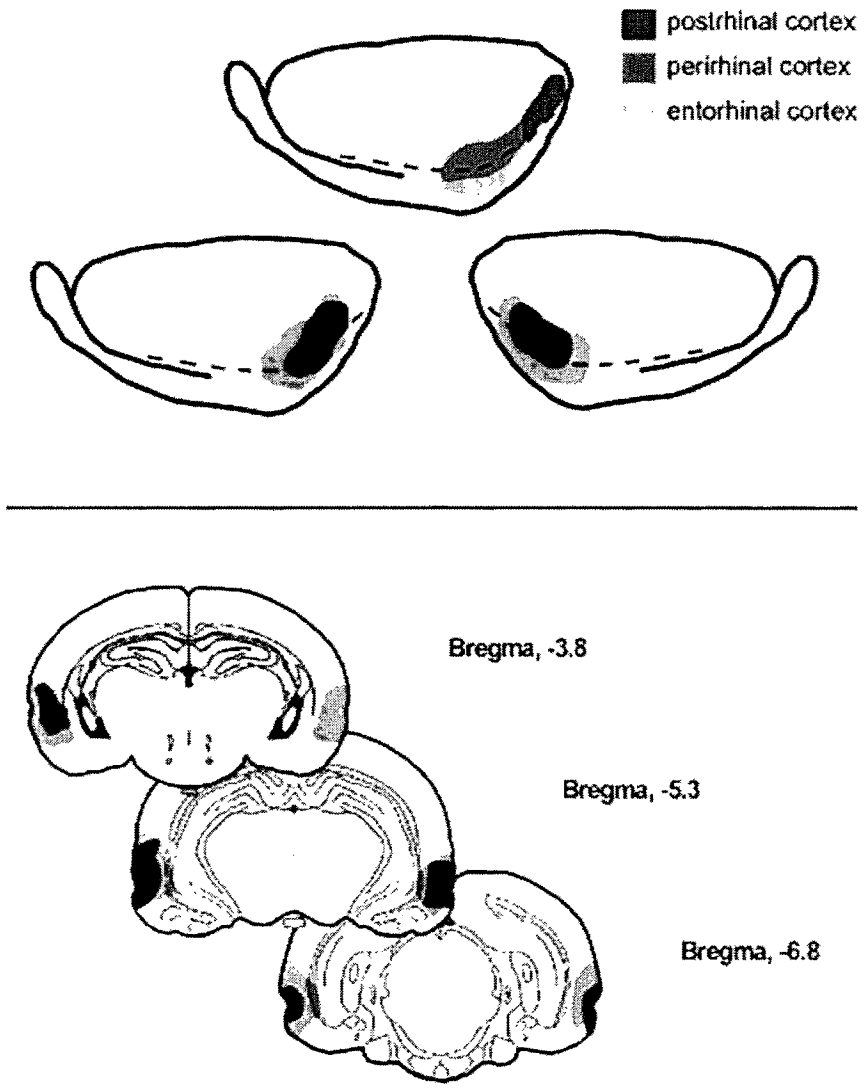


Figure 17. The location and extent of the PRh lesions are shown on the lateral surface of the rat brain in the top panel and in three coronal views in the bottom panel. In each panel, the largest lesion is shown in grey and the smallest lesion is shown in black.

the lateral entorhinal cortex. This structure was approximately 50 percent damaged in the rat with the largest lesion, and 15 percent damaged in the rat with the smallest lesion. This damage also tended to be worse in the posterior extent of the lesions, with more bilateral sparing of the lateral entorhinal cortex in the anterior extent of the lesions.

There was also bilateral damage to the anterior region of the postrhinal cortex in 8 of the 12 rats. This damage was estimated to encompass approximately 10-30 percent of the postrhinal cortex. The other 5 rats had unilateral damage to this area, estimated to include approximately 5-10 percent of the postrhinal cortex. Five of the 8 rats with bilateral postrhinal cortical damage were in the REMOTE group, and the other three were in the RECENT group.

Four of the 12 PRh rats had bilateral damage to the ventral portions of the temporal association cortex, and two had unilateral damage in this region. The rat with the largest lesion sustained approximately 25 percent damage to this area, whereas in the rat with the smallest lesion less than 5 percent was damaged. In two PRh rats there was unilateral damage to the CA1 subfield of the hippocampus, in one rat there was minor, but bilateral damage to this region, and another rat had unilateral damage to the subiculum. One rat had a small amount of bilateral damage to the piriform cortex. There was also slight, unilateral damage to the amygdala in two rats, and bilateral damage in one rat.

2.5.2.2 Behavioural Results

The 4 groups of rats were matched on their latencies to escape onto the hidden platform on the final two trials of training (PRh-REMOTE: $\underline{M}$ = 7.37 s, $\underline{SEM}$ = 0.78; Sham-REMOTE: $\underline{M}$ = 10.83 s, $\underline{SEM}$ = 1.72; PRh-RECENT: $\underline{M}$ = 8.14 s, $\underline{SEM}$ = 1.34; Sham-RECENT: $\underline{M}$ = 7.58 s, $\underline{SEM}$ = 1.57).

Figure 18 shows the average escape latencies of each of the 4 groups. A 2 x 2 x 15 (Lesion x Time of Learning x Trial) revealed a significant interaction between Lesion and Trial ($F[14,238] = 1.935$, $p = .024$). PRh rats that learned the RECENT problem had significantly longer latencies than Sham rats on the first trial of the retention test ($t[9] = -2.236$, $p = .026$); this was not the case for the PRh and Sham rats that learned the REMOTE problem. Due to the longer latency of the PRh rats that learned the REMOTE problem there was also a significant main effect of Trial ($F[14,238] = 2.748$, $p = .001$) and a significant interaction between Trial and Problem ($F[14,238] = 1.943$, $p = .023$). All other main effects and interactions were not significant ($ps > .10$).

The percentage of time spent in each quadrant on the early probe trial by Sham and PRh rats that learned either the REMOTE or RECENT problem is shown in Figure 19. A 2 x 2 x 4 (Lesion x Time of Learning x Quadrant) mixed factorial ANOVA revealed a significant main effect of Quadrant ($F[3,51] = 25.003$, $p = .001$) and significant interactions between Lesion and Quadrant ($F[3,51] = 3.863$, $p = .014$) and Lesion, Time of Learning, and Quadrant ($F[3,51] = 3.236$, $p = .030$). PRh and Sham rats that learned the REMOTE problem spent similar amounts of time in all quadrants, whereas Sham rats that learned the RECENT problem spent significantly more time than PRh rats in the target quadrant ($t[9] = 3.628$, $p = .003$), and significantly less time in the SE and SW quadrants ($t[9] = -2.018$, $p = .037$ and $t[9] = -2.334$, $p = .022$, respectively). All other main effects and interactions were not statistically significant ($ps > .10$).

One-sample t-tests comparing the percent time each of the 4 groups spent in each quadrant also revealed that both groups of Sham rats spent a significant proportion of their swim time in the target quadrant (REMOTE: $t[3] = 3.087$, $p = .027$; RECENT: $t[4] = 7.873$, $p = .001$). This was not the case for the PRh rats; neither group spent a significant

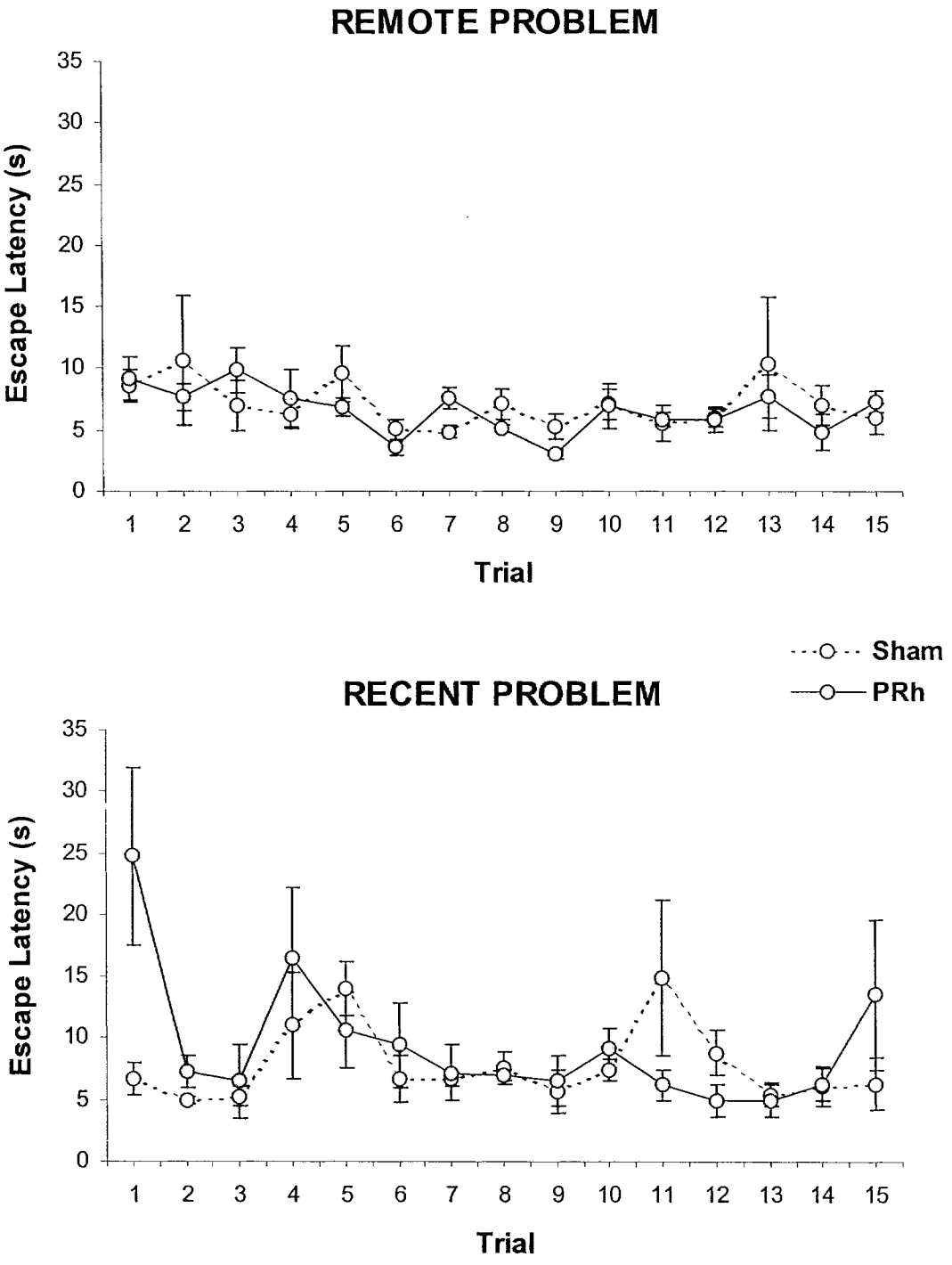


Figure 18. Mean escape latencies during the retention tests for the Sham and PRh rats that learned either the REMOTE or RECENT problem. The error bars represent S.E.M.

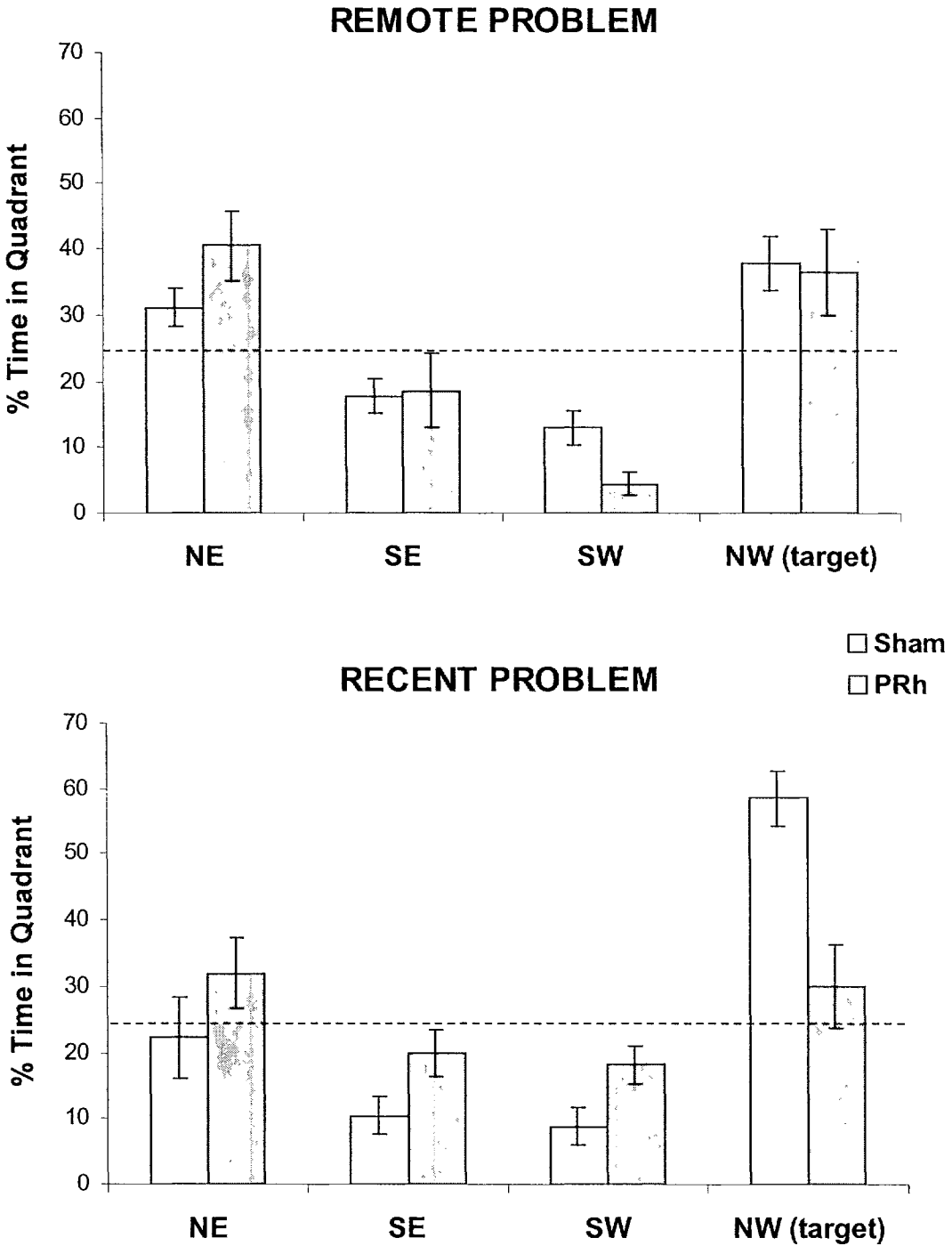


Figure 19. The mean percent time Sham and PRh rats that learned the REMOTE or RECENT problem spent in each quadrant during the early probe trial of the retention test. The error bars represent S.E.M.

proportion of time in the target quadrant. (all ps >.10). PRh rats that learned the REMOTE problem did show a significant preference for the NE quadrant ($t[5] = 2.962$, $p = .016$), and also spent significantly less time in the SW quadrant than would be expected by chance ($t[5] = -11.538$, $p = .001$). PRh rats that learned the RECENT problem spent only as much time in all 4 quadrants as would be expected by chance (ps >.05).

The percentage of time spent in each quadrant on the late probe trial by Sham and PRh rats that learned either the REMOTE or RECENT problem is shown in Figure 20. A 2 x 2 x 4 (Lesion x Time of Learning x Quadrant) mixed factorial ANOVA revealed only a significant main effect of Quadrant ($F[3,51] = 98.610$, $p = .001$). Overall, rats spent the greatest proportion of time in the target quadrant. No other main effects and no interactions were statistically significant (ps >.10).

One sample t-tests confirmed that all 4 groups of rats significantly preferred the target quadrant on late probe trials (PRh-REMOTE: $t[5] = 5.806$, $p = .001$; Sham-REMOTE: $t[3] = 7.951$, $p = .002$; PRh-RECENT: $t[5] = 5.700$, $p = .001$; and Sham-RECENT: $t[4] = 7.881$, $p = .001$). Additionally both the Sham and PRh rats that learned the REMOTE problem spent less time in the other quadrants than would be expected by chance (all ps <.06) and the Sham and PRh rats that learned the RECENT problem spent less time in the SE and SW quadrants (ps <.05) but neither group differed from chance in the amount of time spent in the NE quadrant (p >.05).

2.5.3 Summary

In this experiment, aspiration PRh lesions produced a temporally graded retrograde amnesia for the location of a hidden platform in a water maze. PRh rats that were trained during the week of surgery (RECENT) displayed impaired retention of the platform's

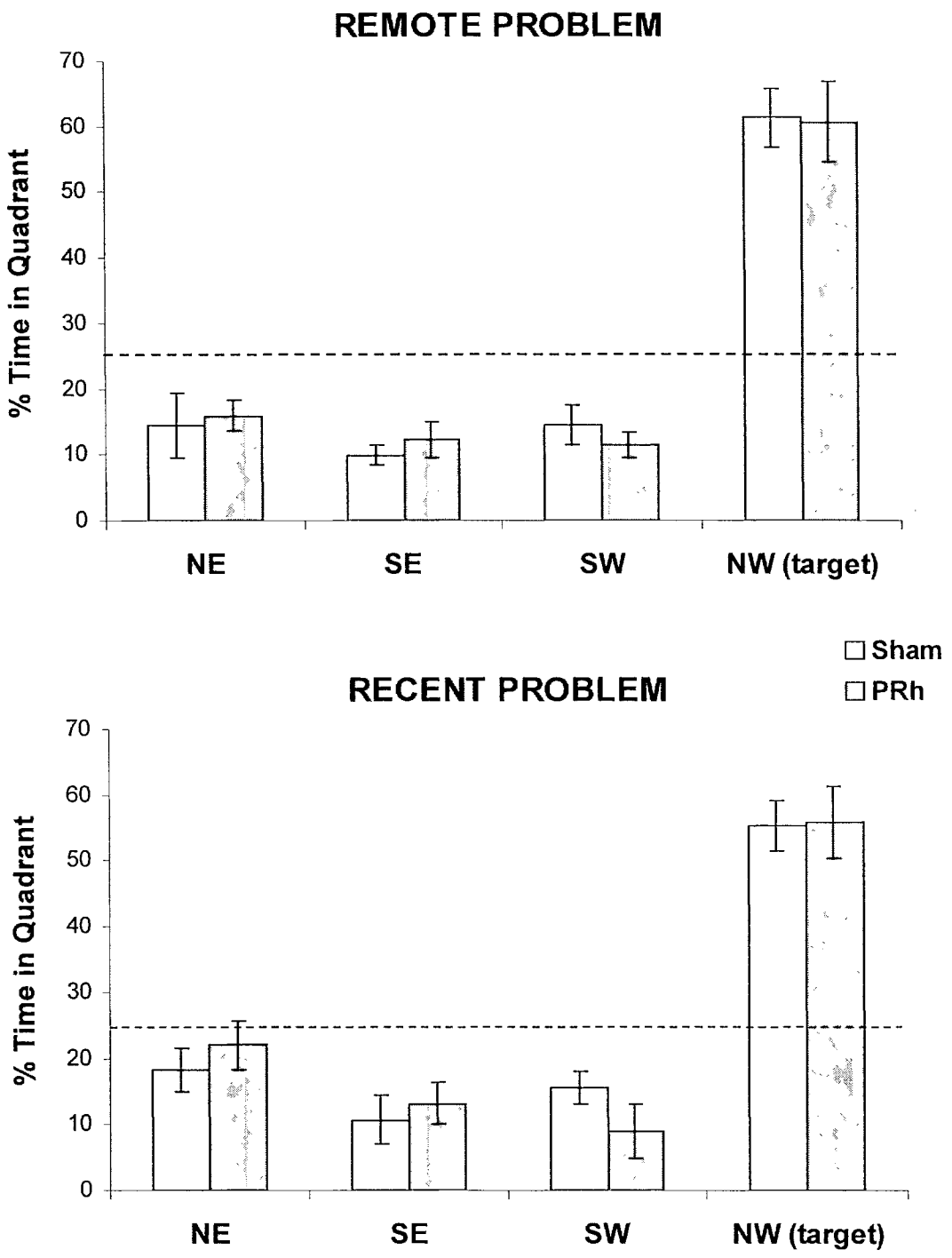


Figure 20. The mean percent time Sham and PRh rats that learned the REMOTE or RECENT problem spent in each quadrant during the late probe trial of the retention test. The error bars represent S.E.M.

location; whereas PRh rats trained 4 weeks prior to surgery (REMOTE) did not. The impairment on the RECENT problem was indexed by a longer latency to find the hidden platform on Trial 1 of the retention test, relative to Sham rats. Additionally, PRh rats failed to show a preference for the target quadrant on the EARLY probe trial of the retention test, whereas Sham rats showed a significant preference for this quadrant. There were no significant differences in escape latency or probe performance between the PRh and Sham rats that learned the REMOTE problem.

These findings are consistent with Experiment 2, in which we observed retrograde amnesia using a water maze task in rats with PRh lesions. Though we did not previously observe evidence of a temporally graded retrograde amnesia on the EARLY probes in that experiment, we did observe that PRh rats had longer escape latencies than Sham rats on the first trial of the RECENT problem, but not the REMOTE problem. Additionally, though PRh rats that learned the REMOTE problem in Experiment 4 did not differ significantly from Sham rats on the EARLY probe, unlike Sham rats, the time PRh rats spent in the target quadrant was not significantly different from chance, nor was it different from the amount of time the PRh rats that learned the RECENT problem spent in the target quadrant. Thus, the pattern of deficits in Experiment 4 could be viewed as a flat gradient of retrograde amnesia. It is, however, difficult to directly compare these two experiments, as both the lesion method and the design were different, but when considered together they suggest that the PRh contributes to the retention of water-maze problems. The precise nature of this contribution in not clear. As in our previous study, the PRh rats in Experiment 4 that displayed retrograde amnesia were able to rapidly relearn the problem during the retention test, as evidenced by their performance on the LATE probe trial and their escape

latencies throughout the session. Thus, it seems unlikely that the retrograde amnesia we observed was due to a spatial navigation deficit.

2.6 General Discussion

Anterograde place memory in rats with aspiration PRh lesions and retrograde place memory in rats with aspiration or electrolytic lesions of the PRh were examined in the present chapter. Aspiration PRh lesions did not impair the ability of rats to learn the location of a hidden, stationary platform in the water maze. Furthermore, rats with this type of lesion only displayed retention deficits for place problems learned before surgery in Experiment 4, but not in Experiment 3. Electrolytic PRh lesions produced retrograde amnesia for places in Experiment 2.

Due to the transient and subtle nature of retrograde amnesia for water maze problems following PRh lesions, it seems likely that the PRh is important for the representation of some aspect of the original learning event that is useful, but not essential, for task performance. One possibility is that the PRh is important for the retention of information about the context in which training occurred. Contextual cues may serve as reminders of the original training experience. Therefore, if this type of information is disrupted prior to the retention test, there may be a minor, but recoverable, impairment in performance. Consistent with this interpretation, Bucci, Phillips, and Burwell (2000) reported that PRh lesions produced both anterograde and retrograde deficits in fear conditioning to the training context, but not to a discrete stimulus. Additionally, Bussey et al. (2001) found that PRh lesions produced an impairment in the ability of rats to acquire a conditional task in which the identity of a rewarded object depended on the context. Thus, PRh rats that show deficits on the initial portions of the retention test may be unable to recognize the context, or certain

features of the context, while retaining the ability to form and flexibly use spatial representations of the environment.

In Experiment 3, we did not observe retrograde amnesia for the platform locations learned before surgery in rats with aspiration lesions of the PRh. This finding was in contrast with the findings of Experiment 2, that there was retrograde amnesia in rats with electrolytic lesions of the PRh, and that experiment used the same mixed design, with time of learning as a within-subjects factor. Thus, the behavioural consequences of electrolytic lesions of the PRh appear to differ from those following aspiration lesions. If the electrolytic lesions were disturbing normal hippocampal functioning, then it is possible that this could result in an impairment in the consolidation of the place information. The absence of a temporal gradient in that case is consistent with the finding that hippocampal lesions impair retrograde memory for places, even problems learned as much as 14 weeks before surgery (Mumby et al., 1999). Additionally, any such disturbance in hippocampal functioning may not be permanent, thus enabling the rapid reacquisition that was observed during retention testing. This hypothesis warrants further investigation.

In Experiment 4, we did observe retrograde amnesia in PRh rats that learned a single water-maze problem approximately 2 days before surgery, but not in PRh rats that learned the same problem 4 weeks before surgery. The main difference between Experiments 3 and 4 was the design; specifically, Time of Learning was a within-subjects factor in Experiment 3 and a between-subjects factor in Experiment 4. A comparison of the findings of Experiments 3 and 4 underscore the importance of considering the design of retrograde amnesia studies. In Experiment 3 we did not observe retrograde amnesia, whereas in Experiment 4 we did. It should be noted that there were differences in the extent of the extra-PRh damage between the two studies: The PRh lesions in Experiment 4, unlike those

in Experiment 3, tended to encroach upon the anterior portion of the postrhinal cortex. We cannot rule out the possibility that this contributed to the differences in the results of the two experiments. However, we did not detect any substantial degree of postrhinal cortical damage to account for the observed deficits in Experiment 2. Also, Bussey et al. (1999; 2000) assessed spatial memory abilities in rats with combined lesions of the PRh and postrhinal cortex and did not observe any deficits in performance using a water maze, radial-arm maze, and a t-maze.

Within-subjects designs are typically selected because it is thought that they most accurately reflect the human syndrome. As previously mentioned, there are disadvantages to using this type of design (Murray & Bussey, 2001). The rate at which animals acquire the first problem will usually be much slower than the rate at which they acquire subsequent problems. In Experiment 3, when time of learning was a within-subjects factor, the average escape latency on the first training session of Problem 1 was 36.10 s, whereas on the first training session of problem 2 the average escape latency was 16.19 s. Accordingly, the researcher must decide whether to equate exposure to each problem, or whether to establish a learning criterion. In the former situation, subsequent problems may be 'over-learned', whereas in the latter situation, subsequent problems will be less familiar. Another concern is equating how each problem is learned. Animals in retrograde memory experiments are still quite dissimilar from human subjects even if they are taught several problems prior to brain damage. It seems unlikely that the animal will not recall prior learning events and attempt to coordinate them with the learning of subsequent problems. Thus, the first problem may be learned in one way, whereas the incorporation of that information when learning other problems may alter the details of what is learned, thus engaging different brain regions than those utilized during the learning of the first problem.

Finally, there is evidence that animals that are given 'reminders' prior to amnestic treatments will display flat gradients of retrograde amnesia, when compared to animals not given reminders, which will display temporally graded retrograde amnesia (Nader, Schafe, & LeDoux, 2000; Sara, 2000a). In a within-subjects study, the subsequent problems may remind animals of previous problems, resulting in a more labile memory for those earlier problems. It has been argued that the activity state of a memory, rather than its age, best predicts its vulnerability (Miller & Matzel, 2000; Sara, 2000a; 2000b). Thus, memories which are in an 'active state' when a trauma occurs will be lost, whereas 'inactive' memories will be spared.

The investigation of putative consolidation processes and retrograde amnesia in human subjects is frequently plagued by interpretational difficulties. For example, it is often impossible to determine exactly when certain memories were acquired, or how well certain information was learned. It may even prove impossible to confirm the reliability or accuracy of certain memories. In cases where the human subject has sustained a brain injury, it is extremely rare for the damage to be circumscribed to a specific brain region. Therefore, many researchers have adopted the use of animal models to investigate retrograde amnesia. The findings from the present study with rats indicate that animal models may also be beset with similar interpretational problems.

Between-groups designs may not accurately reflect the human syndrome. However, the aim of animal studies of retrograde amnesia is not solely to reproduce patterns of memory loss observed in human patients. They also serve to provide clues about the organization of memory, and the brain regions critical for memory consolidation. We provide evidence in the current study that the two types of designs can also yield different results when most other variables are held constant. The apparatus, rooms, and surgical and behavioural

training and testing procedures were the same in both experiments. The lesion technique was also the same and the extent of the PRh lesions was comparable. This clearly points to the design as a critical factor in the differing results. Furthermore, the escape latencies of Sham rats during the retention tests of Experiments 2 and 3 were comparable; approximately 20 s on initial trials for the REMOTE problem and between 10 and 15 s for the RECENT problem. These scores are different from those of the Sham rats in Experiment 4; between 5 and 10 s on initial trials for the REMOTE and RECENT problems. This further supports our interpretation that the lesion method led to the differences between Experiments 2 and 3, whereas the design led to the differences between Experiments 3 and 4 in the present study.

These experiments not only provided more information about the contribution of the PRh to retrograde place memory, they also yielded insight into how certain methodological factors, namely lesion method and design, deserve careful attention in future studies. These factors may bear importantly on the pattern of behavioural deficits that are observed, and could have consequences for future animal studies of retrograde amnesia. As many researchers are currently using excitotoxins to lesion the PRh, and because there is evidence that both excitotoxic and electrolytic lesions of the PRh produce anterograde place deficits (Liu & Bilkey, 1998c), it would be useful to more fully investigate the possible neuropathological and behavioural consequences of these lesion methods.

Chapter 3

Memory for Objects

In Chapter 2, the contribution of the PRh to anterograde and retrograde memory for places was examined. The purpose of the experiments in the present chapter was to examine the contribution of the PRh to anterograde and retrograde memory for objects. The role of the PRh in object-recognition memory is well established (Mishkin & Murray, 1994; Murray, 1996; Murray & Richmond, 2001; but see Buffalo et al., 1998). However, this conclusion is based largely on empirical reports of anterograde amnesia using monkeys and/or variants of DNMS. In the present experiments the effects of aspiration and electrolytic lesions of the PRh in rats on the acquisition and retention of object information using NPT was assessed.

NPT has been used to assess anterograde object memory in rats in previous studies (Bussey et al., 2000; Ennaceur & Aggleton, 1994; Ennaceur et al., 1997; Mumby et al., 2002). However, to our knowledge, it has never been modified to examine retrograde object memory. In Experiment 5 we investigated the effects of PRh lesions on anterograde object versions of NPT and in Experiments 6 through 8 we investigated the effects of PRh lesions on a retrograde object version of NPT. Taken together, these experiments provide a unique evaluation of anterograde and retrograde memory for objects using comparable training procedures.

3.1 General Method

3.1.1 Subjects

As in Chapter 2, the subjects were male, Long-Evans rats weighing between 300 and 350 g at the start of the experiment. Housing and colony conditions were also the same as described in Chapter 2.

3.1.2 Apparatus and Materials

All object encounters occurred in a rectangular, open-field arena (60 x 70 x 70 cm) constructed of grey PVC plastic. A stainless-steel tray (60 x 70 cm), removable through a 6 cm slot along the bottom of one side of the arena, served as the floor and was covered with wood shavings. Two inverted jar lids were attached to the tray; each was positioned 10 cm from opposing corners of the arena. Objects were glued to glass jars and fixed in place by screwing the jars into the lids on the tray.

The objects were miscellaneous household items and toys made of metal, glass, porcelain, or glazed ceramic. There were three copies of each object, which were used interchangeably. They ranged in size from approximately 5 to 10 cm wide and 5 to 15 cm tall. Variations in size and texture were accompanied by variations in shape and colour. Even though objects were counterbalanced across experimental conditions and phases of NPT, the use of objects that rats will explore too much or too little could adversely affect the overall outcome of the test. For that reason, all objects used in this chapter were preference tested, wherein object-naïve rats were placed in the arena with two copies of the same object for 5 minutes and the amount of time spent exploring the objects was recorded. Objects that elicited high or low exploration were excluded from the pool, leaving only objects that elicited moderate amounts of exploration.

For each test of NPT, anterograde or retrograde, 3 copies of two different objects were used as stimuli. For half of each group of rats one object served as the sample and the other as the novel object; the opposite was true for the remaining half of each group.

The objects, the metal tray, and the interior walls of the arena were washed with water, and the wood shavings were changed after every use. A video camera (Sony Handicam 8mm) was positioned on a tripod directly above the arena, and all object encounters were recorded for later analysis.

Object exploration was defined as the amount of time rats spent with their snout oriented at least 45 ° to and within 4 cm of an object. Standing, sitting on, or chewing objects was not considered object exploration. The experimenter scoring object exploration from previously recorded sessions was always blind to the experimental condition of the subjects.

3.1.3 Histology

Histological procedures were as described in Chapter 2.

3.1.4 Statistical Analyses

In all experiments, the time spent exploring each object in the arena was recorded in seconds. During sample sessions there were two identical objects in the arena and the total time spent exploring them was obtained; these data can be viewed in Appendix B. During test sessions there were two different objects in the arena and, in this case, the total times spent exploring the novel and sample objects were obtained in addition to the total time spent exploring objects. The total time spent exploring objects during the test session can also be found in Appendix B.

Unless otherwise noted, only the first 2 minutes of the test session were evaluated. Dix and Aggleton (1999; also see Mumby et al., 2002) found that the maximum discrimination between the novel and sample objects occurred in the first 2 minutes of the test phase. After 2 minutes, normal rats no longer show a preference for the novel object and may even switch their attention to the sample object.

Our primary index of retention was an exploration ratio that was calculated as follows:

$$\frac{\text{Time spent exploring novel object}}{\text{Time spent exploring novel object + time spent exploring sample object}}$$

Exploration ratios above 0.5 indicate more time spent exploring the novel object, whereas ratios below 0.5 indicate more time spent exploring the sample object. Exploration ratios that were not significantly different from the value of 0.5 indicated no preference for either object. All exploration ratios were compared to 0.5 using one-sample t-tests. Rats that did not explore the novel and sample objects for at least 2 seconds during the portion of the test session being examined were excluded from the analysis.

ANOVAs were used to analyze data from sample and test sessions. Factors in these analyses include Lesion (between-subjects factor: Sham versus PRh in Experiments 5-8 and Sham, aspiration PRh, and electrolytic PRh in Experiment 5), Object (within-subjects factor: Novel versus Sample), Session (within-subjects: Sample versus Test), and Time of Learning (between-subjects factor in Experiments 6 and 7: 5, 3, and 1 week in Experiment 6 and REMOTE versus RECENT in Experiment 7; within-subjects factor in Experiment 8: REMOTE versus RECENT). Independent samples t-tests were used to compare groups, where applicable. All statistical tests were evaluated at a significance level of 0.05, but p values between 0.05 and 0.10 were considered noteworthy.

3.2 Experiment 5: Anterograde object memory in rats with lesions of the PRh

As previously discussed, it is well established that the functions of the PRh are important for normal object-recognition memory. Rats and monkeys with lesions restricted to the PRh display delay-dependent deficits on DNMS tasks in which objects or computer-generated images are used (for review see Murray, Bussey, Hampton, & Saksida, 2000). Typically, deficits emerge when the animals with PRh damage must remember the sample object over delays that exceed 15-30 seconds, and their performance becomes progressively worse as the delay period lengthens, falling to chance performance after approximately 1 minute. Monkeys with PRh lesions fail to successfully recognize the sample object even when two sample presentations are provided (Gaffan & Murray, 1992).

Only a few studies have specifically examined anterograde memory for objects using NPT in rats with PRh lesions (Ennaceur et al., 1996; Ennaceur & Aggleton, 1997). As with DNMS, rats with PRh lesions, unlike Sham rats, do not show a preference for the novel object using NPT. However, in these studies (Bussey et al., 1999; 2000) excitotoxic lesions were made. Thus, it is not known whether other lesion methods would lead to similar deficits. Given that deficits on DNMS have been reported using aspiration (Meunier et al., 1993; Glenn & Mumby, 1996; Mumby et al., 1994), and excitotoxic (Baxter & Murrary, 2001) lesions, it is probable that any manner of PRh damage would lead to deficits on NPT. The purpose of this experiment was to determine whether this is the case, particularly since differences between two lesion methods were observed in Chapter 2.

This experiment is divided into 3 sections. Experiment 5a compared the performance of Sham rats and rats with aspiration lesions of the PRh on NPT using a 5-minute retention

delay. Experiment 5b compared the performance of Sham rats and rats with either aspiration or electrolytic lesions of the PRh on NPT using a 15-minute retention delay. Experiment 5c compared the performance of Sham rats and rats with aspiration lesions of the PRh on a modified version of NPT in which exploration of the sample objects was limited to 20 seconds.

3.2.1 Experiment 5a: Anterograde object memory in rats with aspiration lesions of the PRh

In this experiment, rats with aspiration lesions of the PRh were tested on the standard version of the novelty preference task with a 5-minute retention delay.

3.2.1.1 Subjects

Thirty-four rats served as subjects in this portion of the experiment. Of this group, 16 rats were experimentally naïve. The remaining 20 rats served as subjects in either Experiment 7 or 8 prior to participation in this experiment.

3.2.1.2 Procedure

3.2.1.2.1 Surgery. Rats in this portion of the experiment had either bilateral aspiration lesions of the PRh (n=18) or Sham surgery (n=18). The aspiration lesions were conducted as described in Experiment 1. The experimentally naïve rats recovered from surgery for 2 weeks prior to testing. The rats that were subjects in other experiments were tested approximately 3 weeks after surgery.

3.2.1.2.2 Behavioural testing. Prior to anterograde testing, the experimentally naïve rats were habituated to the arena after surgery, singly, in three 15-minute sessions, one per day for three consecutive days. There were no objects present in the arena during habituation. For the naïve rats, anterograde testing was carried out between 48 and 72 hours after the

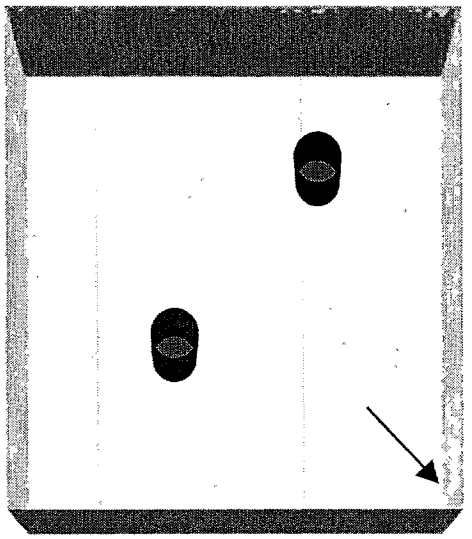
final habituation session. For the remaining rats, habituation to the arena had occurred prior to surgery and no further sessions were given.

NPT consisted of three phases: a 5-minute sample session, a 5-minute retention delay, and either a 5-minute or a 3-minute test phase. The configuration of object placement in the arena is shown in Figure 20. The placement of the novel object during the test session was counterbalanced. Rats were always placed in the arena at the same position, facing the SE corner (also shown in Figure 21). Following the sample session rats were removed from the arena and transported back to their home cage in the colony room for the retention delay. The home cage was not returned to its usual position but remained on a counter in the colony room. During the delay, the arena and metal tray were washed with water, the wood shavings changed, and a third copy of the sample object and a novel object were positioned in the arena. At the end of the retention delay, rats were transported back to the arena for the test session.

3.2.1.3 Results

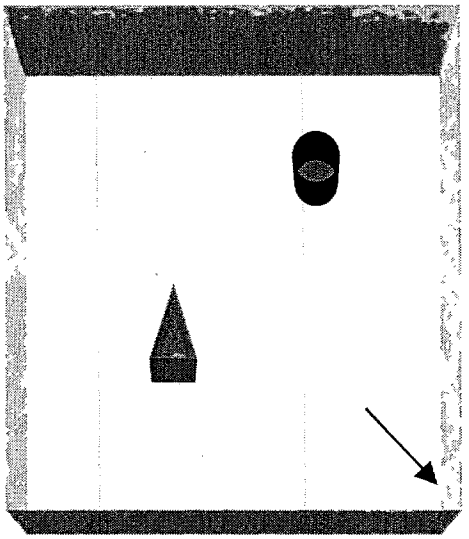
3.2.1.3.1 Histological results. The location and extent of the smallest and largest PRh lesions of the experimentally naïve rats are shown in Figure 22. There was substantial and nearly complete, bilateral damage to the PRh in each lesioned rat. The PRh was 95 percent destroyed in the rat with the largest lesion, and 80 percent destroyed in the rat with the smallest lesion. In the rostral portion of the PRh lesion, there was unilateral sparing of the cells of the PRh in three rats, and bilateral sparing in two rats. In terms of the caudal extent of the lesion, there was unilateral sparing of the PRh tissue in only one rat.

All PRh rats also had bilateral damage to the lateral entorhinal cortex; this damage was primarily in the posterior extent of the lesions, with less, and frequently unilateral, damage evident in the anterior extent of the lesions. The rat with the largest lesion had approximately



Sample Session

---Retention Delay---



Test Session

Figure 21. A schematic diagram showing the location of objects in the arena during the sample and test sessions. The arrow indicates the placement of rats into the open field.

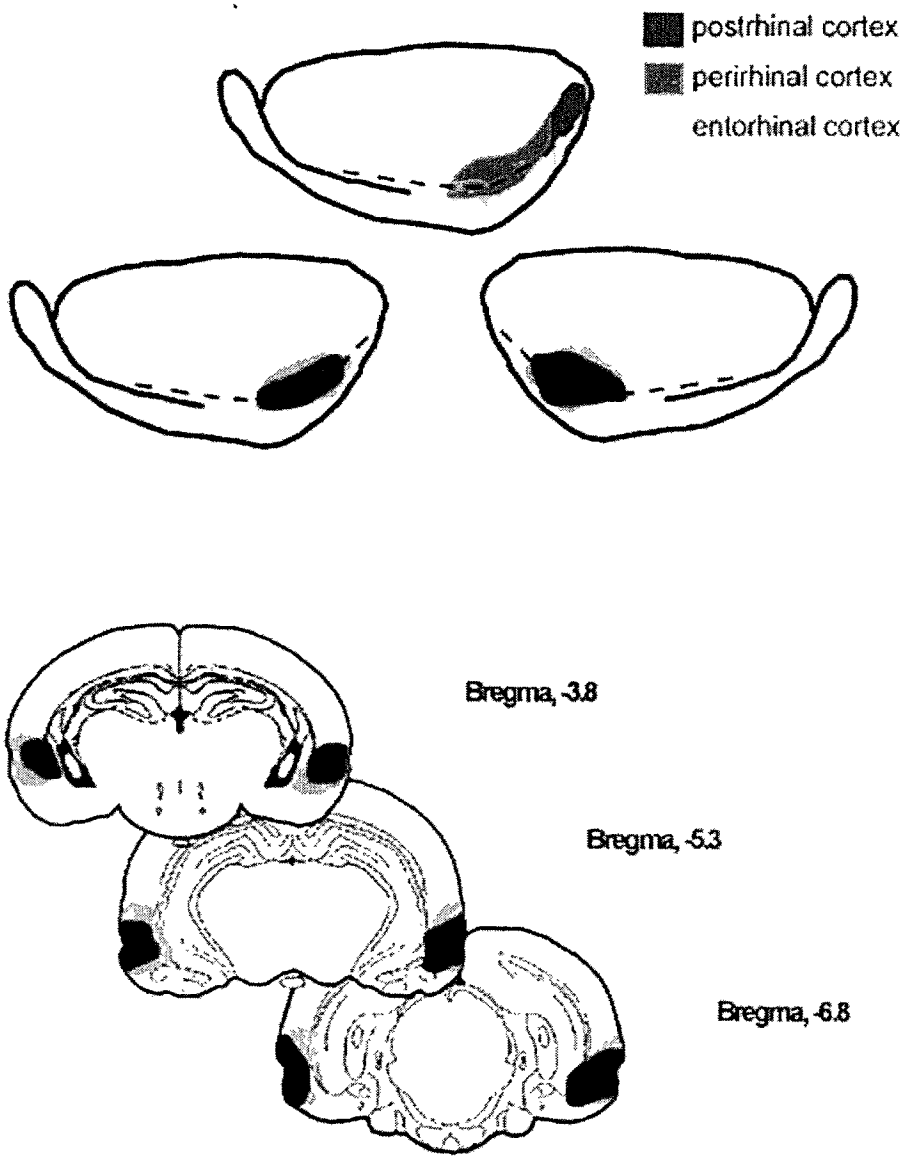


Figure 22. The location and extent of the PRh lesions are shown on the lateral surface of the rat brain in the top panel and in three coronal views in the bottom panel. In each panel, the largest lesion is shown in grey and the smallest lesion is shown in black.

15 percent damage to the lateral entorhinal cortex, whereas the rat with the smallest lesion sustained about 5 percent damage to this region. Damage to the anterior portion of the postrhinal cortex occurred in only 2 PRh rats, and sustained less than 10 percent damage in both cases.

Damage was found unilaterally in the temporal association cortex of four rats and minimal damage was found bilaterally in one rat. The lateral amygdala was damaged unilaterally in four rats and bilaterally in two rats. One rat had significant unilateral damage to the piriform cortex. Three rats sustained unilateral damage to the temporal association cortex and one rat had bilateral damage. Minor damage of the CA1 subfield of the HPC was seen unilaterally in four rats and bilaterally in one rat. The ventral portion of the subiculum was minimally damaged unilaterally in six rats, and none sustained bilateral damage.

The largest and smallest PRh lesions of the rats that first served as subjects in Experiments 7 and 8 can be seen in Figures 30 and 35. Also see Sections 3.4.2.1 and 3.5.2.1 for descriptions of these lesions. Overall, there was good concordance in the location and extent of the PRh lesions.

3.2.1.3.2 Behavioural results. Figure 23 shows the mean exploration ratios for Sham and PRh rats during the first 2 minutes of the test session. Due to not meeting the criterion of at least 2 second of exploration with both objects, two Sham rats were excluded from this analysis. There was no significant difference between the groups ($t[30] = -.298$, $p = .384$) and both Sham and PRh rats displayed a significant preference for the novel object ($t[14] = 3.029$, $p = .005$ and $t[16] = -.315$, $p = .005$, respectively).

Figure 23 also shows the amount of time Sham and PRh rats spent exploring the novel and sample objects during the first 2 minutes of the test session. One Sham rat and one PRh

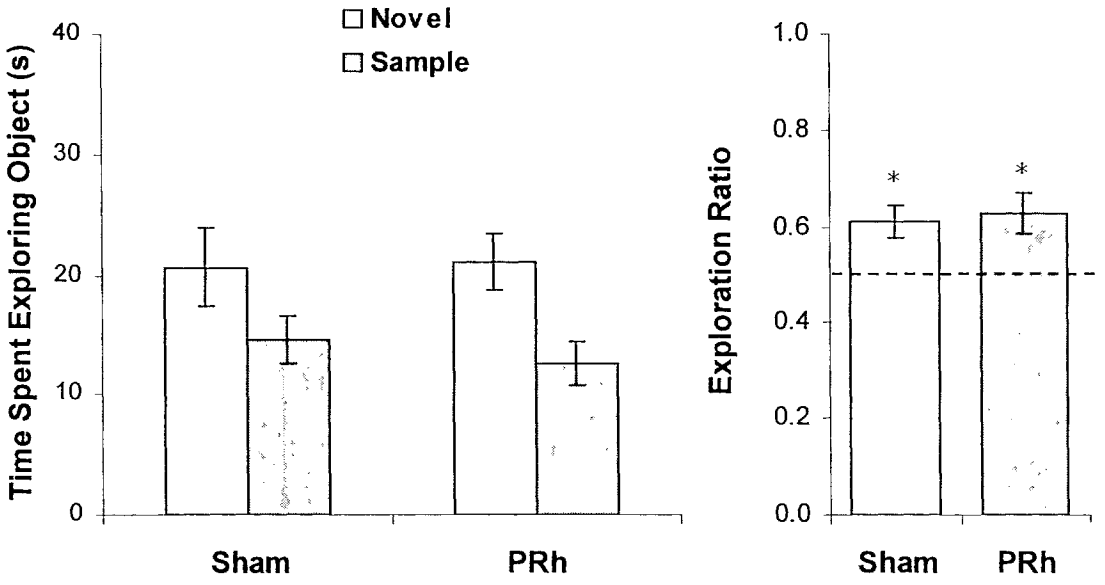


Figure 23. Mean times Sham and PRh rats spent exploring the novel and sample object during and their mean exploration ratios from the first 2 minutes of the test session. The error bars represent S.E.M. and the dashed line indicates chance exploration (no object preference). Asterisks denote mean ratios that are significantly higher than chance level (one-sample t-test, $p < .05$).

rat did not explore objects during the sample and test sessions and were excluded from all analyses. A 2 x 2 (Lesion x Object) mixed-factorial ANOVA revealed a significant main effect of Object ($F[1,32] = 10.172$, $p = .003$). Overall, rats spent more time exploring the novel object during the test session. The main effect of Lesion and the interaction between Lesion and Object were not statistically significant (ps $>.10$).

3.2.1.4 Summary

Rats with aspiration lesions of the PRh did not display anterograde amnesia for objects. PRh rats spent as much time as Sham rats exploring the novel object during the test session. This suggests that PRh rats were able to remember the sample object as well as Sham rats when a 5-minute retention delay was interposed between investigation of the sample objects and the test with the novel object. Additionally, the total amount of time Sham and PRh rats spent exploring objects in the sample and test sessions was not significantly different, nor were any significant differences in their patterns of object exploration over each of the 5 minutes of the sample and test sessions (see Appendix B).

Over the last 10 years it has been repeatedly demonstrated that PRh lesions in monkeys and rats lead to a inability to remember objects over retention delays as brief as 15 seconds. The majority of these studies used DNMS to test object-recognition. It is, therefore, possible that DNMS and NPT do not tax object memory in the same way. For example, in DNMS, rats view the sample object for only a few seconds. In NPT, rats are in an arena with two copies of the sample object for 5 minutes. Both Sham and PRh rats spent approximately 80 seconds exploring the sample objects. Thus, it is possible that NPT with a 5-minute retention delay is not sufficiently taxing, and this is why PRh rats remember the sample object. However, deficits on NPT have been reported in rats with excitotoxic PRh lesions with delays of 15 minutes (Ennaceur et al., 1996). In Experiments 2 and 3, we found

differences in the behavioural outcome of PRh lesions made electrolytically or by aspiration. Therefore, it is also possible that aspiration PRh lesions have fewer adverse consequences for learning and memory in general. These two possibilities were addressed in Experiments 5a and 5c.

3.2.2 Experiment 5b: Anterograde object memory in rats with either electrolytic or aspiration lesions of the PRh

In this experiment, the performance of rats with electrolytic or aspiration lesions of the PRh on the object version of NPT was examined. The findings from Experiments 2, 3 and 4 provide evidence that electrolytic PRh lesions may have a more detrimental impact on place memory than aspiration PRh lesions. Furthermore, in Experiment 5a we failed to detect an impairment on the object version of NPT in rats with aspiration PRh lesions, a finding that is inconsistent with an extensive literature demonstrating the importance of the PRh to normal object memory. In the preceding summary we explored the possibility that the 5-minute retention delay was not sufficiently taxing, thus, in this section of the experiment, we increased the retention delay to 15 minutes. Thus, the purpose of this part of the experiment was twofold: to determine whether rats with electrolytic lesions display anterograde amnesia for objects, and to assess the performance of PRh rats on NPT using a longer delay than on our previous test.

3.2.2.1 Subjects

Seventy-one rats served as subjects in this portion of the experiment. Rats were not experimentally naïve, nor were they all tested at the same time (see section 3.2.2.2.2 for details). Thirty-four rats had previously served as subjects in Experiment 6 and had extensive object experience. Twenty rats had previously served as subjects in Experiment 4 and 8 and

had minimal object experience. The remaining 17 rats had previously served as subjects in Experiment 3 and did not have object experience.

3.2.2.2 Procedure

3.2.2.2.1 Surgery. Rats in this portion of the experiment had either bilateral aspiration lesions of the PRh (n=21), bilateral electrolytic lesions of the PRh (n=16) or Sham surgery (n=34). The aspiration lesions were conducted as described in Experiment 1. The electrolytic lesions were conducted as described in Experiment 2. As all rats were previously in other experiments, they were tested between 3 and 4 weeks after surgery.

3.2.2.2.2 Behavioural testing. All behavioural testing was the similar to the first section of this experiment (see section 3.2.1.2.2). In this case, however, all rats received a 5-minute sample session, a 15-minute retention delay, and a 5-minute test session.

Nine of the rats with aspiration PRh lesions and 8 of the Sham rats were tested first. These rats had no prior object experience. The 16 rats with electrolytic PRh lesions and 18 of the Sham rats were tested a few months later. The remaining rats with aspiration PRh lesions and Sham rats were tested last.

3.2.2.3 Results

3.2.2.3.1 Histological results. The location and extent of the largest and smallest electrolytic PRh lesions are shown in Figure 24. Two rats sustained significant sparing of the PRh bilaterally and were therefore excluded from analysis. One rat was in the 3-week group, and the other was in the 1-week group. The PRh was nearly completely destroyed in the remaining rats. The rat with the largest lesion sustained approximately 95 percent damage to the PRh and the rat with the smallest lesion sustained approximately 75 percent damage to the PRh. In rats with smaller lesions, the anterior portions of the PRh were spared in at least one hemisphere.

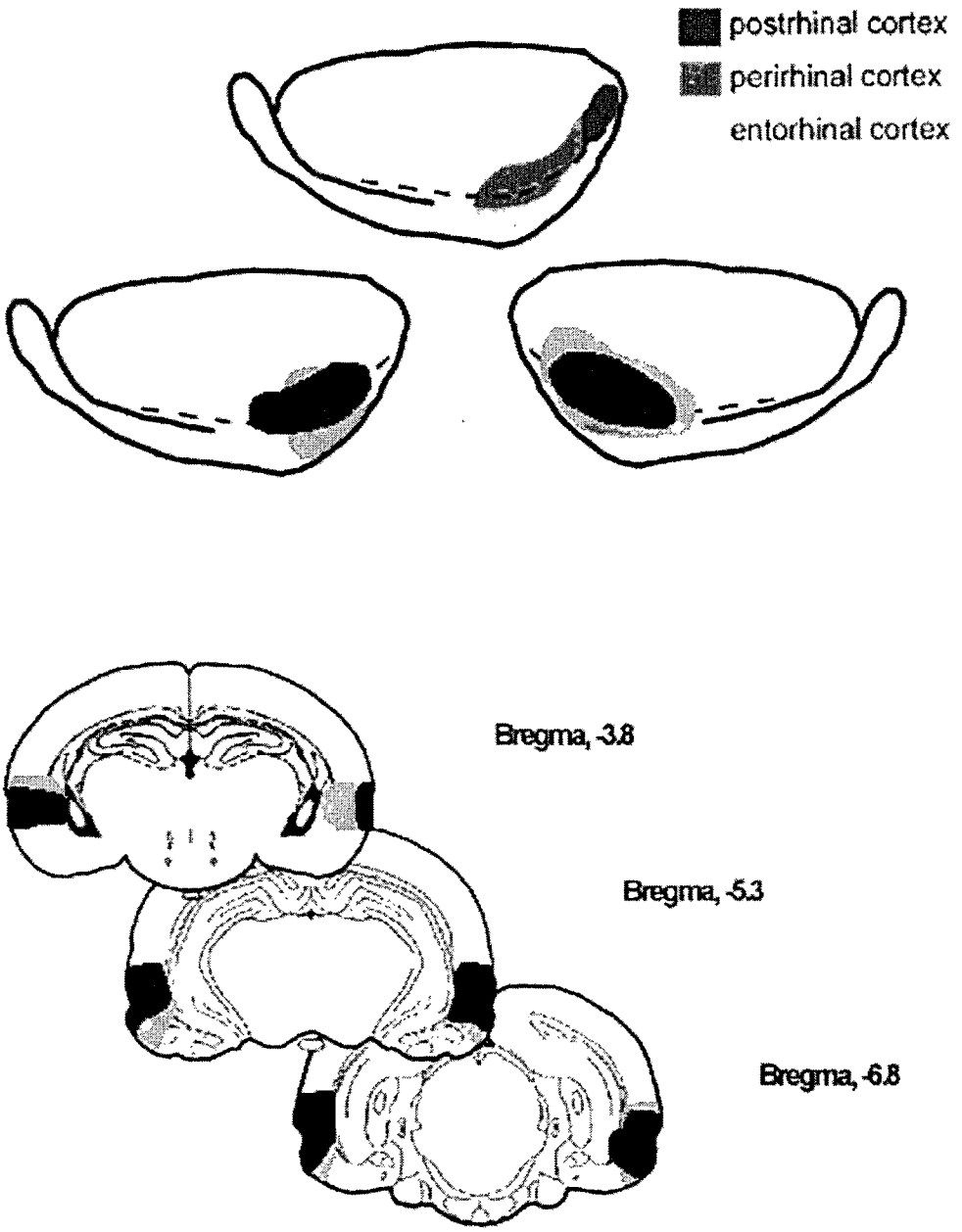


Figure 24. The location and extent of the PRh lesions are shown on the lateral surface of the rat brain in the top panel and in three coronal views in the bottom panel. In each panel, the largest lesion is shown in grey and the smallest lesion is shown in black.

All PRh lesions encroached bilaterally into the lateral entorhinal area, this damage was estimated to be between 20 and 50 percent (smallest and largest lesion, respectively). The anterior portion of the postrhinal cortex was also damaged in all rats. The rat with the smallest lesion sustained less than 10 percent damage to this region in one hemisphere only. Other rats with small lesions sustained less than 10 percent damage bilaterally. The rat with the largest lesion sustained approximately 35 percent damage in one hemisphere and 20 percent damage in the other.

All lesions included slight to moderate damage to the ventral portions of temporal association cortex. Four lesions included minor, unilateral damage to the ventral subiculum and CA1 cell field.

The location and extent of the largest and smallest aspiration PRh lesions can be seen in Figures 13 and 17. Also see sections 2.4.2.1 and 2.5.2.1 for a description of those lesions.

3.2.2.3.2 Behavioural results. The performance of Sham rats tested at the three different time points was compared by conducting a one-way ANOVA on the exploration ratios calculated based on the first 2 minutes of the test session. There were no significant differences between the groups ($F < 1$); therefore their data were combined to form a single Sham group. The performance of the two groups of rats with aspiration PRh lesions was also not significantly different ($t[19] = -.272$, $p = .789$), and their data were combined to form a single PRh-aspiration group.

Figure 25 shows the mean exploration ratios for each group during the first 2 minutes of the retention test. Two rats with aspiration PRh lesions failed to explore either object during the retention test and were excluded from this analysis. One-sample t-tests revealed that Sham rats and rats with aspiration PRh lesions had exploration ratios that were significantly higher than chance ($t[33] = 1.671$, $p = .052$ and $t[18] = 2.898$, $p = .005$,

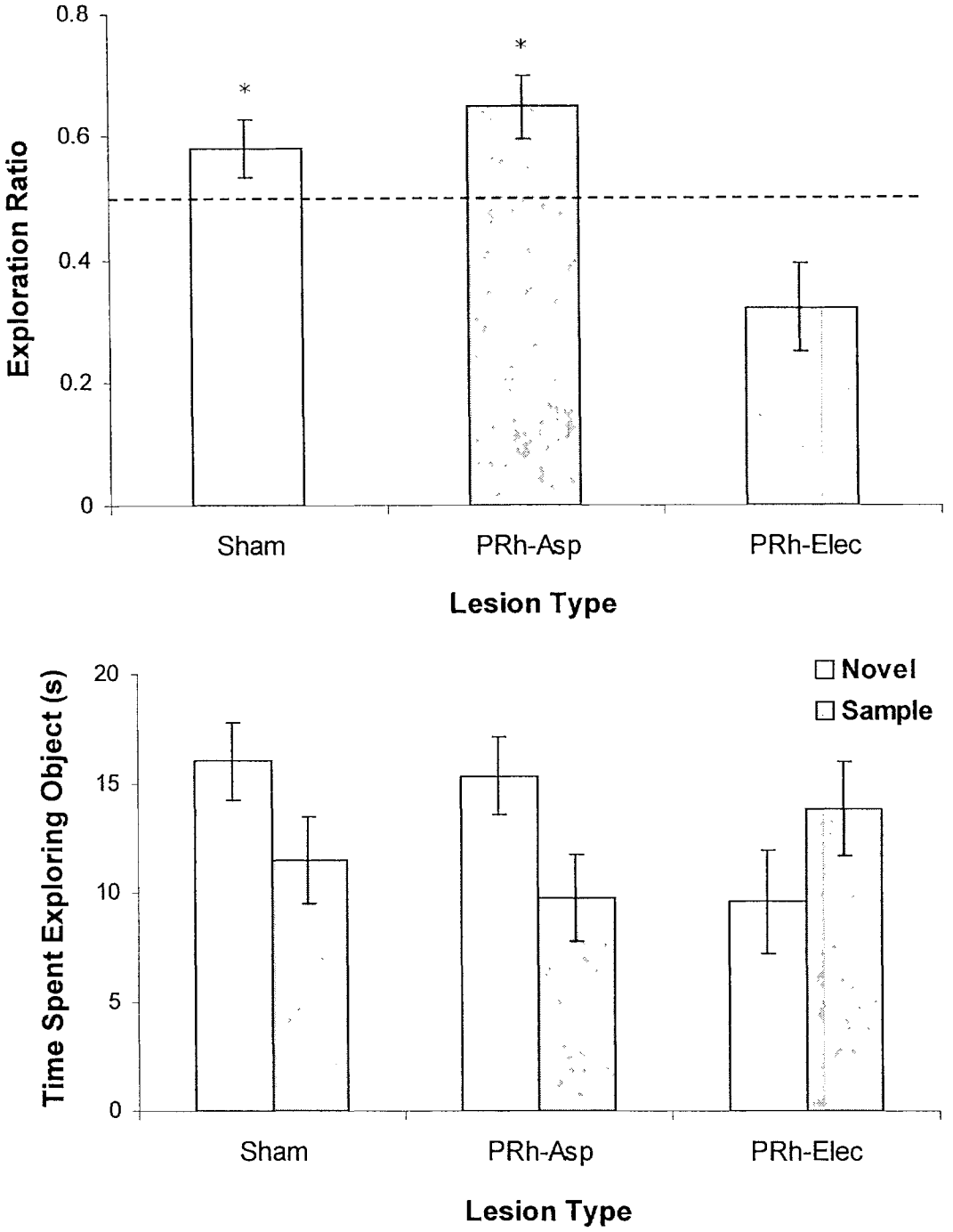


Figure 25. Mean exploration ratios (top panel) and the mean time Sham and PRh rats spent exploring the sample and novel objects (bottom panel) during the first 2 minutes of the test session. The error bars represent S.E.M. and the dashed line indicates chance performance (no object preference). Asterisks denote mean ratios that are significantly higher than chance level (one-sample t-test, $p < .05$).

respectively), whereas rats with electrolytic PRh lesions had exploration ratios that were significantly lower than chance ($t[15] = -2.504$, $p = .024$). A one-way ANOVA revealed that the groups were significantly different ($F[2,66] = 7.387$, $p = .001$). Follow-up tests revealed that the exploration ratios of both Sham rats and rats with aspiration PRh lesions were significantly higher than those of rats with electrolytic lesions ($t[48] = 3.048$, $p = .004$ and $t[33] = 3.803$, $p = .001$, respectively), but not significantly different from each other ($t[51] = -.911$, $p = .183$).

The amount of time Sham and PRh rats spent exploring the sample and novel objects during the first 2 minutes of the test session is also shown in Figure 25. A 3 x 2 (Lesion x Object) mixed factorial ANOVA revealed nonsignificant main effects of Lesion and Object ($ps > .10$), but the interaction between Lesion and Object approached statistical significance ($F[2,68] = 2.618$, $p = .080$). As can be seen in Figure 25, both Sham rats and rats with aspiration lesions of the PRh spent more time exploring the novel object, but rats with electrolytic lesions of the PRh spent more time exploring the sample object.

Some rats in each group explored only one of the two objects during the first 2 minutes of the retention test. Rats that only explored the sample object obtained an exploration ratio of 0, whereas rats that only explored the novel object obtained an exploration ratio of 1. These values were used in the preceding analysis. Of the rats that fell into one of these two categories, there were 2 Sham rats and 5 rats with electrolytic lesions of the PRh that only explored the sample object, and 3 Sham rats and 1 rat with an aspiration lesion of the PRh that only explored the novel object. Figure 26 shows the mean exploration ratios for each group with the data from these animals removed. One-sample t-tests indicated that both Sham rats and rats with aspiration PRh lesions still had exploration ratios higher than chance ($t[28] = 1.873$, $p = .036$ and $t[16] = 2.264$, $p = .019$, respectively), whereas rats with

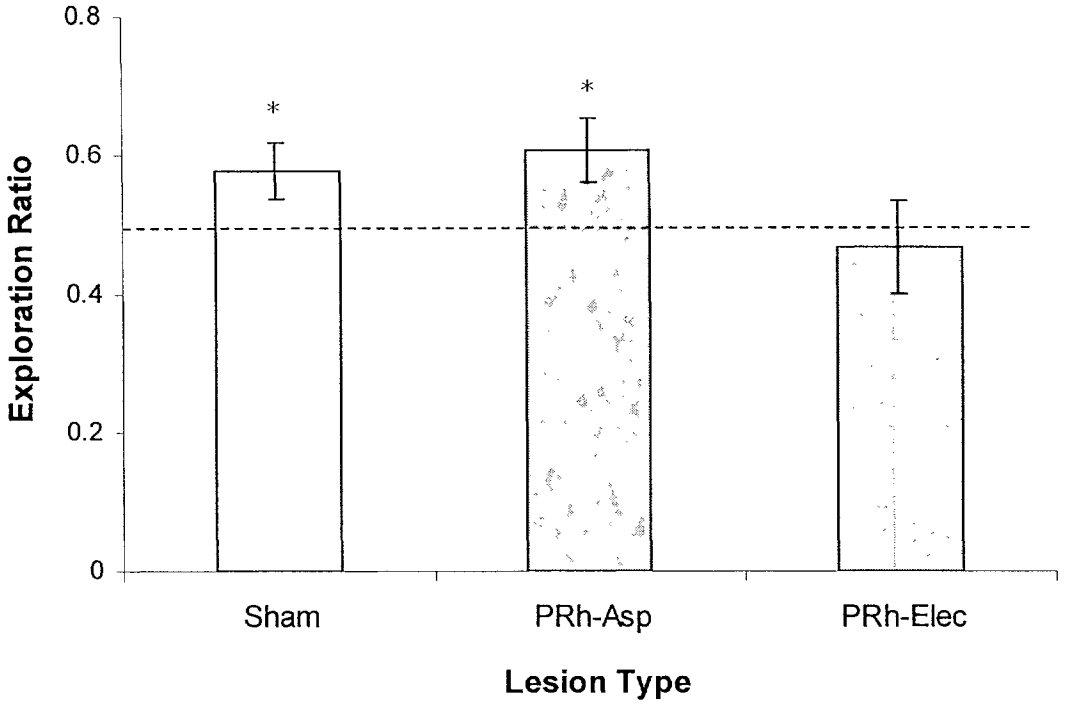


Figure 26. The mean exploration ratios of each group following the removal of rats that spent time with only one object or neither. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object). Asterisks denote mean ratios that are significantly higher than chance level (one-sample t-test, $p < .05$).

electrolytic lesions of the PRh were not significantly different from chance ($t[10] = -.507$, $p = .312$). However, a one-way ANOVA conducted on the revised data revealed a nonsignificant effect of Lesion ($p > .10$).

3.2.2.4 Summary

Rats with electrolytic lesions of the PRh showed anterograde amnesia for objects, but rats with aspiration lesions of the PRh did not. When all the data for the rats with electrolytic PRh lesions were examined they displayed a significant preference for the sample object. However, 5 of these rats scored an exploration ratio of 0 because they only spent time exploring the sample object. When the data were analyzed with these scores removed the exploration ratios from this group did not differ from chance, indicating no preference for either object. Contrary to these findings, rats with aspiration PRh lesions, like Sham rats, displayed a significant preference for the novel object.

The finding that electrolytic lesions of the PRh impaired object recognition is consistent with previous studies. The absence of deficits in rats with aspiration PRh lesions is not consistent with previous studies, but is consistent with the findings from Experiment 5a. Additionally, this pattern of impaired and spared object memory in rats with electrolytic and aspiration PRh lesions, respectively, is consistent with the findings from Experiments 2, 3, and 4 using the water maze and provides further evidence that the lesion method may play a substantial role in whether memory deficits are observed. Both lesion methods destroy neurons in the PRh, as well as fibers passing through or adjacent to the PRh. The aspiration and electrolytic lesions were comparable in location and size. Therefore, the magnitude or position of the lesion does not adequately account for the discrepancies in performance between the PRh-lesioned rats.

Aspiration lesions are frequently used in monkey models of anterograde amnesia, and PRh lesions in those instances produce object memory deficits, evaluated using DNMS. As well, we previously found that aspiration PRh lesions impaired object recognition in rats using DNMS (Glenn & Mumby, 1996). Based on the findings so far obtained in the present experiment, aspiration PRh lesions do not disrupt object memory with delays of 5 or 15 minutes using NPT. It is likely that the differences between NPT and DNMS are still an important consideration and this is addressed in the next and final section of this experiment.

3.2.3 Experiment 5c: Anterograde object memory in rats with aspiration lesions of the PRh: Limited exposure to the sample objects

The main goal of Experiment 5c was to determine whether rats with aspiration lesions of the PRh would discriminate between the sample and novel objects during the test session if the amount of time they spent with the sample objects during the sample session was limited to only 20 seconds. Rats in Experiments 5a and 5b tended to spend approximately 80 seconds with the sample objects. Reducing the amount of time rats spend with the sample object should render the task more difficult, and more like DNMS in the amount of exposure rats have to the sample object than the version of NPT previously used. Rats with excitotoxic lesion of the PRh were impaired when a similarly modified version of NPT was used in previous studies (Ennaceur & Aggleton, 1997).

3.2.3.1 Subjects

Seventeen rats served as subjects in this experiment. These rats had some prior object experience as they had previously served as subjects in Experiments 9 and 10.

3.2.3.2 Procedure

3.2.3.2.1 Surgery. Rats had either bilateral aspiration lesions of the PRh (n=9) or Sham surgery (n=8). The aspiration lesions were conducted as described in Experiment 1.

3.2.3.2.2 Behavioural testing. For this, modified, version of NPT, the sample phase was terminated once rats had explored the sample objects for 20 seconds. The experimenter placed the rat in the arena for the sample session, and proceeded to an adjacent room that contained a television attached to the video camera in the testing room. The experimenter scored the object exploration as it was occurring and when the cumulative time spent exploring both objects was 20 seconds, the experimenter immediately returned to the testing room and removed the rat from the arena. All rats received a 5-minute retention delay and a 3-minute test session.

3.2.3.3 Results

3.2.3.3.1 Histological results. The location and extent of the largest and smallest PRh lesions are shown in Figure 46 and described in Section 4.3.2.1. Five of these PRh rats were not tested in the present experiment. However, the rats with the largest and smallest lesions were in the present group.

3.2.3.3.2 Behavioural results. Figure 27 shows the mean exploration ratios of Sham and PRh rats during the first 2 minutes of the test session and the amount of time each group spent exploring the novel and sample objects during that same portion of the The exploration ratios of Sham and PRh rats were not significantly different ($t[15] = -.246$, $p = .405$). However, neither Sham nor PRh rats displayed exploration ratios that were

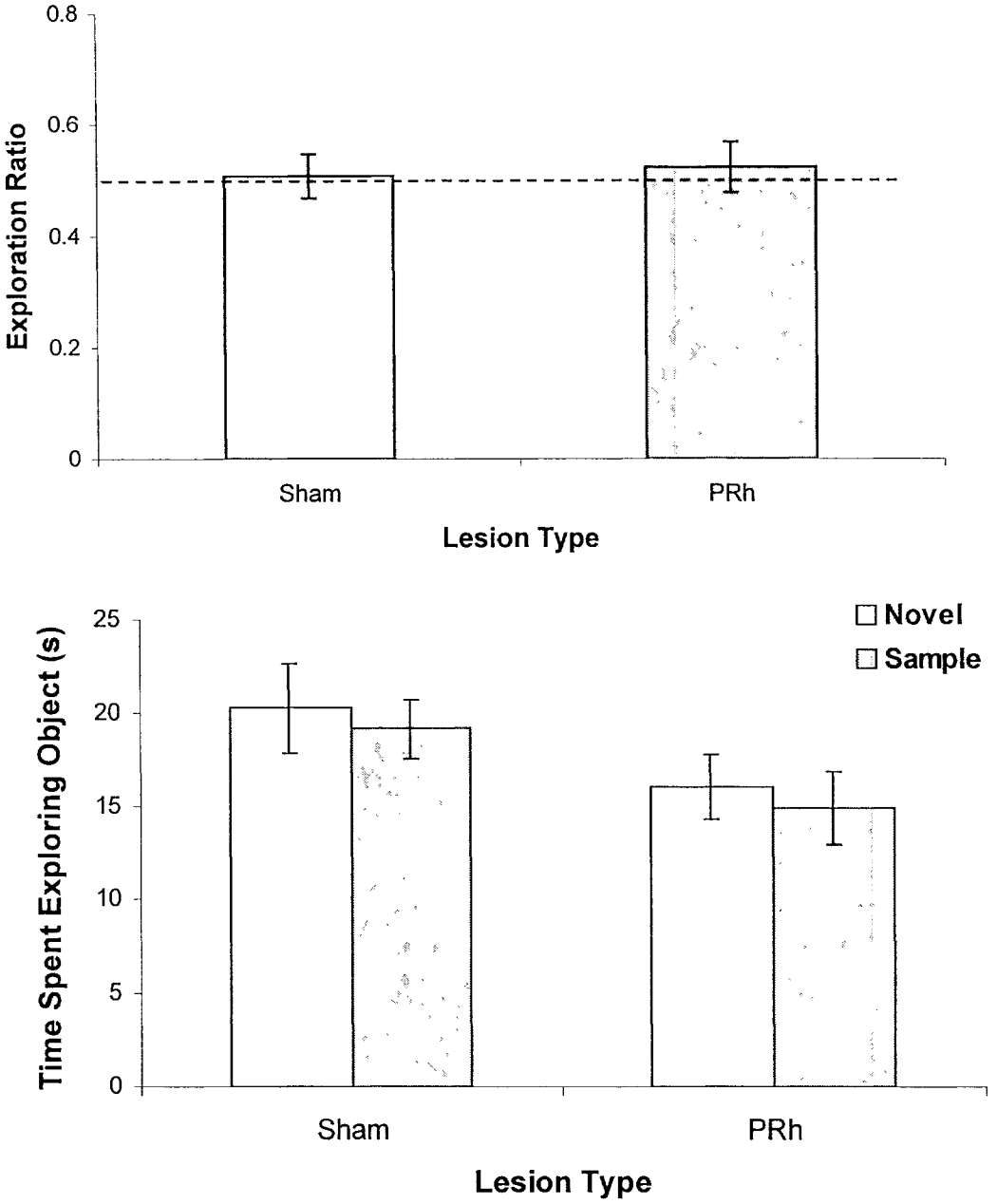


Figure 27. Mean time Sham and PRh rats spent exploring the novel and sample objects during the first 2 minutes of the test session and their mean exploration ratios for that same portion of the test. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object).

significantly different from chance ($t[7] = .158$, $p = .440$ and $t[8] = .470$, $p = .326$, respectively). A 2 x 2 (Lesion x Object) mixed-factorial ANOVA revealed a significant main effect of Lesion ($F[1,15] = 4.969$, $p = .042$). Overall, Sham rats spent more time exploring objects than PRh rats (see Appendix B). The main effect of Object and the interaction between Lesion and Object were not statistically significant (ps $>.10$).

Since neither Sham nor PRh rats displayed a significant preference for the novel object during the first 2 minutes of the retention test the first minute only was examined. Figure 28 shows mean exploration ratios of Sham and PRh rats during the first minute of the test session and the amount of time they spent exploring the novel and sample objects for that portion of the test. There was a tendency for Sham rats to have higher exploration ratios than PRh rats ($t[15] = 1.494$, $p = .087$) and Sham rats displayed a significant preference for the novel object ($t[7] = 1.998$, $p = .043$), whereas PRh rats did not ($t[8] = -.112$, $p = .457$). A 2 x 2 (Lesion x Object) mixed-factorial ANOVA conducted on time spent exploring objects failed to reveal any significant effects (ps $>.10$).

3.2.3.4 Summary

Aspiration lesions of the PRh produced anterograde object memory deficits on NPT when exploration of the sample objects was limited to 20 seconds. Neither Sham or PRh rats discriminated between the novel and sample objects when the first 2 minutes of the test session was examined. However, when the first minute of the retention test was examined, Sham rats, but not PRh rats, displayed a significant preference for the novel object. It seems that limiting rats' exploration of the sample objects made the test more difficult than when rats receive a 5-minute sample session (during which they explore the sample objects for approximately 80 seconds). This was evident from the poor performance of Sham rats during the first 2 minutes of the test session. Thus, novelty preference is more

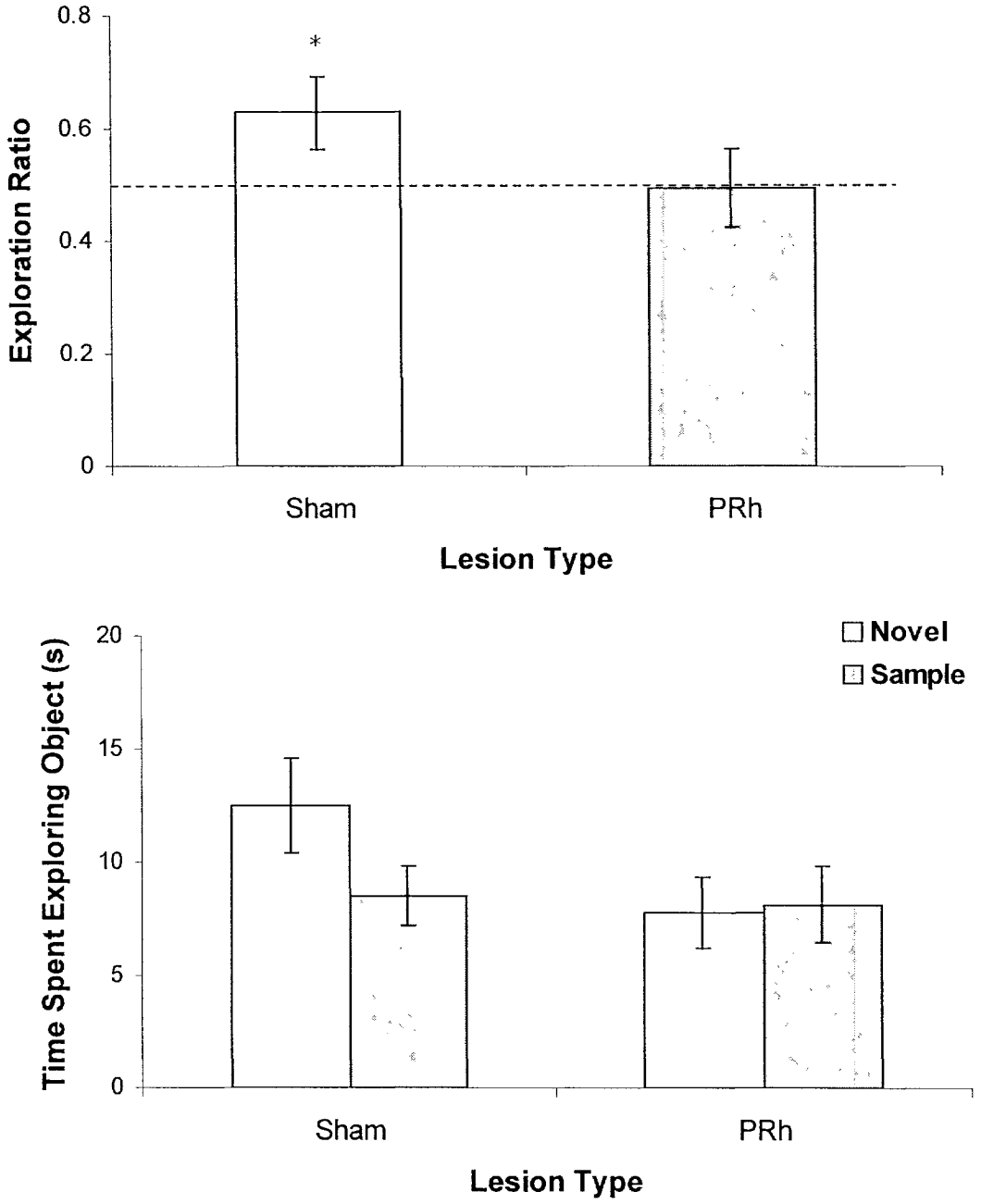


Figure 28. Mean exploration ratios and mean time Sham and PRh rats spent exploring the novel and sample objects during the first minute of the test session. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object). Asterisk denotes mean ratio that was significantly higher than chance level (one-sample t-test, $p < .05$).

difficult to detect in this modified version of NPT. Even so, PRh rats, unlike Sham rats did not show a preference for the novel object during the first minute of the test session. This finding suggests that the previous use of the standard version of NPT in Experiments 5a and 5b, was not sufficiently taxing to require the integrity of the PRh.

3.2.4 Summary of Experiment 5

The main findings of Experiments 5a, 5b, and 5c were that both electrolytic and aspiration lesions of the PRh impaired object recognition using NPT. However, the circumstances under which these lesions led to behavioural deficits were not the same. Rats with electrolytic PRh lesions were impaired with a 15-minute retention delay that followed a 5-minute sample session. Rats with aspiration PRh lesions were not impaired with a 5- or 15-minute retention delay that followed a 5-minute sample session, and instead only displayed a deficit in object recognition with a 5-minute retention delay when exploration of the sample objects was limited to 20 seconds.

The discrepant results from rats with electrolytic and aspiration lesions of the PRh cannot be accounted for by differences in the extent or location of the damage to PRh. In both cases, the PRh sustained nearly complete bilateral damage and structures adjacent to the PRh, including the lateral entorhinal and postrhinal cortices and the HPC, were also damaged to a similar, minor extent. In some cases, aspiration lesions of the PRh included more damage to adjacent structures than the electrolytic PRh lesions, but this group also tended to have spared object recognition abilities. The simplest interpretation of these findings is that the electrolytic lesion method exacerbates the effects of PRh ablation. These lesions were made by delivering a 1.5 mA current to 5 sites per hemisphere through the extent of the PRh. It is possible that this disrupts normal function in structures outside of the damaged region that may contribute in some way to normal anterograde object memory.

The lack of anterograde object memory deficits in rats with aspiration PRh lesions on the standard version of NPT with delays as long as 15 minutes was unexpected and inconsistent with other findings in the literature (e.g. Ennaceur et al., 1996). However, we did find that limiting the exploration of the sample objects to 20 seconds rendered the task sensitive to PRh lesions. It is possible that the objects we used are more easily distinguished from each other than those used by others and this makes our task less dependent on the object-processing functions of the PRh. Therefore, a deficit was only revealed when rats had little time to explore the sample objects. Also, that this manipulation was necessary to reveal an impairment in rats with aspiration PRh lesions, whereas rats with electrolytic PRh lesions were impaired on the standard version, further suggests that the electrolytic lesion method produces more widespread neurological dysfunction.

3.3 Experiment 6: Retrograde object memory following electrolytic lesions of the PRh: Between-subjects design

The main goal of this experiment was to examine retrograde memory for objects in rats with electrolytic PRh lesions. Few studies have examined the contribution of the PRh to retrograde memory. Thornton et al. (1997) found that monkeys with PRh lesions displayed a complete retrograde amnesia for object discrimination problems learned up to 15 weeks before surgery, while Wiig et al. (1996) found that rats with PRh lesions displayed a temporally graded retrograde amnesia for object discrimination problems learned up to 8 weeks before surgery. By contrast, Mumby et al. (2002) failed to detect retrograde amnesia for object discrimination problems learned singly or concurrently in rats with PRh lesions. Additionally, as previously discussed, PRh lesions do not impair the ability of animals to acquire object discrimination problems (Bussey et al., 1999; Glenn & Mumby, 1996; Mumby et al., 1994; 2002; Thornton et al., 1997; Wiig et al., 1996). Thus, it is difficult to understand the contribution of the PRh to anterograde and retrograde object memory when the tasks on which deficits are observed are dissimilar (anterograde: DNMS, retrograde: object discrimination).

This experiment used the NPT task to assess retrograde object memory. Ennaceur & Delacour (1988) reported that normal rats showed poor discrimination after 24 hours in the standard version of NPT. Therefore, it was necessary to modify the NPT procedures in a way that would permit detection of object recognition after delays of several weeks. In a pilot study (Glenn & Mumby, unpublished data; Khoury, 1997) the administration of 5 5-minute sample sessions, in which rats encountered the same two identical, sample objects, produced adequate retention on a test session conducted 6 weeks later. This variant of NPT

was used in this, and the other retrograde object memory experiments described in the remainder of this chapter.

In Experiment 2, there was evidence that electrolytic lesions of the PRh retrograde amnesia for place problems learned 4 weeks and 2 days before surgery. Additionally, rats with electrolytic PRh lesions displayed a deficit in anterograde object memory in Experiment 5b. Thus, this experiment assessed the effects of electrolytic lesions of the PRh on retrograde object recognition. A between-subjects design was used, in which rats were familiarized with a single pair of identical sample objects either 5, 3, or 1 weeks prior to surgery.

3.3.1 Method

3.3.1.1 Subjects

Thirty-seven rats served as subjects in this experiment: 11 rats encountered objects 5 weeks before surgery (PRh n=6, Sham n=5), 16 rats encountered objects 3 weeks before surgery (PRh n=8, Sham n=8), and 12 rats encountered objects during the week before surgery (PRh n=5, Sham n=7). All rats in this experiment were part of another study in which they learned object discrimination problems during the weeks in which they were not being tested for the present experiment.

3.3.1.2 Procedure

3.3.1.2.1 Presurgery familiarization. All rats first received three 15-minute habituation sessions, one per day for three consecutive days. Rats were habituated in pairs for the first two sessions, and singly in the final session. There were no objects present in the arena during habituation. Approximately 48 hours after the last habituation session rats received 5 5-minute sample sessions, one per day for 5 consecutive days. In each session, the same pair of identical sample objects was present in the arena.

3.3.1.2.2 Surgery. Rats received surgery either 5 weeks, 3 weeks, or between 24 and 48 hours after the final sample phase session. Bilateral electrolytic lesions of the PRh and sham surgery were conducted as described in Experiment 2. Rats recovered from surgery for two weeks prior to retention testing.

3.3.1.2.3 Postsurgery retention testing. One hour before the test session, all rats received a single 5-minute re-habituation session in the arena. For the test session, the sample and a novel object were positioned in the arena. The test was 5 minutes in duration.

3.3.2 Results

3.3.2.1 Histological results

The location and extent of the largest and smallest PRh lesions are shown in Figure 24 and are described in Section 3.2.3.2.1.

3.3.2.2 Behavioural results

Figure 29 shows the mean exploration ratios for the first 2 minutes of the test session for the Sham and PRh rats at each time point. The exploration ratios from one PRh rat in the 5-week, one PRh rat in the 3-week group, and one Sham rat in the 1-week group were not included in this analysis because they failed to explore the objects for the minimum of 2 seconds. One-sample t-tests comparing the exploration ratios of each of the 6 groups to chance revealed that neither Sham nor PRh rats in the 5-week group displayed a significant preference for an object ($t[4] = -1.341$, $p = .126$ and $t[4] = .02$, $p = .493$, respectively). Sham rats in the 3- and 1-week groups displayed a significant preference for the novel object ($t[7] = 2.193$, $p = .032$ and $t[5] = 2.344$, $p = .033$, respectively), whereas PRh rats in the 3- and 1-week groups did not ($t[5] = -.066$, $p = .475$ and $t[3] = -.521$, $p = .319$, respectively). However, A 2 x 3 (Lesion x Time of Learning) completely between-subjects ANOVA failed to reveal any significant effects ($ps > .10$).

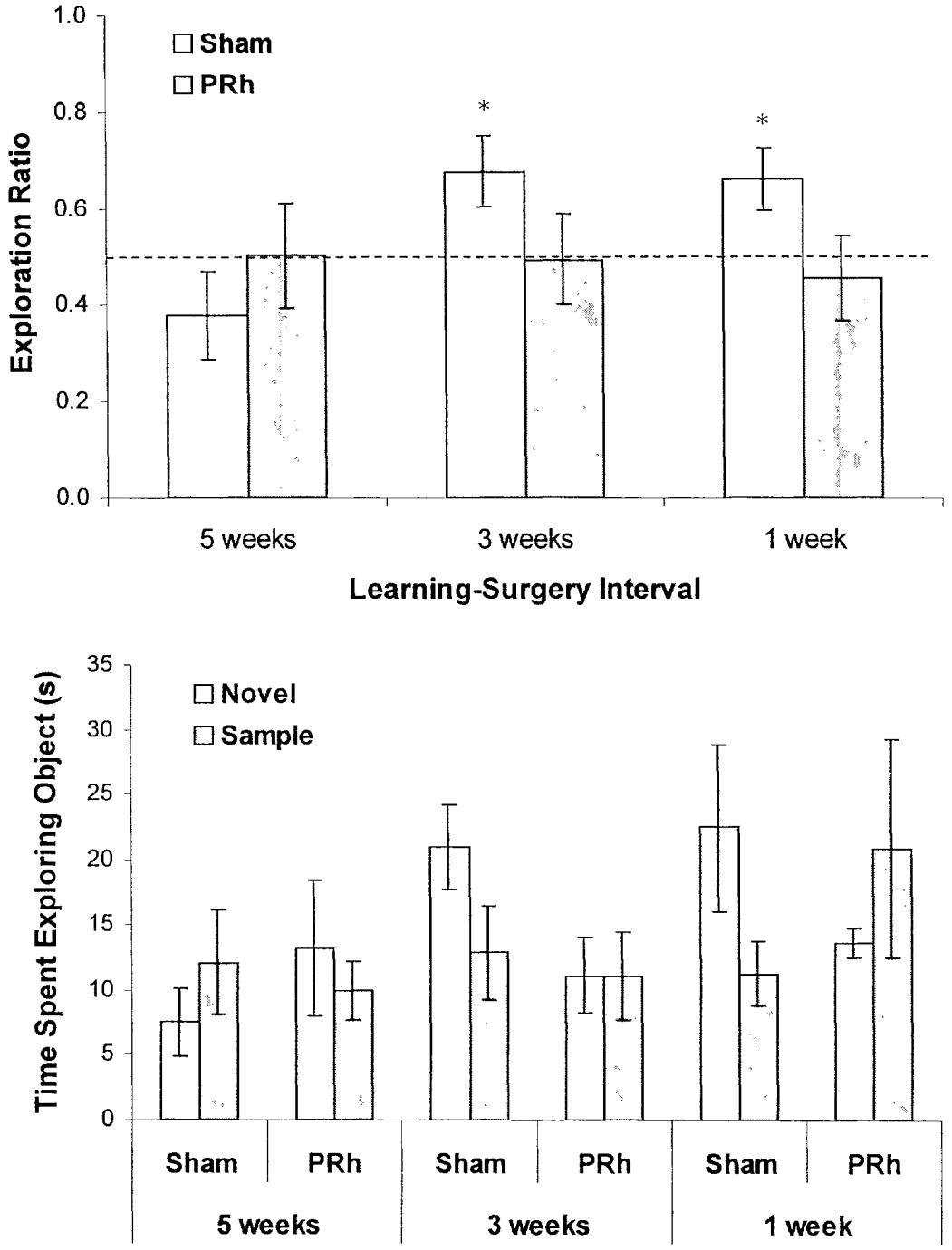


Figure 29. The top panels shows the mean exploration ratios for each group during the first 2 minutes of the test session. The bottom panel shows the mean times Sham and PRh rats from each time point spent exploring the novel and sample objects during the same portion of the test. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object). Asterisks denote mean ratios that were significantly higher than chance level one-sample t-test, $p < .05$).

Figure 29 also shows the amount of time Sham and PRh rats spent exploring the novel and sample objects during the first 2 minutes of the test session. A 2 x 3 x 2 (Lesion x Time of Learning x Object) mixed factorial ANOVA revealed that the interaction between Lesion, Time of Learning, and Object approached statistical significance ($F[2,31] = 2.515$, $p = .097$). As can be seen in Figure 29, Sham rats in the 5-week group tended to spend more time with the sample object, but Sham rats in the 3- and 1-week group spent more time with the novel object. PRh rats in the 5-week group also tended to spend more time with the sample object, but PRh rats in the 3-week group spent similar amounts of time with both objects, and PRh rats in the 1-week group tended to spend more time with the sample object. All main effects and all other interactions were not statistically significant ($ps > .10$).

3.3.3 Summary

In this experiment electrolytic PRh lesions produced retrograde amnesia, without a temporal gradient, for objects. Sham and PRh rats that were familiarized with identical sample objects 5 weeks before surgery failed to discriminate between the novel and sample object during the test session conducted 2 weeks after surgery. Because the Sham rats were unable to recognize the sample object they encountered 5 weeks before surgery, it was not possible to draw conclusions about the performance of PRh rats at that time point. However, Sham rats familiarized with the sample objects 3 weeks and during the week of surgery displayed a significant preference for the novel object during the test session, whereas PRh rats familiarized with sample objects at both of these time points did not display a preference for either object.

It is widely believed that the PRh is critical for normal anterograde object memory (for review see Murray & Richmond, 2001). The present findings are consistent with this view, and bolster a scant literature on the contribution of the PRh to retrograde object memory.

Additionally, the same rats that displayed retrograde amnesia in the present experiment also displayed anterograde amnesia in Experiment 5. Thus, it seems that the abilities of rats to acquire new object information and later recall it are both dependent on the integrity of the PRh. This comparison and interpretation was possible because features of the learning events in Experiment 5 and in the present experiment were similar.

Unfortunately, the experiments on place memory (in Chapter 2) and object-recognition (Experiment 5b) demonstrated that the behavioural outcome following PRh damage is different following electrolytic versus aspiration lesions. Thus, the effects of aspiration lesions of the PRh on retrograde object memory were investigated in the following experiments.

3.4 Experiment 7: Retrograde object memory following aspiration lesions of the PRh: Between-subjects design

In Chapter 2, electrolytic PRh lesions led to retrograde amnesia for places; the same pattern observed following HPC lesions (Mumby et al., 1999). In Section 2.6, we explored the interpretation that the electrolytic lesion may be disturbing normal function in the HPC, an efferent of the PRh. Gaskin, Tremblay, & Mumby (2003) found that excitotoxic HPC lesions produced retrograde amnesia, without a temporal gradient, using the same, modified, version of NPT used in Experiment 6. Thus, it is possible that the electrolytic lesion method itself was in part, or wholly responsible for the retrograde amnesia observed in Experiment 6. In Experiment 5, aspiration lesions of the PRh did not impair anterograde object-recognition unless exposure to the sample objects was limited to 20 seconds. Thus, the present experiment assessed the effects of aspiration PRh lesions on retrograde object recognition.

Rats were familiarized with a pair of identical sample objects either 4 weeks before surgery, or during the week before surgery. These learning-surgery intervals were chosen for two reasons: First, in Experiment 6, Sham rats displayed no evidence of recognizing the sample objects from 5 weeks before surgery. Second, 4-week and 1-week intervals were used in the retrograde place memory experiments in Chapter 2. This provides an opportunity for a direct comparison of retrograde memory for object and place information after similar learning-surgery intervals and similar PRh lesions. In the present experiment a between-subjects design was used, as in Experiment 6, to provide an opportunity to compare retrograde object-recognition abilities following aspiration and electrolytic PRh lesions.

3.4.1 Method

3.4.1.1 Subjects

Thirty-three rats served as subjects in this experiment: Seventeen rats encountered objects 4 weeks before surgery (PRh n=9, Sham n=8), and 16 rats encountered objects during the week before surgery (PRh n=9, Sham n=7). Twenty-one of the rats were subjects in Experiment 4. They either learned a place problem 4 weeks before surgery if they were in the 1-week group in this experiment (PRh n=6, Sham n=4), or during the week before surgery in they were in the 4-week group in this experiment (PRh n=6, Sham n=5). The remaining 12 rats were experimentally naïve (PRh n=6, Sham n=6).

3.4.1.2 Procedure

3.4.1.2.1 Presurgery familiarization. All procedures were the same as described in Experiment 7, except rats received sample sessions either 4 weeks (REMOTE) or during the week (RECENT) before surgery.

3.4.1.2.2 Surgery. Rats received surgery either 4 weeks or between 48 and 24 hours after the final sample phase session. Bilateral aspiration lesions of the PRh and Sham surgery were conducted as described in Experiment 1.

3.4.1.2.3 Postsurgery retention testing. All procedures were the same as in Experiment 6.

3.4.2 Results

3.4.2.1 Histological results

The lesions of the 12 PRh rats that first served as subjects in Experiment 4 are described in section 2.5.2.1, and the largest and smallest lesions of this group are shown in Figure 17. All rats in this group sustained substantial, bilateral damage to the PRh.

For the remaining 6 PRh rats the location and extent of the largest and smallest PRh lesions are shown in Figure 30. There was substantial and nearly complete, bilateral damage to the PRh in each lesioned rat. The PRh was 95 percent destroyed in both the largest and smallest lesions. Thus, there was good concordance in the amount of PRh damage with these lesions and those of the rats from Experiment 4.

Also consistent with the lesions of the rats from Experiment 4, portions of the lateral entorhinal cortex was damaged bilaterally in the additional 6 PRh rats. The rat with the largest lesion sustained approximately 15 percent loss of tissue in this region and the rat with the smallest lesion sustained approximately 10 percent loss. This amount of damage was slightly less than in the lesions of the Experiment 4 rats. Similarly, there was less damage to the postrhinal cortex in the 6 additional lesions; there was less than 5 percent damage and it was unilateral in all rats.

Bilateral damage to temporal association cortex was evident in 3 of the 6 PRh rats, while the remaining 3 rats sustained mostly unilateral damage to this area. Two rats sustained bilateral damage to the ventral portions of the CA1 cell field and the subiculum. Damage to these regions was unilateral in the remaining PRh rats. One lesion included bilateral damage to the lateral amygdala, and two lesions included unilateral damage to this area.

3.4.2.2 Behavioural results

Figure 31 shows the mean exploration ratios for the first 2 minutes of the test session for each of the 4 groups. One Sham and one PRh rat from the RECENT group were excluded from the analysis for failing to explore either object during this portion of the test session. For the REMOTE group, Sham rats tended to have higher exploration ratios than PRh rats ($t[15] = 1.454$, $p = .084$), whereas the exploration ratios of the Sham and PRh rats

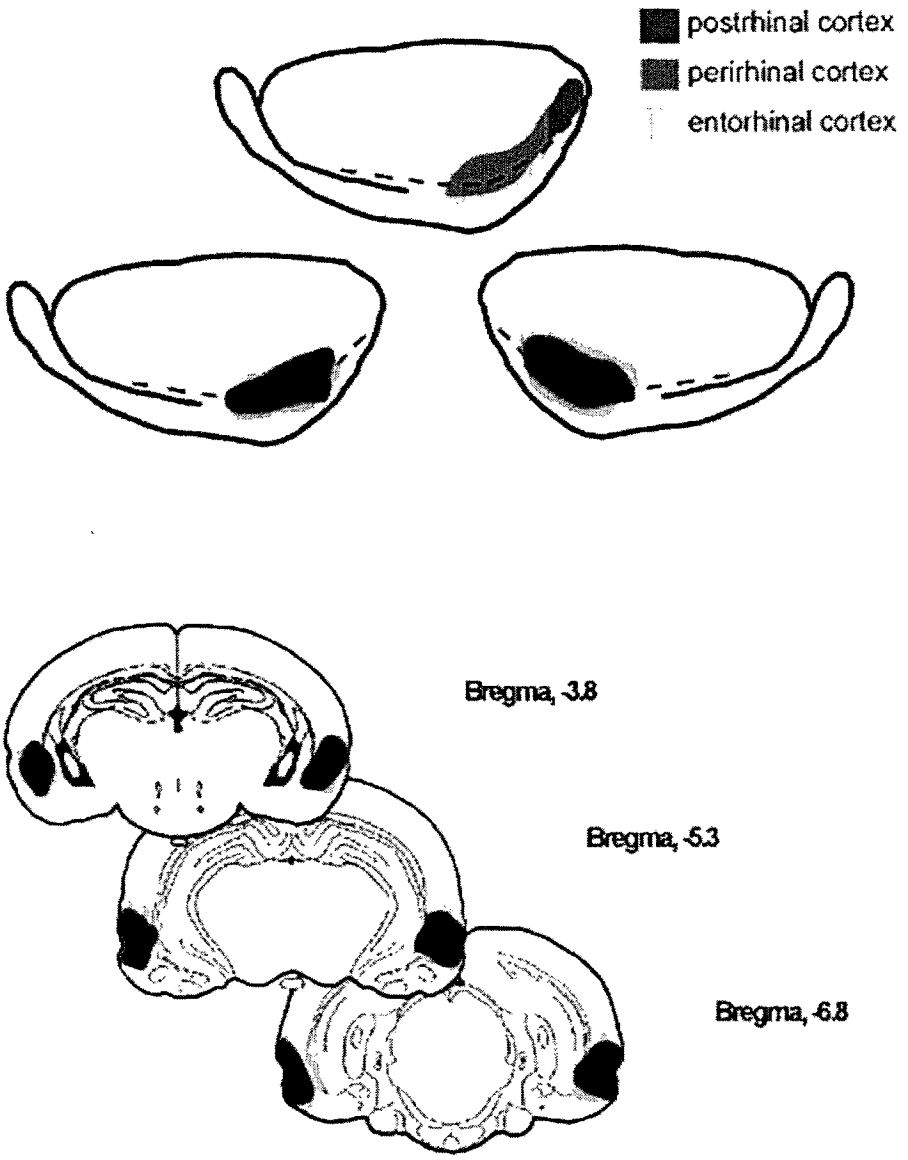


Figure 30. The location and extent of the PRh lesions are shown on the lateral surface of the rat brain in the top panel and in three coronal views in the bottom panel. In each panel, the largest lesion is shown in grey and the smallest lesion is shown in black.

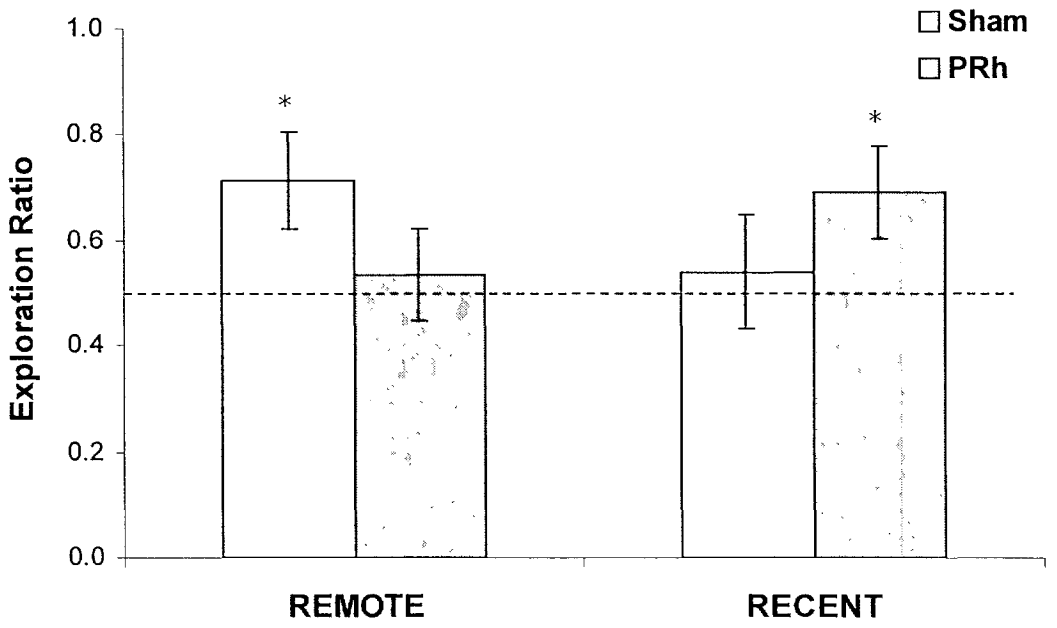


Figure 31. Mean exploration ratios of Sham and PRh rats from the first 2 minutes of the REMOTE and RECENT test sessions. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object). Asterisks denote mean ratios that were significantly higher than chance level one-sample t-test, $p < .05$).

in the RECENT group were not significantly different ($t[12] = -.993$, $p = .170$). One sample t-tests confirmed that, for the REMOTE group, Sham rats had exploration ratios that were significantly higher than chance ($t[7] = 2.363$, $p = .025$) and PRh rats had exploration ratios that did not differ significantly from chance ($t[8] = .372$, $p = .360$). For the RECENT group, the exploration ratios of Sham rats did not differ significantly from chance ($t[5] = .373$, $p = .362$) and the exploration ratios of PRh were significantly higher than chance ($t[7] = 2.043$, $p = .040$). A 2 x 2 (Lesion x Time of Learning) completely between-subjects ANOVA revealed that the interaction between Lesion and Time of Learning approached statistical significance ($F[1,27] = 2.921$, $p = .099$), but neither of the main effects were statistically significant ($ps > .10$).

Figure 32 shows the amount of time Sham and PRh rats spent exploring the novel and sample objects during the first 2 minutes of the test session. A 2 x 3 x 2 (Lesion x Time of Learning x Object) mixed factorial ANOVA revealed only a significant main effect of Object ($F[1,29] = 8.374$, $p = .007$). Overall, rats spent more time exploring the novel object. None of the other main effects or interactions were statistically significant ($ps > .10$).

Because the Sham rats in the RECENT group, unlike those in the REMOTE group, did not show a preference for the novel object during the first 2 minutes of the test session, the first 3 minutes of the retention test were examined (the pattern of exploration in the first minute of the retention test was similar to the data shown here for the first 2 minutes). Figure 33 shows the mean exploration ratios for each group based on the first 3 minutes of the test session. One-sample t-tests revealed that the exploration ratios of the Sham rats from the REMOTE group were not significantly different from chance ($t[7] = 1.089$, $p = .156$), however the comparison of the exploration ratios of the PRh rats from the REMOTE group to chance approached statistical significance ($t[8] = 1.749$, $p = .059$). For the

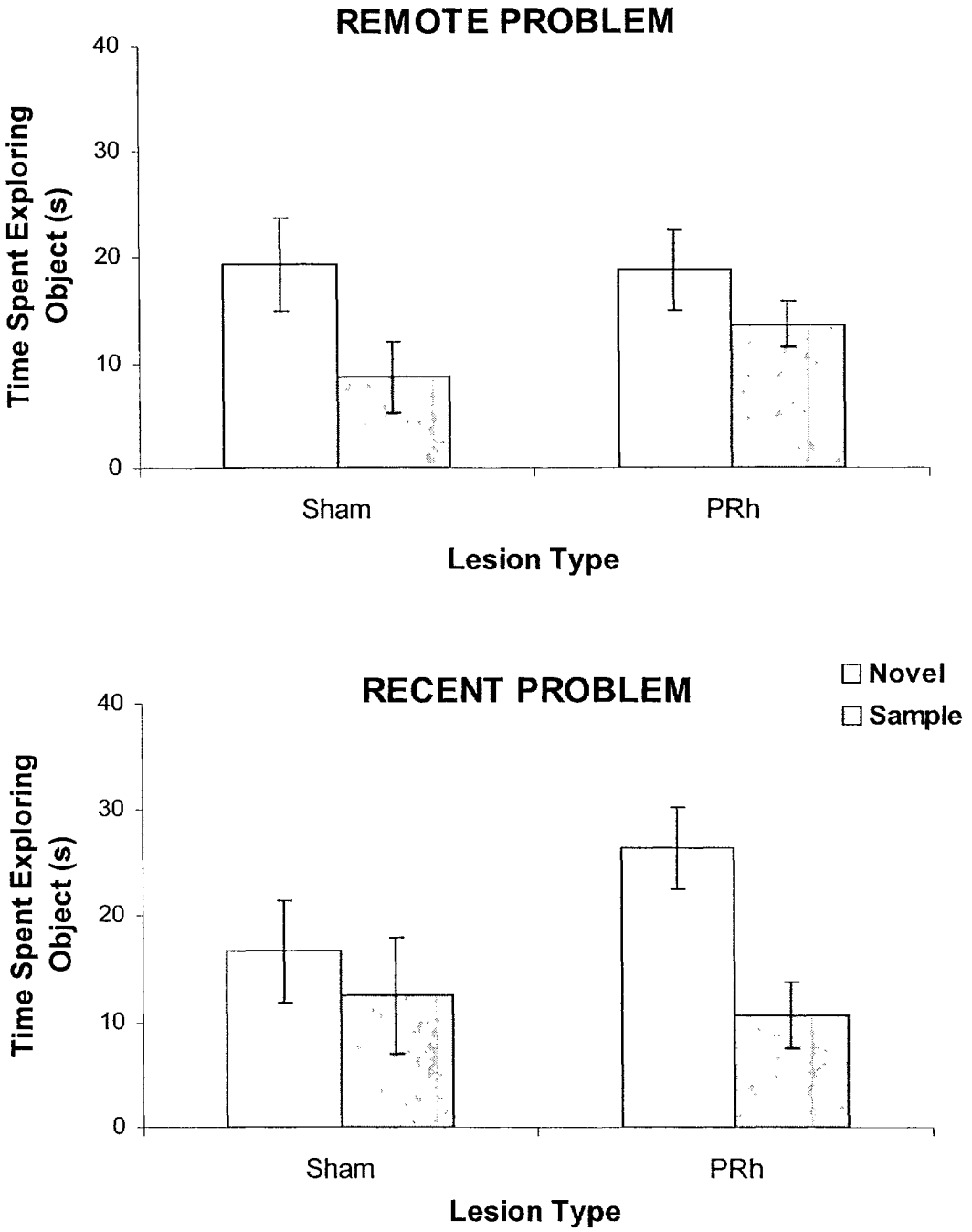


Figure 32. Mean times spent exploring the novel and sample objects during the first 2 minutes of the REMOTE and RECENT test sessions. The error bars represent S.E.M.

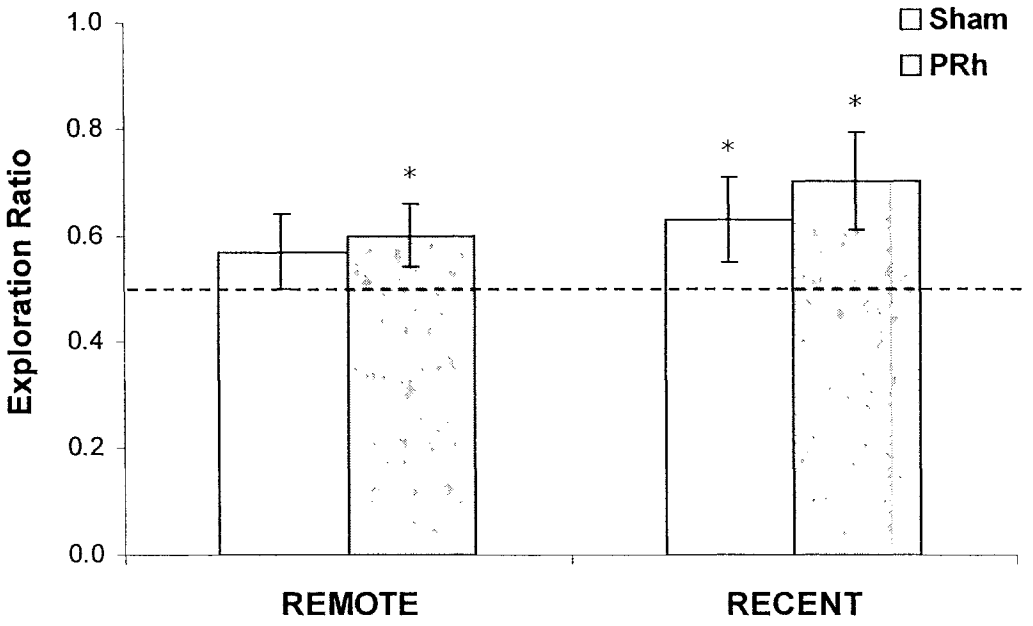


Figure 33. Mean exploration ratios of Sham and PRh rats from the first 3 minutes of the REMOTE and RECENT test sessions. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object). Asterisks denote mean ratios that were significantly higher than chance level one-sample t-test, $p < .10$).

RECENT group, the exploration ratios of the Sham rats tended to be higher than chance ($t[5] = 1.705$, $p = .075$) and the exploration ratios of the PRh rats were significantly higher than chance ($t[8] = 2.385$, $p = .025$). A 2 x 2 (Lesion x Time of Learning) between-subjects ANOVA failed to reveal any statistically significant effects (all $ps > .10$).

The mean times each of the 4 groups spent exploring the sample and novel objects during this portion of the test session are also shown in Figure 34. The same two rats excluded above were also excluded from this analysis for still failing to explore the objects in the third minute. A 2 x 3 x 2 (Lesion x Time of Learning x Object) mixed factorial ANOVA revealed only a significant main effect of Object ($F[1,29] = 8.374$, $p = .007$). As with the first 2 minutes, rats again spent more time exploring the novel object, overall. None of the other main effects or interactions were statistically significant ($ps > .10$).

3.4.3 Summary

Aspiration lesions of the PRh did not produce retrograde amnesia for objects in rats. During the first 2 minutes of the retention test, Sham rats, but not PRh rats, in the REMOTE (-4 week) condition displayed a significant preference for novel object relative to the sample object, whereas PRh rats, but not Sham rats, in the RECENT (-1 week) condition displayed a significant preference for novel object relative to sample object. However, if the first 3 minutes of the retention test was considered, the rats in all 4 conditions displayed a novelty preference which was statistically significant in all groups except Sham-REMOTE.

Overall, the data showed no evidence of a deficit in the PRh rats and this is in contrast with the findings from Experiment 6, in which rats with electrolytic PRh lesions familiarized with objects at similar time points prior to surgery displayed retrograde

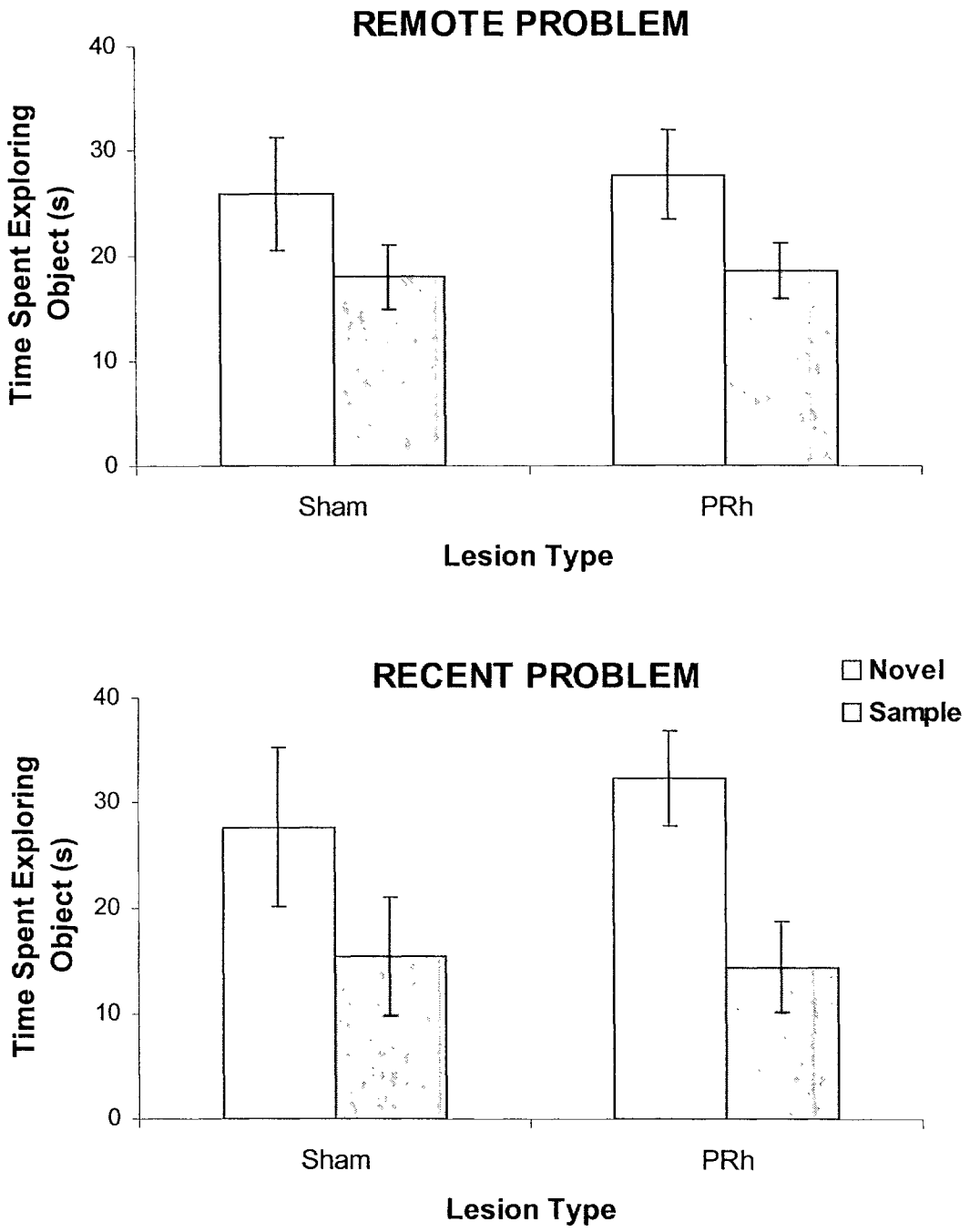


Figure 34. Mean times spent exploring the novel and sample objects during the first 3 minutes of the REMOTE and RECENT test sessions. The error bars represent S.E.M.

amnesia. However, the combined findings from Experiments 6 and 7 are consistent with the combined findings from Experiments 2 and 3 in Chapter 2. In both cases, there was retrograde amnesia following electrolytic, but not aspiration lesions. These findings provide compelling evidence that the electrolytic lesion method itself is a contributing factor to retrograde amnesia. As previously discussed, HPC lesions also produce retrograde amnesia for places (Mumby et al., 1999) and objects (Gaskin et al., 2003), therefore the electrolytic PRh lesions may be disturbing normal function in this region. We explored this hypothesis in Chapter 5.

The lack of retrograde amnesia in the rats with aspiration PRh lesions in the present experiment is not consistent with the prevalent view that the PRh is important for normal object-recognition memory. It is possible that the functions of the PRh are primarily important for working memory for objects, and not the consolidation of long-term memories about objects. In Experiment 5, aspiration PRh lesions impaired anterograde recognition memory when exploration of the sample objects was limited to 20 seconds, but not when rats were permitted to explore the sample objects for 5 minutes, which resulted in approximately 80 seconds of object exploration (see Appendix B). Thus, the modified version of NPT used in this experiment to assess retrograde object memory may similarly allow too much exploration of the sample objects to make recognition dependent on the PRh (approximately 300 seconds, see Appendix B). Perhaps with longer exposure there is recruitment of other brain regions to form long-lasting memories of the sample objects. The PRh may also be recruited, but subsequent damage to it does not abolish all representations of the object. However, it might be possible to reveal PRh-dependency by increasing the number of object pairs rats encounter before surgery (Hampton & Murray, 2002). This possibility was examined in the next experiment.

3.5 Experiment 8: Retrograde object memory following aspiration lesions of the PRh: Within-subjects design

The absence of retrograde amnesia for objects in Experiment 7 following aspiration lesions of the PRh is both puzzling and intriguing. Conventional theories of consolidation and long-term memory formation typically assert that brain regions important for these functions are also needed for information acquisition (Alvarez & Squire; 1994; Squire, 1991). However, the combined findings from Experiment 5 and 7 suggest that this is not the case. In Experiment 5, aspiration lesions of the PRh produced anterograde amnesia for objects, but in Experiment 7, aspiration lesions of the PRh did not produce retrograde amnesia for objects. One possible explanation is that the PRh is important for the intermediate-term storage of object representations, but not for consolidation or the formation of long-term memories about objects. Another explanation is that the task parameters used in Experiment 7 were not sufficiently taxing to require the PRh for normal performance. Previous studies have found that PRh involvement in object recognition is most evident when the demand to recognize an object or discriminate objects is very high, such as when multiple problems are presented concurrently (Easton & Gaffan, 2000), large sets of objects are used (Eacott et al., 1994), physical discriminability of objects are reduced (Eacott et al., 1994), or when complex visual stimuli are used and animals must recognize multiple features (Eacott, Machin, & Gaffan, 2001).

In the present experiment, the task demands were increased by making the Time of Learning variable a within-subjects factor. Consequently, rats encountered two pairs of identical sample objects prior to surgery; one pair was encountered 4 weeks before surgery and the other during the week before surgery. This design is typically preferred in retrograde

memory experiments as it more closely mimics the human condition where the same individual is tested for memory of information acquired at different time points prior to brain injury. In most animal experiments, however, there are inherent difficulties with teaching animals multiple problems. For example, in Chapter 2, the rats in Experiments 2 and 3 learned two place memory problems in the water maze prior to surgery. Rats develop a learning set during presentation of the first problem and subsequently acquire later problems more rapidly. This was evident in Experiments 2 and 3. In these experiments, exposure to the problem was equated. An alternative is to establish a learning criterion in which facility with the problem is equated, even though this may mean that exposure to successive problems decreases because they are learned faster.

Our retrograde version of NPT is, in fact, relatively unaffected by these potential difficulties. The rats are not required to learn rules or contingencies, as there is no problem, per se, to solve. Therefore, in this case we also equated exposure to the objects: all rats received 5 5-minute sample phases sessions with each pair of sample objects. It was hypothesized that this would place more demands on an object-recognition system. If the PRh is an integral component in such a system, and if this system participates in the formation of long-term memories about objects than rats with PRh lesions should show retrograde amnesia.

3.5.1 Method

3.5.1.1 Subjects

Fourteen experimentally naïve rats served as subjects in this experiment.

3.5.1.2 Procedure

3.5.1.2.1 Presurgery familiarization. The procedures for familiarizing rats with objects prior to surgery were the same as in Experiments 6 and 7. However, in this case, all rats

received 5 5-minute sample phase sessions, one per day for 5 consecutive days, 4 weeks prior to surgery, and another 5 sample phase sessions during the week of surgery. Two different pairs of identical sample objects were used, one for each time point, counterbalanced.

3.5.1.2.2 Surgery. Rats received either bilateral aspiration lesions of the PRh (n=7) or Sham surgery (n=7) between 24 and 48 hours after the final sample phase session. Surgical procedures were as described in Experiment 1. Rats recovered from surgery for two weeks prior to retention testing.

3.5.1.2.3 Postsurgery retention testing. Like in Experiments 6 and 7, all rats received one 5-minute re-habituation session, except in the present experiment this occurred approximately 24 hours prior to the retention test. Unlike in Experiments 6 and 7, rats in the present experiment received two retention tests, one for each of the sample object pairs they encountered before surgery. Each retention test was conducted as in the previous experiments: 5 minutes in the arena with one of the sample objects and a novel object. There was 1 retention test per day for two consecutive days, and the presentation of each sample object was counterbalanced over the two days.

3.5.2 Results

3.5.2.1 Histological results

The location and extent of the largest and smallest PRh lesions are shown in Figure 35. There was substantial and nearly complete, bilateral damage to the PRh in each lesioned rat. The PRh was approximately 95 percent destroyed in the rat with the largest lesion and 85 percent destroyed in the rat with the smallest lesion.

All lesions included bilateral damage to the lateral entorhinal cortex. This damage was estimated to be approximately 15 percent in the rat with the largest lesion and approximately 5 percent in the rat with the smallest lesion. Unlike previous studies, the lesions in this

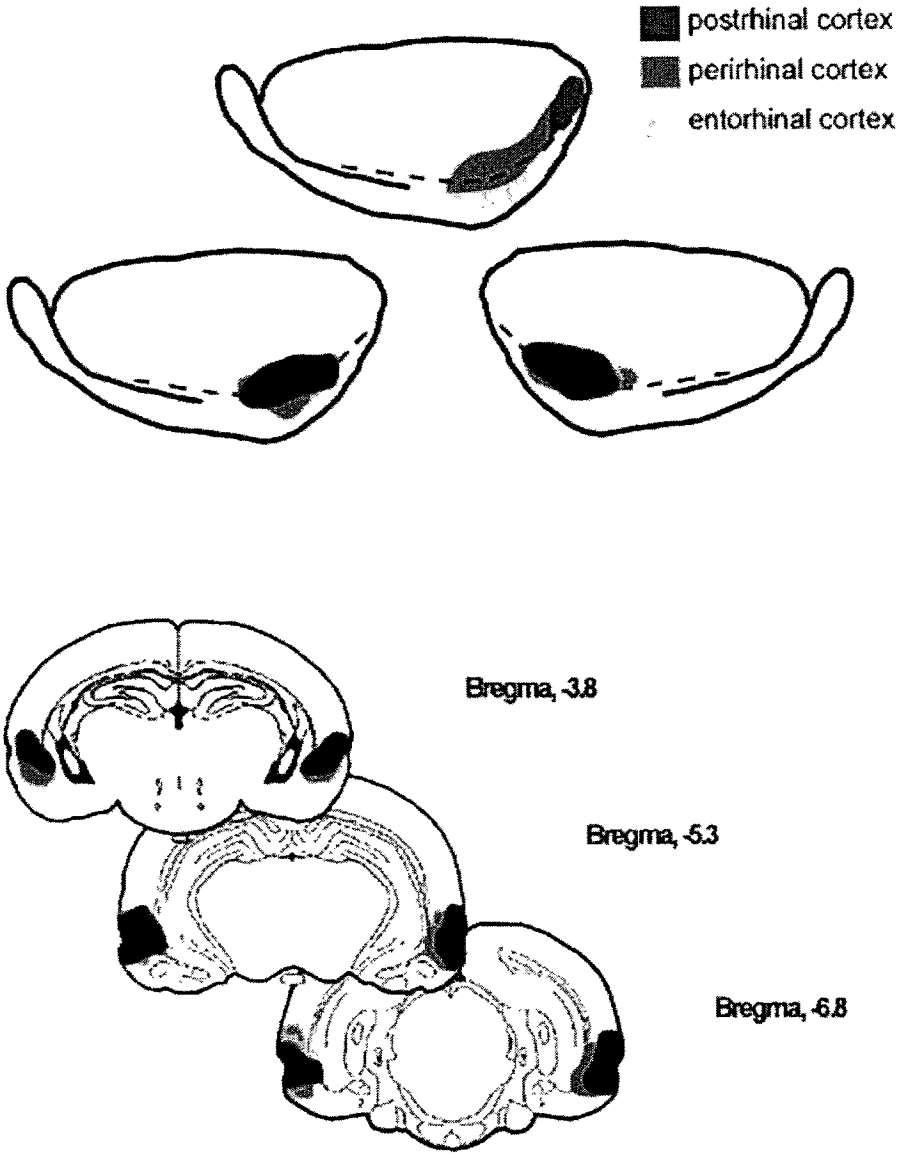


Figure 35. The location and extent of the PRh lesions are shown on the lateral surface of the rat brain in the top panel and in three coronal views in the bottom panel. In each panel, the largest lesion is shown in grey and the smallest lesion is shown in black.

experiment included very little damage to the postrhinal cortex. Only one of the seven rats sustained damage in this region and, though bilateral, it was estimated to include only about 5 percent of the postrhinal cortex.

All lesions included damage to portions of the ventral temporal association cortex. However, this damage was minimal in all rats. Four rats sustained bilateral damage to this region, while the remaining three rats sustained unilateral damage. There was bilateral damage to the ventral portions of the CA1 cell field and subiculum in 2 of the 7 rats, and an additional 4 rats sustained unilateral damage to this area. Five lesions included portions of the lateral amygdala, in 2 rats this damage was bilateral and in 3 it was unilateral.

3.5.2.2 Behavioural results

In Experiment 7, exploration ratios were calculated for the first 2 minutes and the first 3 minutes of the test session. Thus, to facilitate comparisons, the same was done in this experiment. One Sham failed to explore objects during the RECENT test session and was excluded from that analysis.

Figure 36 shows the mean exploration ratios of Sham and PRh during the first 2 minutes of the REMOTE and RECENT test sessions. One sample t-tests revealed that neither Sham nor PRh rats displayed a significant preference for the novel object during the first 2 minutes of the REMOTE test session ($t[6] = -.252$, $p = .405$ and $t[6] = .941$, $p = .192$, respectively). During the RECENT test session, Sham rats displayed a significant preference for the novel object ($t[5] = 2.813$, $p = .019$), but PRh rats did not ($t[6] = 1.302$, $p = .121$). A 2 x 2 (Lesion x Time of Learning) mixed-factorial ANOVA failed to reveal any significant effects ($ps > .10$).

Figure 37 shows the mean times Sham and PRh rats spent exploring the novel and sample objects during the first 2 minutes of the REMOTE and RECENT test sessions. A 2

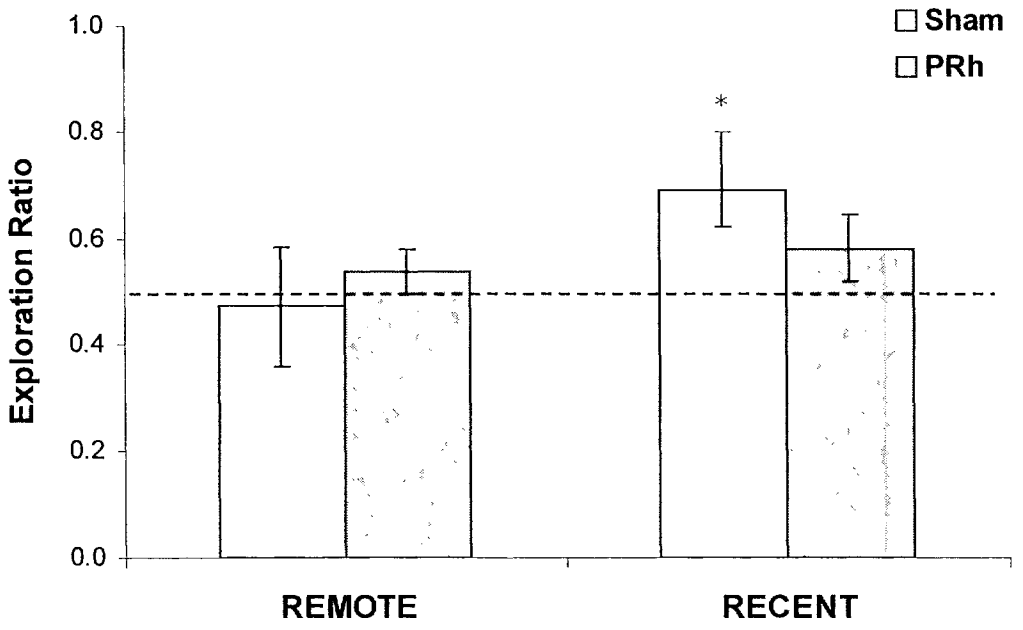


Figure 36. Mean exploration ratios for Sham and PRh rats from the first 2 minutes of the REMOTE and RECENT test sessions. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object). Asterisk denotes mean ratio that was significantly higher than chance level one-sample t-test, $p < .05$).

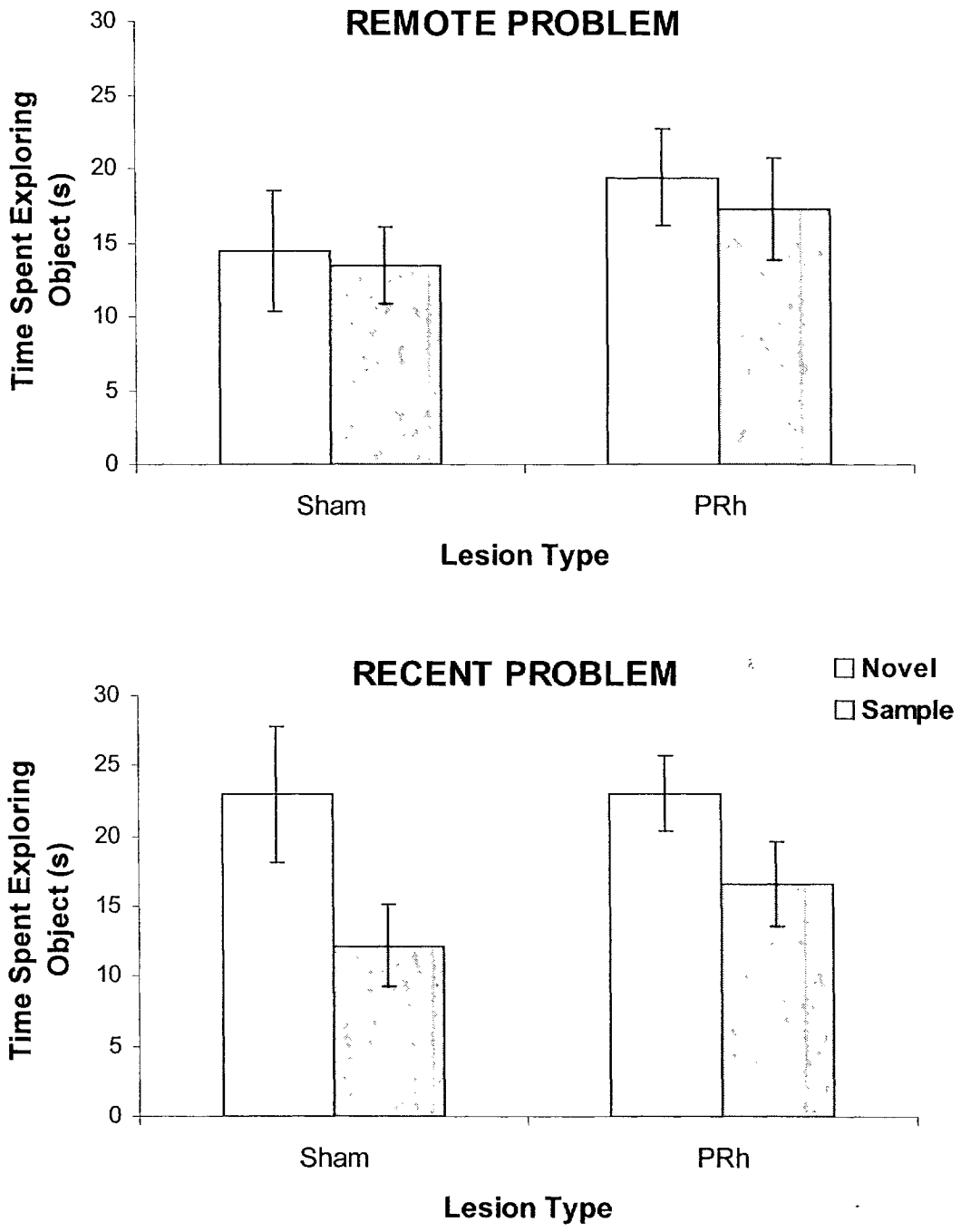


Figure 37. Mean times Sham and PRh rats spent exploring the sample and novel objects during the first 2 minutes of the REMOTE and RECENT test sessions. The error bars represent S.E.M.

x 2 x 2 (Lesion x Time of Learning x Object) mixed-factorial ANOVA revealed only a significant effect of Object ($F[1,11] = 9.687$, $p = .010$), indicating that, overall, rats spent more time exploring the novel object compared to the sample object. No other main effects and no interactions were statistically significant ($ps > .10$).

Figure 38 shows the mean exploration ratios of Sham and PRh rats during the first 3 minutes of the REMOTE and RECENT test sessions. One-sample t-tests revealed a tendency for PRh rats to prefer the novel object during the REMOTE test session ($t[6] = 1.592$, $p = .081$), whereas the exploration ratios of Sham rats were not significantly different from chance ($t[6] = -.145$, $p = .445$). Both Sham and PRh rats displayed a significant preference for the novel object during the RECENT test session ($t[5] = 4.052$, $p = .005$ and $t[6] = 1.901$, $p = .053$, respectively). As with the first 2 minutes, a 2 x 2 (Lesion x Time of Learning) mixed-factorial ANOVA failed to reveal any significant effects ($ps > .10$).

The mean times spent by Sham and PRh exploring the novel and sample objects during the first 3 minutes of the REMOTE and RECENT test sessions are shown in Figure 39. A 2 x 2 x 2 (Lesion x Time of Learning x Object) mixed-factorial ANOVA also revealed a significant main effect of Object ($F[1,11] = 15.442$, $p = .002$), and no other main effects and no interactions were statistically significant ($ps > .10$). As with the analysis of the first 2 minutes of the retention test, rats spent more time, overall, exploring the novel object.

3.5.3 Summary

Aspiration lesions of the PRh did not produce retrograde amnesia for objects when a within-subjects design was used. When the first 2 minutes of REMOTE test session were

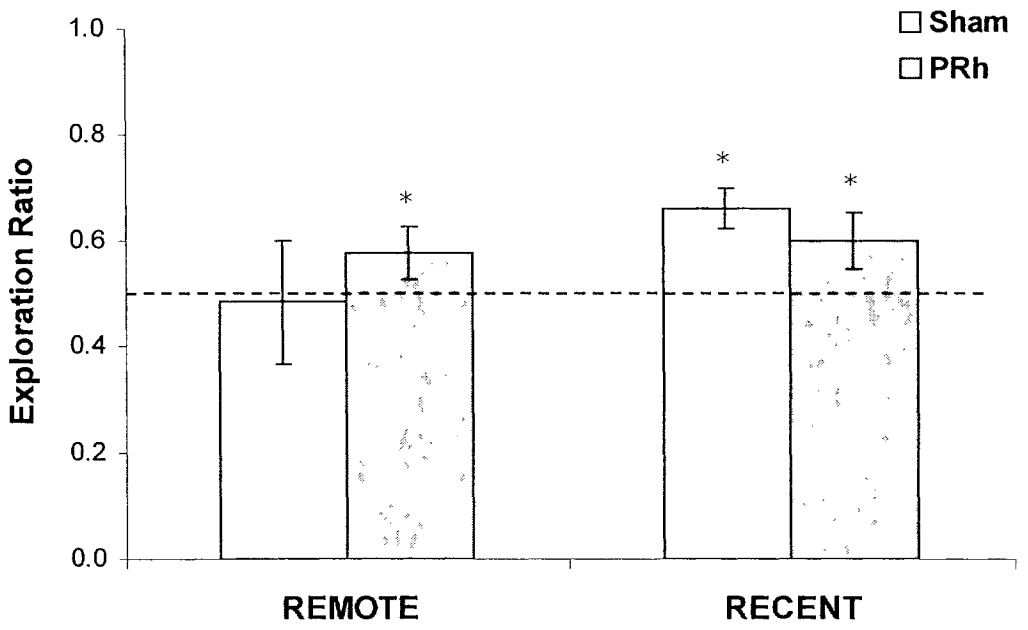


Figure 38. Mean exploration ratios for Sham and PRh rats from the first 3 minutes of the REMOTE and RECENT test sessions. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object). Asterisks denote mean ratio that was significantly higher than chance level one-sample t-test, $p < .10$).

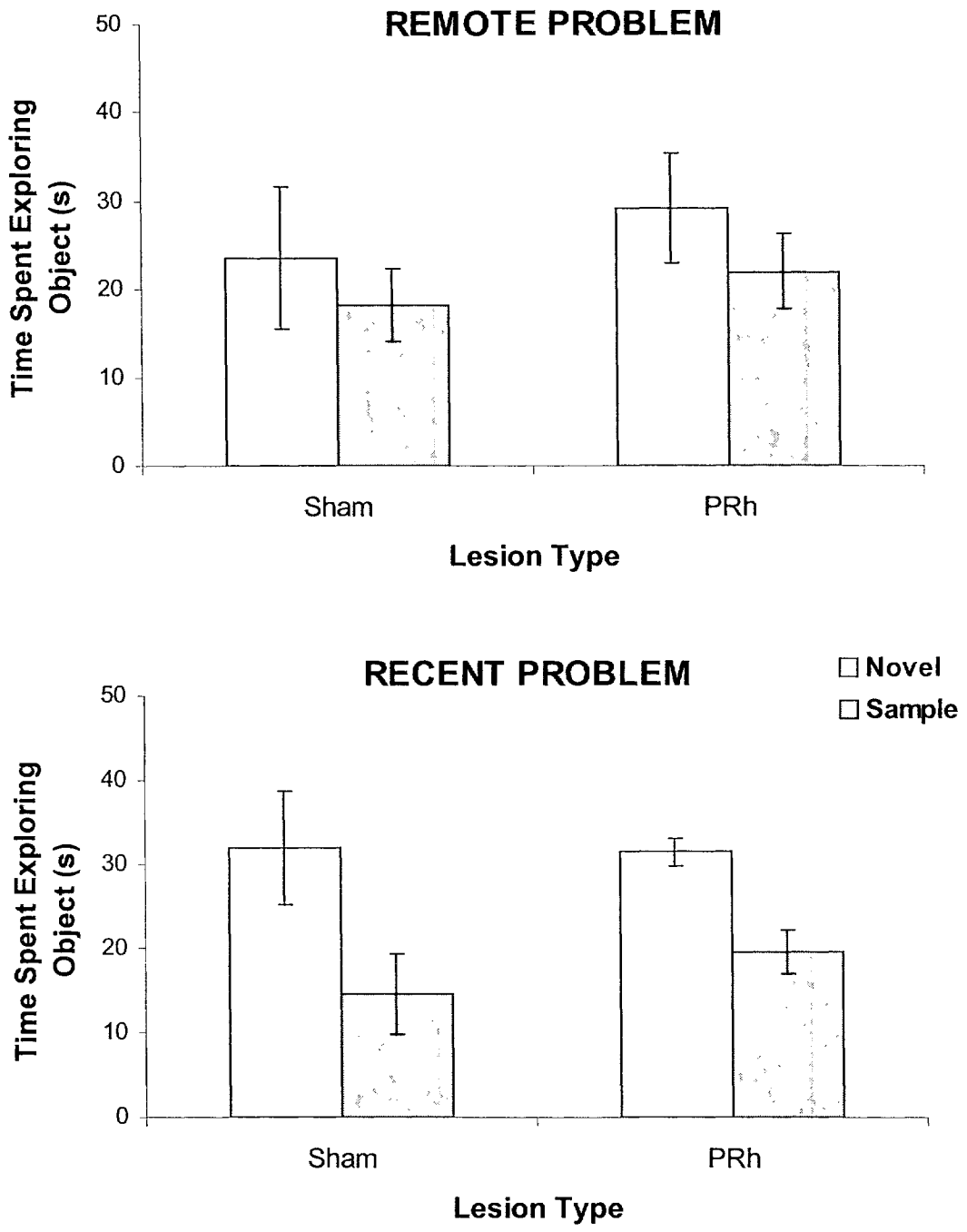


Figure 39. Mean times Sham and PRh rats spent exploring the sample and novel objects during the first 3 minutes of the REMOTE and RECENT test sessions. The error bars represent S.E.M.

analyzed neither Sham nor PRh rats displayed a significant preference for the novel object; when the first 3 minutes of this session were analyzed Sham rats still did not display a significant preference for the novel object, whereas PRh rats displayed a marginally significant preference. When the first 2 minutes of the RECENT test session were analyzed only Sham rats displayed a significant preference for the novel object, but when the first 3 minutes of this session were analyzed both Sham and PRh rats displayed a significant preference for the novel object. Thus, PRh lesions did not appear to interfere with normal retrograde object memory.

The primary goal of this experiment was to determine whether increasing the task demands by presenting rats with 2 pairs of sample objects before surgery would lead to retrograde amnesia following aspiration PRh lesions. The Sham rats in this experiment displayed poor retention of the REMOTE sample objects, whereas the Sham rats in Experiment 7 displayed adequate retention of the REMOTE sample objects. Thus, the presentation of 2 pairs of sample objects in the present experiment appears to be more difficult than the presentation of a single pair of sample objects. Nonetheless, the PRh rats in the present experiment showed a tendency to prefer the novel object during the REMOTE retention test. This time point is difficult to interpret due to the poor retention in Sham rats, but the detection of some retention in PRh rats is not consistent with retrograde amnesia. Additionally, PRh rats, like Sham rats, showed good retention of the RECENT sample objects.

The lack of retrograde amnesia in rats with PRh lesions is consistent with the findings from Experiment 7 and suggests that the ability of rats to form long-term memories about objects does not rely on the integrity of the PRh. Models of memory consolidation tend to imply that brain regions important in learning will also be necessary for the formation of

long-term memories. Additionally, it has been suggested that the PRh is part of a system important for the formation of semantic memories. The nature of the NPT task is such that information about sample objects could conceivably become integrated into an existing semantic framework about objects and the PRh is an ideal candidate for this transition. If this is the case, then the lack of retrograde amnesia for objects in the PRh rats in Experiment 7 and the present experiment suggests that this process is complete by the time of surgery and the PRh is not necessary for the retrieval of that information.

It is possible that presenting rats with more objects prior to surgery may increase the probability that retrograde amnesia will be observed. However, it seems that NPT is not suitable for this. As is evident from Experiment 7 and the present experiment, the likelihood of detecting a preference for novelty can be dubious and the lack of a preference may, in fact, not indicate that rats do not remember the objects. That previous studies found retrograde amnesia for object discrimination problems following PRh lesions (Thornton et al., 1997) suggests that tasks with reward contingencies may be more appropriate for assessing retrograde memory for objects.

3.6 Discussion

Anterograde and retrograde object memory in rats with aspiration and electrolytic lesions of the PRh were examined in the present chapter. The overall findings were that electrolytic PRh lesions produced anterograde and retrograde amnesia for objects, whereas aspiration PRh lesions produced anterograde, but not retrograde amnesia for objects. Discrepant behavioural results after electrolytic and aspiration lesions of the PRh were also observed in Chapter 2. Thus, the combined findings from rats with electrolytic PRh lesions presented so far in this thesis provide compelling evidence that this lesion method, itself, has consequences for normal learning and memory. That there may be abnormal activation in structures efferent to the PRh following electrolytic, but not aspiration lesions is a hypothesis addressed by the experiment in Chapter 5.

The use of NPT to evaluate both anterograde and retrograde object memory afforded an opportunity to assess the ability of rats to learn and remember information about objects in a manner that was relatively free from excessive training and reward contingencies. The findings that aspiration PRh lesions impaired anterograde, but not retrograde object memory suggest that the PRh does not play a fundamental role in the consolidation of long-term memories about objects. However, this interpretation must be tempered with the possibility that the presentation of one or two sample object pairs before surgery may not require a sophisticated object-processing system, of which the PRh has been proposed to be a central component (Bussey et al., 2002; Murray & Richmond, 2001).

The ability of rats with aspiration PRh lesions to discriminate between novel and sample objects on the standard version of NPT with retention delays of 5 and 15 minutes and their inability to do so on the modified version further suggest that the use of NPT may not be suitable for revealing the object recognition functions of the PRh. If the integrity of the PRh

is only required when the demands on object recognition are high, as in the modified version of NPT in which exploration of the sample objects is limited to 20 seconds, then it follows that the ability to remember objects following the repeated and prolonged exposure in the retrograde version of NPT (25 minutes in total, typically resulting in approximately 300 seconds of sample object exploration) is unlikely to depend on the PRh. It is also possible that the PRh is not essential for object recognition and the findings from Experiments 5a, 5b, 7, and 8 support this interpretation.

Thornton et al. (1997) observed retrograde amnesia for object discrimination problems in monkeys with PRh lesions. These monkeys learned two sets of 60 object discrimination problems prior to surgery. It is difficult to compare this with the ability of rats to detect that one or two objects encountered for a total of 25 minutes each before surgery are familiar. However, we recently taught rats two sets of 5 object discrimination problems prior to PRh surgery and failed to observe retrograde amnesia (Mumby et al., 2002). This suggests that the differences between object discrimination problems and NPT cannot wholly account for the lack of retrograde amnesia in the present PRh rats. Unfortunately, the performance of rats on the object discrimination problems was merely adequate. Therefore, increasing the number of discriminations is apt to create a floor effect rendering the task unsuitable for evaluating retrograde amnesia.

The problems associated with studying retrograde memory for objects in rats do not appear to be resolved with the present series of experiments. However, the lack of retrograde amnesia in rats with aspiration lesions of the PRh following familiarization with one (Experiment 7) or two (Experiment 8) pairs of sample objects are novel findings that suggest that the functions of the PRh is not essential to the recall of information about them. Based mainly on primate literature it is believed that, as an extension of the visual-processing

stream, the PRh is critical for processing the visual features of objects (e.g. Bussey et al., 2002). It is not clear whether this is also the case in rats as their visual system is less sophisticated and subordinate to olfaction and the use of their vibrissae to collect tactile information. In monkeys it is possible to design tasks where monkeys rely entirely on visual memory. This is achieved by presenting them with images on a touch screen. By contrast, the rats in the present experiments encounter actual objects and are able to collect visual, olfactory, and tactile information about them. Because different copies of objects are used in each session of NPT olfactory information may not be a reliable feature for subsequent recognition. However, the olfactory system of rodents is quite sophisticated and it is possible that they are able to detect odors associated with the material matter of the object. If the rodent PRh is also important for visual object memory, then their subsequent ability to discriminate the novel and sample objects during the test session may simply reflect their ability to remember non-visual features of the object. In future experiments, it would be beneficial to directly investigate the contribution of the rat PRh to the processing of modality-specific information.

Overall, the conclusions from the anterograde and retrograde tests of object memory are that aspiration PRh lesions have little effect on the ability of rats to recognize objects, except when they have little time to explore them during their initial encounter. These lesions also had little effect on the ability of rats to recognize objects encountered before surgery. Electrolytic PRh lesions, on the other hand, disrupted the ability of rats to recognize objects.

Chapter 4

Memory for Object Location

The experiments in the preceding two chapters examined the contribution of the PRh to anterograde and retrograde memory for objects and places. Overall, the findings were that electrolytic lesions of the PRh produced retrograde amnesia for places and anterograde and retrograde amnesia for objects, whereas aspiration lesions of the PRh produced retrograde, but not anterograde amnesia for places, and anterograde, but not retrograde amnesia for objects. Unlike rats with electrolytic PRh lesions, rats with aspiration PRh lesions displayed a temporally graded retrograde amnesia for places. Additionally, the inability of the rats with aspiration PRh lesions to remember the location of a stationary, hidden platform was only evident when they learned a single problem during the week of surgery and not when they learned two problems: one 4 weeks before surgery and one during the week of surgery. This latter finding suggests that the PRh is involved in the consolidation of place information for a limited time prior to surgery.

The experiments in this chapter, sought to further explore the contribution of the PRh to anterograde and retrograde memory for place information, using the place version of NPT. This task relies on the ability of rats to detect that an object has been moved within the arena. Normal rats can detect this displacement, and spend more time exploring the moved object (Dix & Aggleton, 1999). Knowing where objects are located in a familiar environment is a central tenet of the cognitive mapping theory of hippocampal function (O'Keefe and Nadel, 1978), and there is evidence that rats with lesions of the hippocampus (Mumby et al., 2002) or fornix (Bussey et al., 2000) fail to discriminate a moved object from an unmoved object when tested on the place version of NPT.

Few studies have examined retrograde memory for places in rats with PRh lesions. Thus, it was considered important to augment the water-maze findings from Experiment 4 by determining whether PRh lesions would lead to retrograde amnesia for place information using a different behavioural task. In Experiment 10, rats were familiarized with 2 identical objects in an open-field arena during 5 5-minute sample sessions during the week immediately preceding surgery. Only this, RECENT, time point was used because PRh rats in Experiment 4 displayed retrograde amnesia for place information acquired during this time. Aspiration lesions of the PRh were used because of previous evidence that the electrolytic lesions were the most likely to yield an effect.

4.1 General Method

4.1.1 Subjects

The subjects were male, Long-Evans rats weighing between 300 and 350 g at the start of experiments. Housing and colony conditions were also the same as in previous experiments.

4.1.2 Apparatus and Materials

The arena, objects, and recording equipment were the same as in Chapter 3. Unlike in Chapter 3, only a single pair of identical objects was required; rats saw the same two, identical objects during the sample and test phase sessions.

4.1.3 Histology

Histological procedures were as described in Chapter 2.

4.1.4 Statistical Analyses

The primary dependent measure for the place version of NPT was the time, in seconds, rats spent exploring the objects. The criterion for identifying object exploration was the same as in the object version used in previous experiments. For the sample sessions, the total time each rat spent exploring objects was determined, and these data are described in Appendix C. For the tests sessions, the total time each rat spent with the moved and unmoved objects during the first 2 minutes was determined, unless otherwise noted. The total time spent exploring objects during this period is also described in Appendix C. The exploration ratios, in this case, were based on the time spent exploring the object that remained in the same position and the time spent exploring the object that was moved to a new position, as follows:

$$\frac{\text{Time spent exploring moved object}}{\text{Time spent exploring moved object + time spent exploring unmoved object}}$$

Exploration ratios were compared to chance performance (0.5) using one-sample t-tests.

ANOVAs were also used to analyze data. Factors for these analyses included Lesion (between-subjects factor: Sham versus PRh), Object (within-subjects factor: Moved versus Unmoved), and Sample (within-subjects factor: Sample versus Test). Independent sample t-tests were used to make between group comparisons, where applicable. All statistical tests were evaluated at a significance level of 0.05, but p values between 0.05 and 0.10 were also noted.

4.2 Experiment 9: Anterograde memory for object location in rats with aspiration lesions of the PRh

In Experiment 1, rats with aspiration lesions of the PRh were taught a water-maze problem as quickly as Sham rats, and when retrograde amnesia was detected in Experiment 4, PRh rats were able to rapidly relearn the location of the hidden platform during the retention test. These findings suggest that the PRh is not essential for normal place memory and are contrary to other findings in the literature (Wiig & Bilkey, 1994; Liu & Bilkey, 1998a, 1998b, 1998c, 1999, 2001). In the present experiment, rats with aspiration lesions of the PRh were tested on the place version of NPT to obtain further evidence regarding the effects of PRh lesions on anterograde place memory.

4.2.1 Method

4.2.1.1 Subjects

Fifty-four rats served as subjects in this experiment. Twenty-six of the rats were tested on the standard version of NPT (PRh n=14, Sham n=12). These rats had some, though minimal, object experience and had not previously been tested on the place version of NPT. Twenty-eight rats were tested on the modified version of NPT (PRh n=14, Sham n=14). Ten rats from each group served as subjects in Experiment 10 prior to testing for this experiment. The remaining eight rats had no prior object experience (PRh n=4, Sham n=4).

4.2.1.2 Apparatus and Materials

In addition to the arena used in Chapter 3, an oval Formica arena with a circumference of approximately 207 cm was constructed and used in the present experiment. The cylinder was positioned inside the stainless-steel tray used as the floor of the usual arena.

Approximately half of the rats were tested in our original apparatus and half were tested in the new arena.

4.2.1.3 Procedure

4.2.1.3.1 Surgery. Rats received either bilateral aspiration lesions of the PRh or sham surgery. Surgical procedures were as described in Experiment 1. All rats were permitted to recover for a minimum of two weeks prior to testing.

4.2.1.3.2 Behavioural testing. Prior to testing, all rats were habituated to the arena, singly, in three 15-minute sessions, one per day for three days. There were no objects present and the inverted jar lids were removed from the tray during habituation sessions. During sample phase sessions, objects were always located in the same position within the arena for all rats. During test sessions, either object could be moved to one of two locations, but only two test configurations were used. They are shown in Figure 40 and were counterbalanced across groups.

4.2.1.3.2.1 Standard NPT test. For the sample session of the standard version, rats were placed in the arena for 5 minutes with two identical objects. At the end of the session, rats were returned to their home cage in the colony for a 5-minute retention delay. During this time the objects, arena, and tray were cleaned, the wood shavings were changed, and the same two, identical, objects were positioned for the test phase session. One object was positioned in the same place as during the sample phase sessions. The other object was positioned in a new place within the arena. Rats were then returned to the arena for the 5-minute test session.

4.2.1.3.2.2 Modified NPT test. For the modified version of NPT, the sample phase was terminated after rats had accumulated a total of 20 seconds of exploration time with the

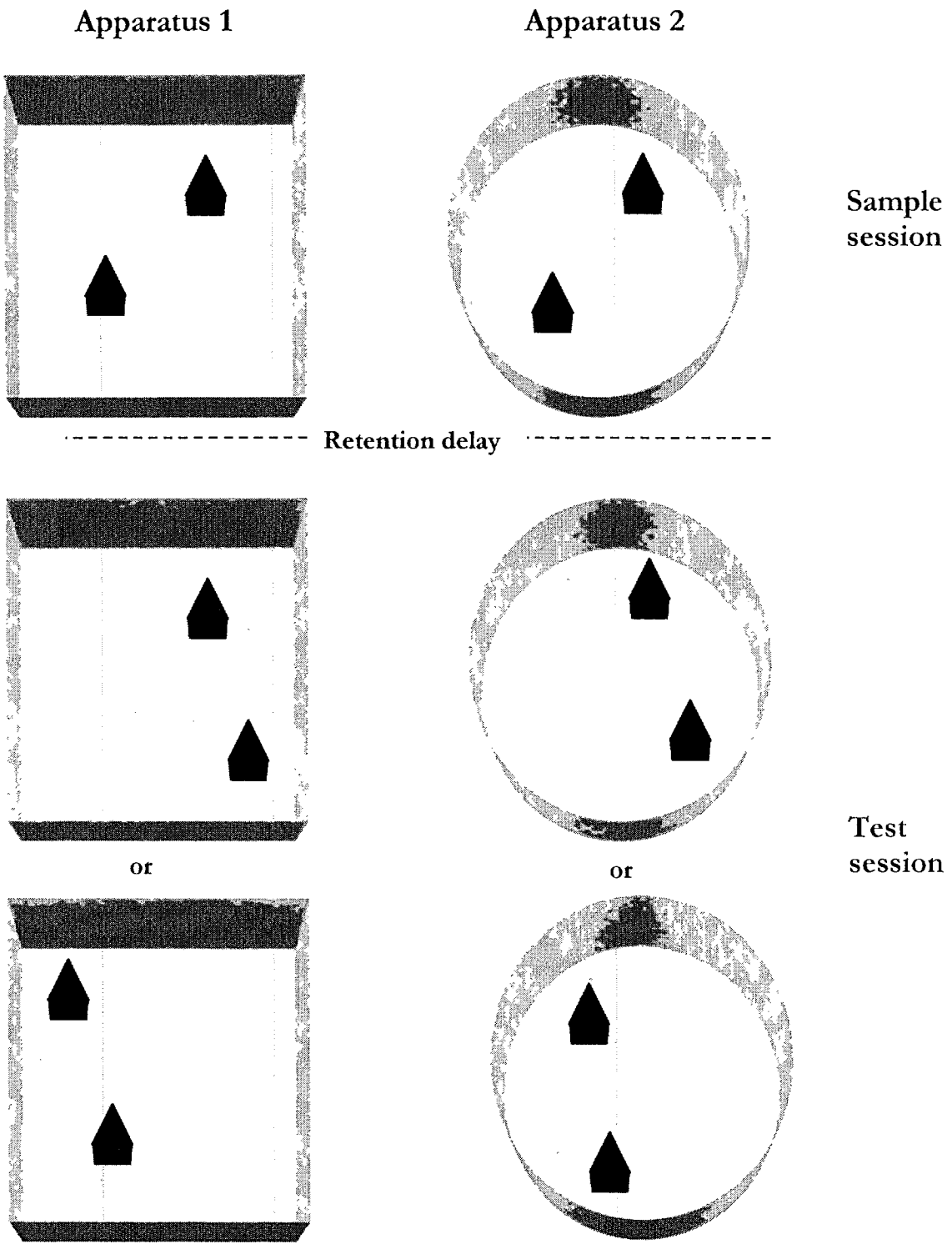


Figure 40. Schematic diagram indicating the position of objects in the two arenas during sample and test sessions.

sample objects. The procedure for this was as described in Chapter 3 (section 3.2.3.2.2). In this case it was also required that rats spend at least 5 seconds with each of the objects to ensure that they gathered sufficient information about their placement within the arena. None of the rats tested required additional time with the sample objects, beyond 20 seconds, as they all explored both objects during their accumulation of that amount of exploration time.

4.2.2 Results

4.2.2.1 Histological results

The location and extent of the largest and smallest PRh lesions of those rats tested on the standard version are shown in Figure 22 and see Section 3.2.1.3.1 for a description of these lesions. The location and extent of the largest and smallest PRh lesions of those rats tested on the modified version that also served as subjects in Experiment 10 are shown in Figure 46 and described in Section 4.3.2.1.

The largest and smallest lesions of the remaining 4 PRh rats that had no prior object experience are shown in Figure 41. Overall, there was good concordance in the amount of PRh and extra-PRh damage in these lesions and those described in Experiments 5 and 10. Each of these lesions included substantial, bilateral damage to the PRh. The largest and smallest PRh lesions included 95 and 85 percent damage to the PRh, respectively. The lateral entorhinal cortex was damaged bilaterally in all rats. This damage was not extensive, however, and was estimated to be about 15 percent damaged in the rat with the largest PRh lesion and 5 percent damaged in the rat with the smallest lesion. The postrhinal cortex sustained minor, but bilateral damage in 3 rats and was estimated to include approximately 5-10 percent in the anterior portion. The remaining rat sustained minor, unilateral damage to this area.

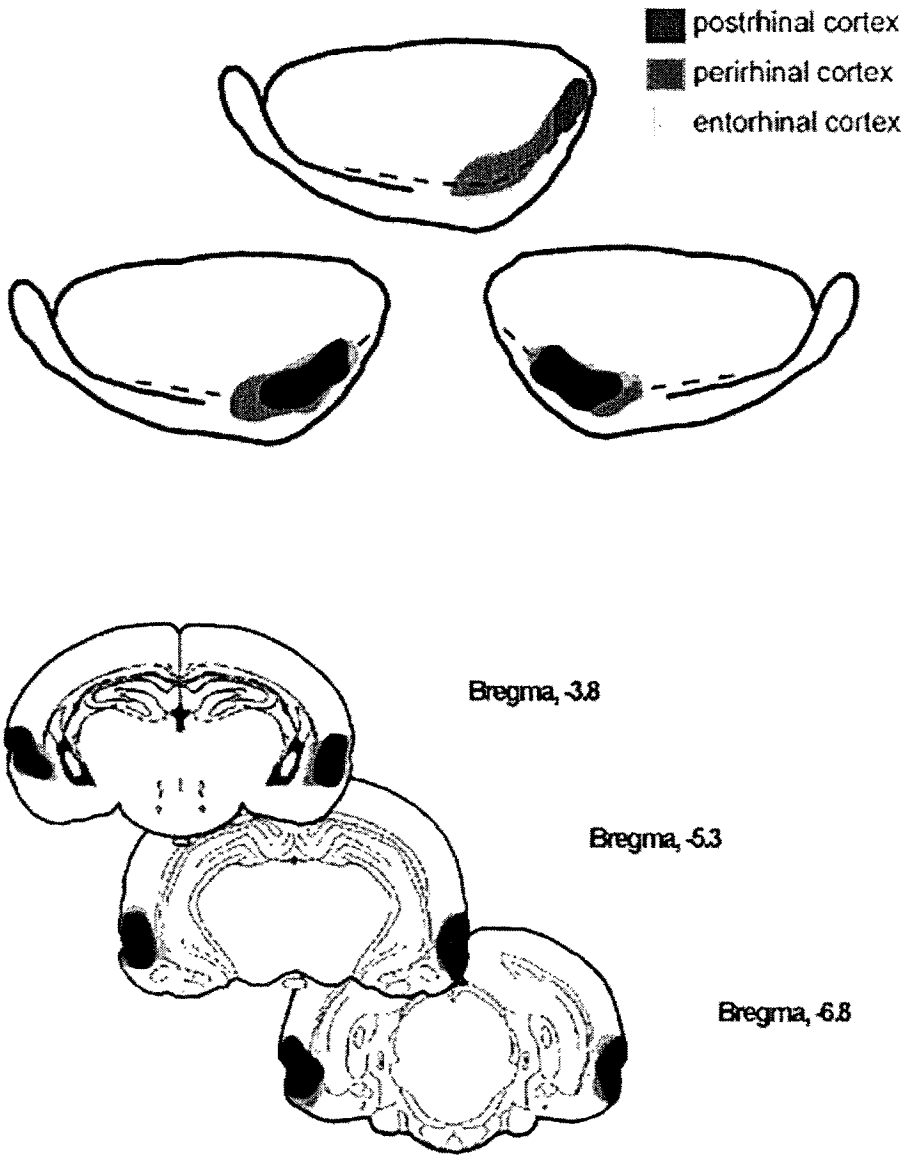


Figure 41. The location and extent of the PRh lesions are shown on the lateral surface of the rat brain in the top panel and in three coronal views in the bottom panel. In each panel, the largest lesion is shown in grey and the smallest lesion is shown in black.

Only 1 rat of the 4 PRh rats sustained bilateral damage to the temporal association cortex. There was little or no damage in the remaining 3 rats. There was unilateral damage to the CA1 cell field of the HPC and subiculum in 3 of the rats. One rat sustained no damage to these area and one rat sustained bilateral damage to the subiculum. There was minor, though bilateral damage to the amygdala in 2 rats.

4.2.2.2 Behavioural Results

4.2.2.2.1 Standard NPT test. Figure 42 shows the mean exploration ratios of Sham and PRh rats and the mean amount of time each group spent exploring the moved and unmoved objects during the first 2 minutes of the retention test. There was no statistically significant difference between the exploration ratios of Sham and PRh rats ($t[24] = -0.22$, $p = .492$). In addition, one-sample t-tests revealed that both Sham and PRh rats showed a significant preference for the moved object ($t[11] = 3.004$, $p = .006$ and $t[13] = 3.112$, $p = .004$, respectively). A 2 x 2 (Lesion x Object) mixed factorial ANOVA revealed a significant main effect of Object ($F[1,24] = 12.537$, $p = .002$), indicating that, overall, rats spent more time exploring the moved object than the unmoved object. The main effect of Lesion and the interaction between Lesion and Object were not statistically significant ($ps > .10$).

4.2.2.2.2 Modified NPT test. Figure 43 shows the mean exploration ratios of Sham and PRh rats and the mean amount of time each group spent exploring the moved and unmoved objects during the first 2 minutes of the retention test. There were was not a statistically significant difference between the groups ($t[26] = .648$, $p = .523$). However, one sample t-tests comparing each groups' exploration ratios to chance revealed that Sham rats significantly preferred the moved object ($t[13] = 2.616$, $p = .010$), but PRh rats did not ($t[13] = 1.212$, $p = .124$). A 2 x 2 (Lesion x Object) mixed factorial ANOVA revealed a significant main effect of Object ($F[1,26] = 4.511$, $p = .043$) indicating that, overall, rats spent more time

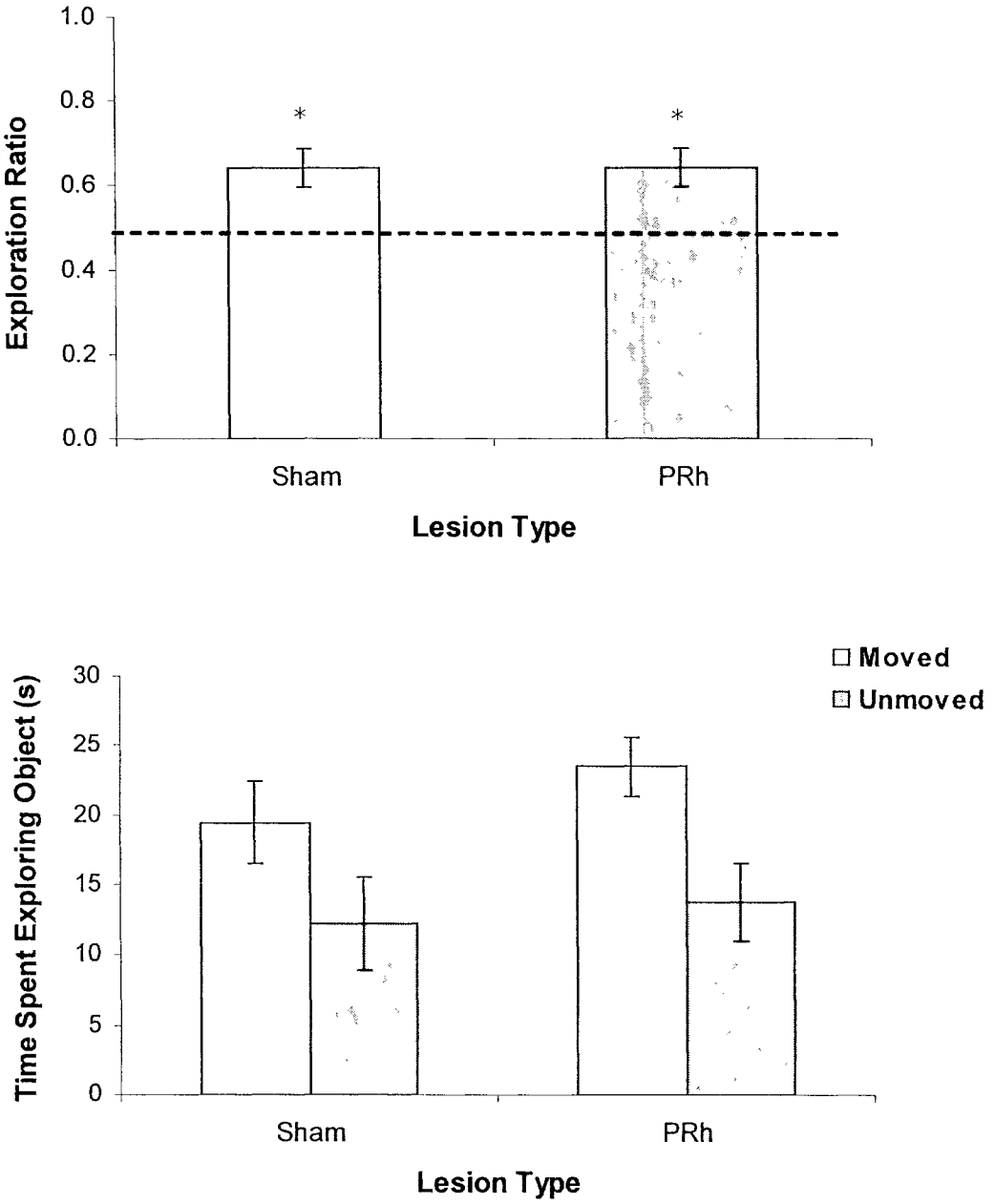


Figure 42. Mean exploration ratios of Sham and PRh rats (top panel) and the mean time each group spent exploring the moved and unmoved objects (bottom panel) during the first 2 minutes of the test session. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object). Asterisks denote mean ratios that are significantly higher than chance level (one-sample t-test, $p < .05$).

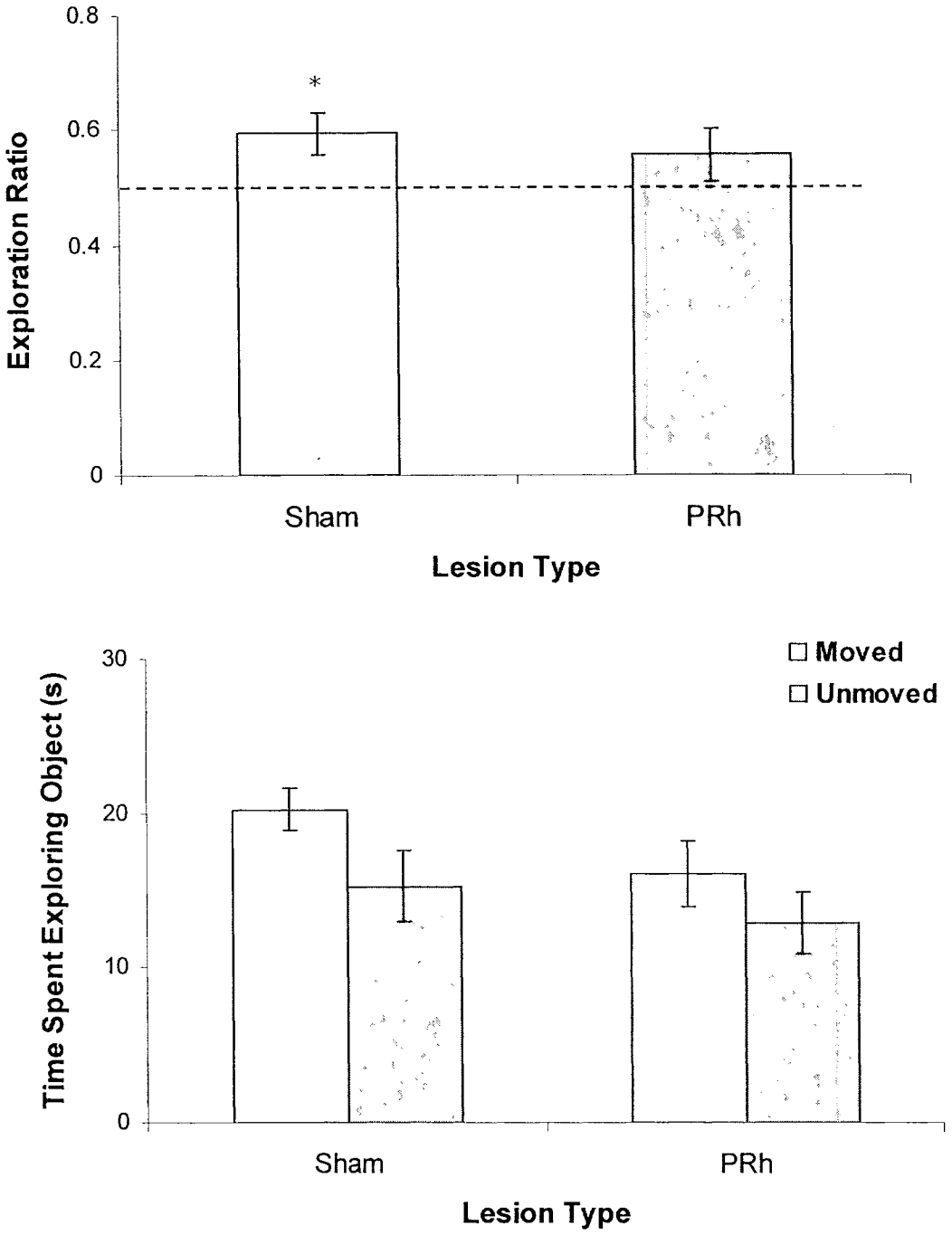


Figure 43. Mean exploration ratios of Sham and PRh rats (top panel) and the mean time each group spent exploring the moved and unmoved objects (bottom panel) during the first 2 minutes of the test session. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object). Asterisks denote mean ratios that are significantly higher than chance level (one-sample t-test, $p < .05$).

exploring the moved object than the unmoved object. The main effect of Lesion and the interaction between Lesion and Object were not statistically significant (ps >.10).

In Experiment 5 of Chapter 3, a preference for the novel object was only detected during the first minute of the retention test with the modified object version of NPT. For this reason, the first minute of the present retention test was also examined. Figure 44 shows the mean exploration ratios of Sham and PRh rats and the mean amount of time each group spent exploring the moved and unmoved objects during the first minutes of the retention test. The exploration ratios of Sham and PRh rats were not statistically significant ($t[26] = -.488$, $p = .315$), and one sample t-tests comparing each groups' exploration ratios to chance revealed that both Sham and PRh rats significantly preferred the moved object ($t[13] = 2.133$, $p = .027$ and $t[13] = 2.64$, $p = .010$). Thus, in this portion of the test session, PRh rats clearly displayed a preference for the moved object.

A 2 x 2 (Lesion x Object) mixed factorial ANOVA on time spent exploring the moved and unmoved objects revealed a significant main effect of Object ($F[1,26] = 9.254$, $p = .005$) and Lesion ($F[1,26] = 5.516$, $p = .027$). Overall, rats spent more time exploring the moved object than the unmoved object and Sham rats spent more time exploring objects than PRh rats (see Appendix C). The interaction between Lesion and Object was not statistically significant ($F < 1$).

4.2.3 Summary

Aspiration PRh lesions did not impair memory for the placement of objects when rats were tested on either the standard or modified place versions of NPT. Both Sham and PRh rats displayed a preference for the moved object 5 minutes after a 5-minute sample session and when they were only permitted to explore the sample objects for a total of 20 seconds. Intact anterograde memory for object location in rats with PRh lesions is consistent with the

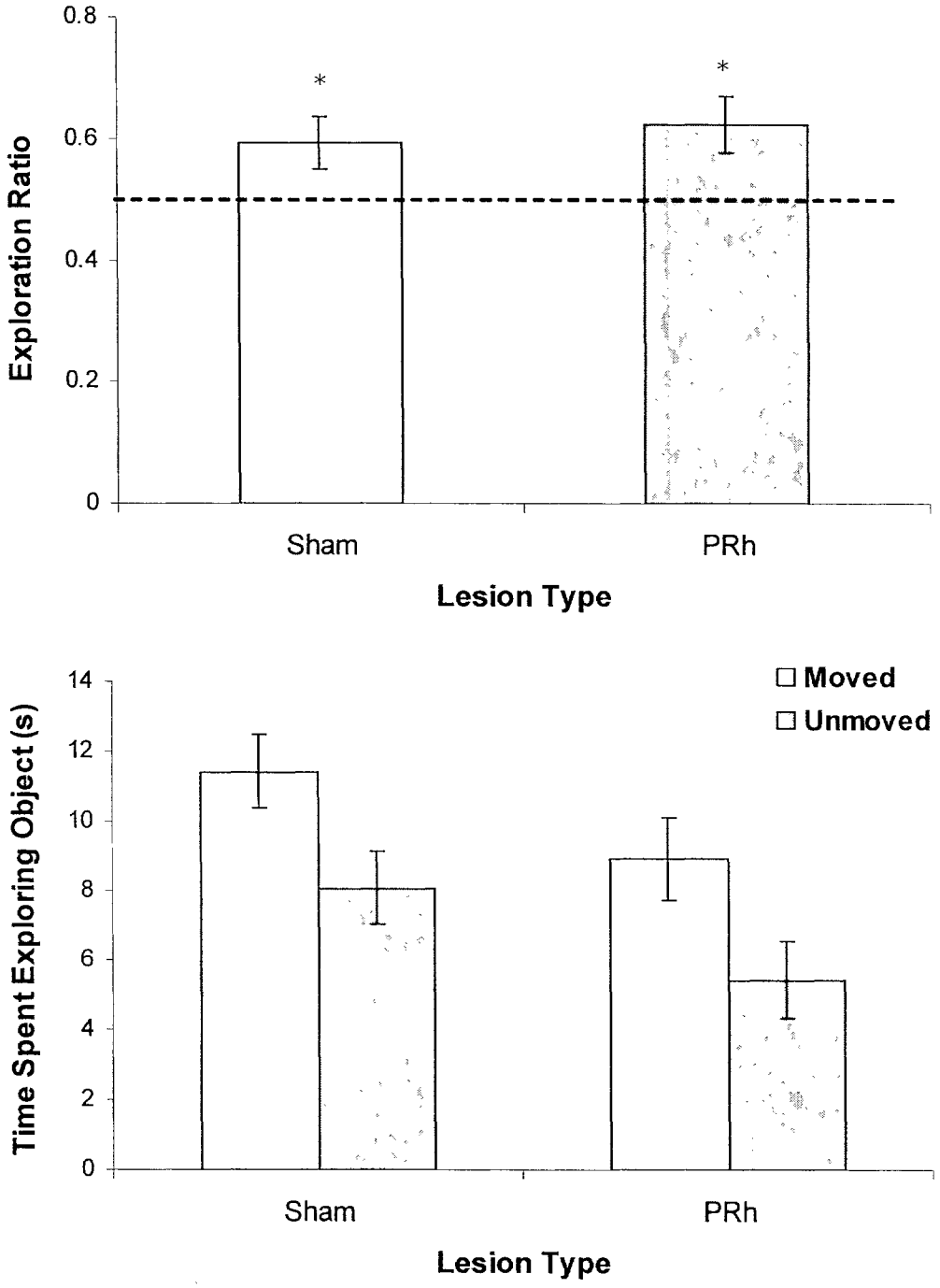


Figure 44. Mean exploration ratios of Sham and PRh rats (top panel) and the mean time each group spent exploring the moved and unmoved objects (bottom panel) during the first minute of the test session. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object). Asterisks denote mean ratios that are significantly higher than chance level (one-sample t-test, $p < .05$).

findings of Experiment 1 in Chapter 2 in which rats with the same type of lesion were able to learn the location of a hidden, stationary platform in a water maze. These findings are also consistent with other reports in which PRh lesions did not impair the ability of rats to acquire place information on a working memory version of the water maze (Glenn & Mumby, 1996; 1998) or on the radial-arm maze (Machin et al., 2002).

Numerous prior reports in which PRh lesions impair place memory in the water maze provide some support for the interpretation that under specific circumstances the PRh participates in the acquisition of place information. These studies were all conducted by the same investigators under ostensibly equivalent training conditions. Thus, certain features of their methodology, such as rat strain, maze and/or room size, trial duration, intertrial interval, water temperature, lighting conditions, number and quality of extramaze cues, or levels of arousal and motivation in the rats, may contribute to the inability of those PRh rats to learn the problem as quickly as Sham rats. It should be noted, however, that the PRh rats do learn the problem, but appear to do so at a slower rate than Sham rats. And, in all of their experiments PRh lesions were made electrolytically or using excitotoxins. Thus, it is possible that the lesion method and/or any combination of the other training parameters or conditions mentioned above led to the deficiencies in anterograde place memory observed.

4.3 Experiment 10: Retrograde memory for object location following aspiration lesions of the PRh

In Experiment 9, rats with aspiration lesions of the PRh were able to recognize that an object was moved to a new location after a 5-minute retention delay. These findings are consistent with the intact anterograde memory for places observed in Experiment 1 and in our previous work using the water maze (Glenn & Mumby, 1998). Combined, these findings strongly suggest that the PRh does not play a critical role in the acquisition of place information. Additionally, the place memory functions of the HPC do not appear to rely on projections from the PRh.

The main purpose of the present experiment was to assess retrograde amnesia for object location in rats with aspiration lesions of the PRh, using the place version of NPT. If this task and the water maze task tax the same type of place memory abilities, then PRh lesions should produce retrograde amnesia. Rats were familiarized with 2 identical objects in the open field during the week before surgery because retention deficits for information acquired during this time point were observed in both Experiments 2 and 4. It was not possible to assess a time-course of memory loss with the single time point. However, due to the influence of design factors contributing to temporal patterns of memory loss in Chapter 2 (Experiments 3 and 4), the use of additional time points was not likely to be informative unless both within and between designs were utilized. This was viewed to be beyond the scope of the present investigation.

4.3.1 Method

4.3.1.1 Subjects

Twenty-eight experimentally naïve rats (PRh n=14, Sham n=14) served as subjects.

4.3.1.2 Procedure

4.3.1.2.1 Presurgery familiarization. Rats received three 15-minute habituation sessions prior to testing. There were no objects present in the apparatus and the lids were removed from the metal floor during habituation. Rats were habituated in pairs for the first two sessions, and singly in the last session. The administration of sample phase sessions began approximately 48 hours after the final habituation session. Rats received 5 5-minute sessions, one per day for five consecutive days. In addition to the configurations of object placement used in Experiment 9, the objects were positioned in other configurations during the sample and test sessions, as shown in Figure 45.

4.3.1.2.2 Surgery. Between 24 and 48 hours after the final sample phase session rats received either bilateral aspiration lesions of the PRh or sham surgery. Surgical procedures were the same as described in Experiment 1. Rats were permitted to recover for two weeks prior to retention testing.

4.3.1.2.3 Postsurgery retention testing. All rats received a single re-habituation session approximately 24 hours prior to the retention test. The retention test consisted of one 5-minute session in which one sample object was located in the same position within the arena and the other object was moved to a novel location.

4.3.2 Results

4.3.2.1 Histological results

The location and extent of the largest and smallest PRh lesions are shown in Figure 46. All rats sustained substantial and bilateral damage to the PRh. The largest and smallest lesions were estimated to include approximately 95 and 80 percent of the PRh, respectively. The lateral entorhinal cortex was damaged bilaterally in all rats. In most rats, this area was approximately 10-20 percent damaged. The postrhinal cortex, on the other hand, sustained

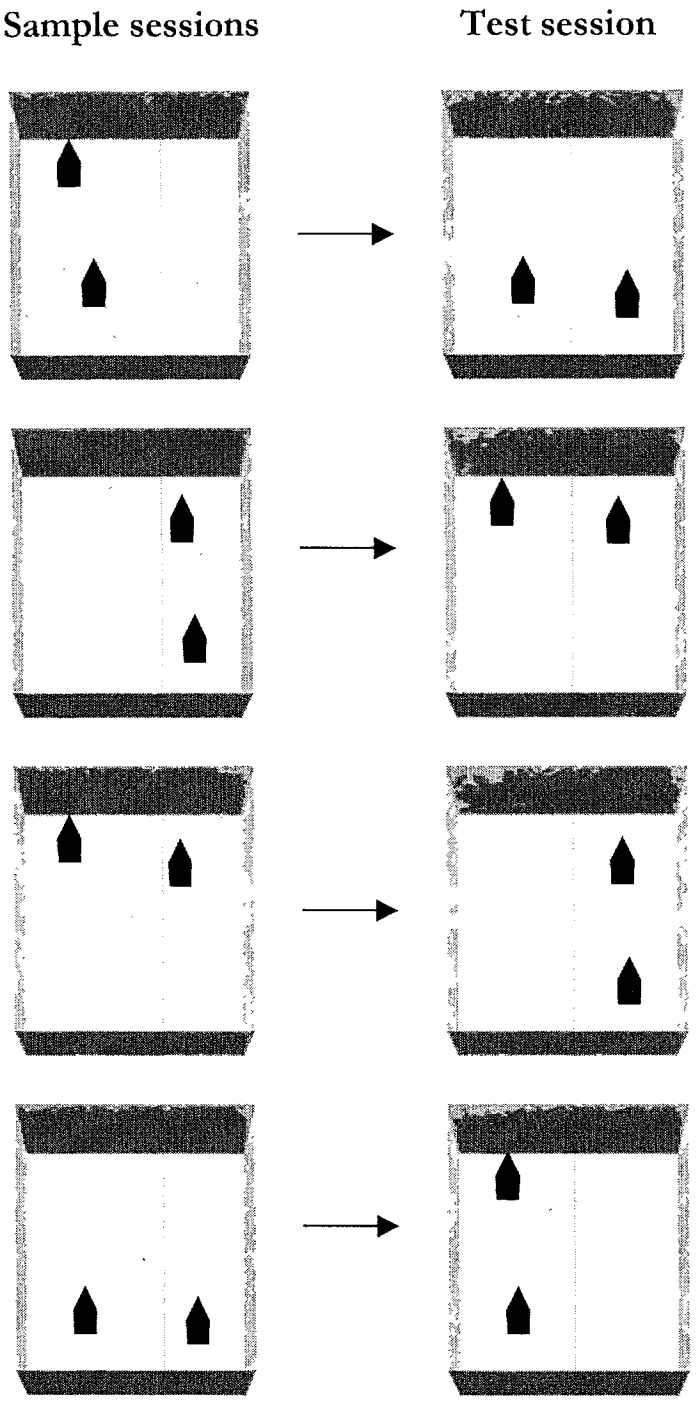


Figure 45. Schematic diagram indicating the position of objects in the two arenas during sample and test sessions.

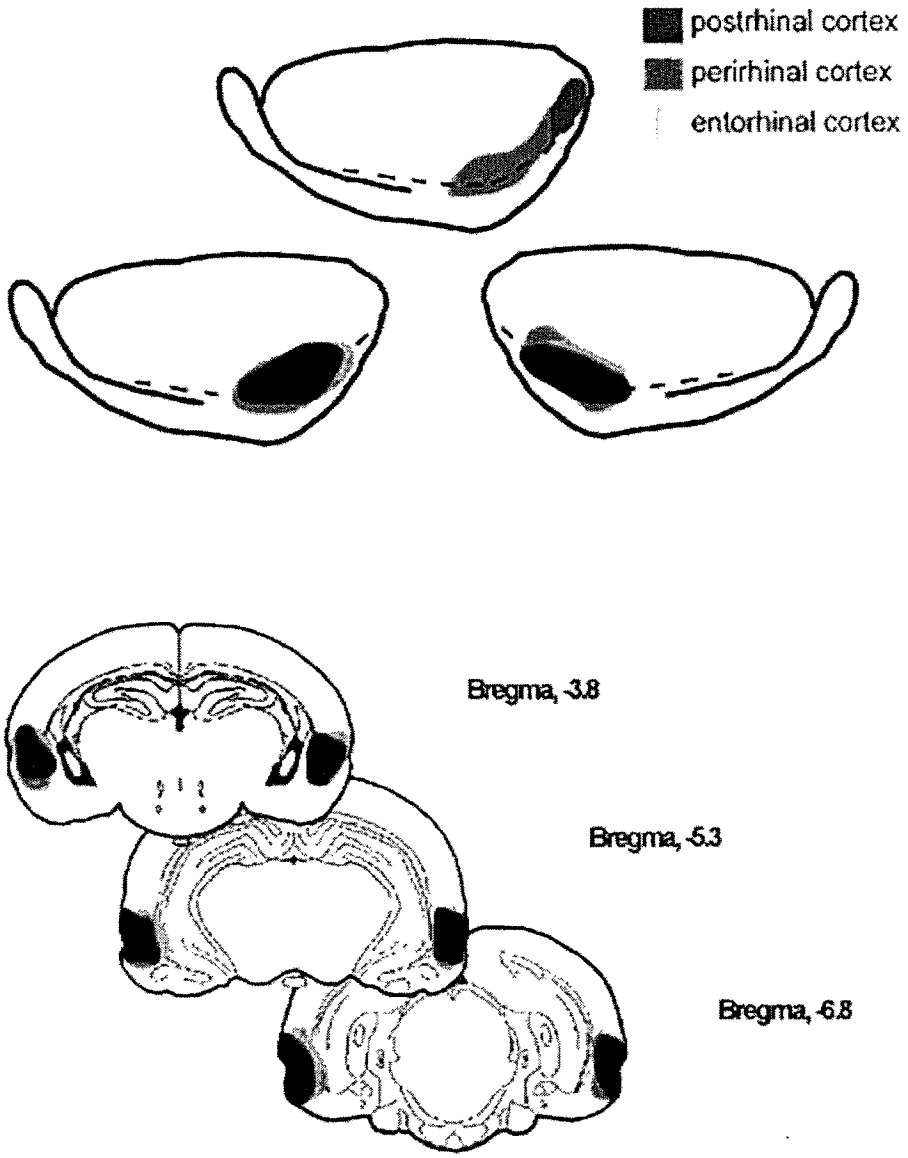


Figure 46. The location and extent of the PRh lesions are shown on the lateral surface of the rat brain in the top panel and in three coronal views in the bottom panel. In each panel, the largest lesion is shown in grey and the smallest lesion is shown in black.

very little damage overall. Only 6 of the 14 lesions included bilateral damage to this area, between approximately 5 and 10 percent. Two PRh rats sustained unilateral damage to this area, and, in the remaining 6 rats, it was spared. Similarly, the temporal association cortex was minimally damaged in most rats. Bilateral damage to this region was found in 6 PRh rats, and in the remaining 8 rats the damage was primarily unilateral.

In 2 PRh rats there was bilateral damage to the ventral portions of the CA1 cell field. Six PRh rats sustained unilateral damage to this region. One rat sustained bilateral damage to the subiculum and another sustained significant, but unilateral damage. Five lesions included minor, unilateral damage to this area. There was bilateral damage to the amygdala in 2 of the 14 rats. One rat sustained significant, unilateral damage to the lateral amygdala and 4 other rats sustained minor, unilateral damage to the dorsolateral amygdala.

4.3.2.2 Behavioural results

Figure 47 shows the mean exploration ratios of Sham and PRh rats and the mean amount of time they spent exploring the moved and unmoved objects during the first 2 minutes of the test session. Sham rats had a significantly higher exploration ratio than PRh rats ($t[26] = 2.708$, $p = .006$). Consistent with this finding, one sample t-tests comparing each groups' exploration ratios to chance revealed that Sham rats showed a significant preference for the moved object ($t[13] = 2.547$, $p = .012$), but PRh rats did not ($t[13] = -1.189$, $p = .128$).

A 2 x 2 (Lesion x Object) mixed factorial ANOVA conducted on time spent with each object revealed a significant main effect of Lesion ($F[1,26] = 7.609$, $p = .010$); overall Sham rats spent less time exploring objects during the first two minutes of the test session than PRh rats (see Appendix C). There was also a significant interaction between Lesion and Object ($F[1,26] = 6.689$, $p = .016$). Sham rats spent less time than PRh rats exploring the

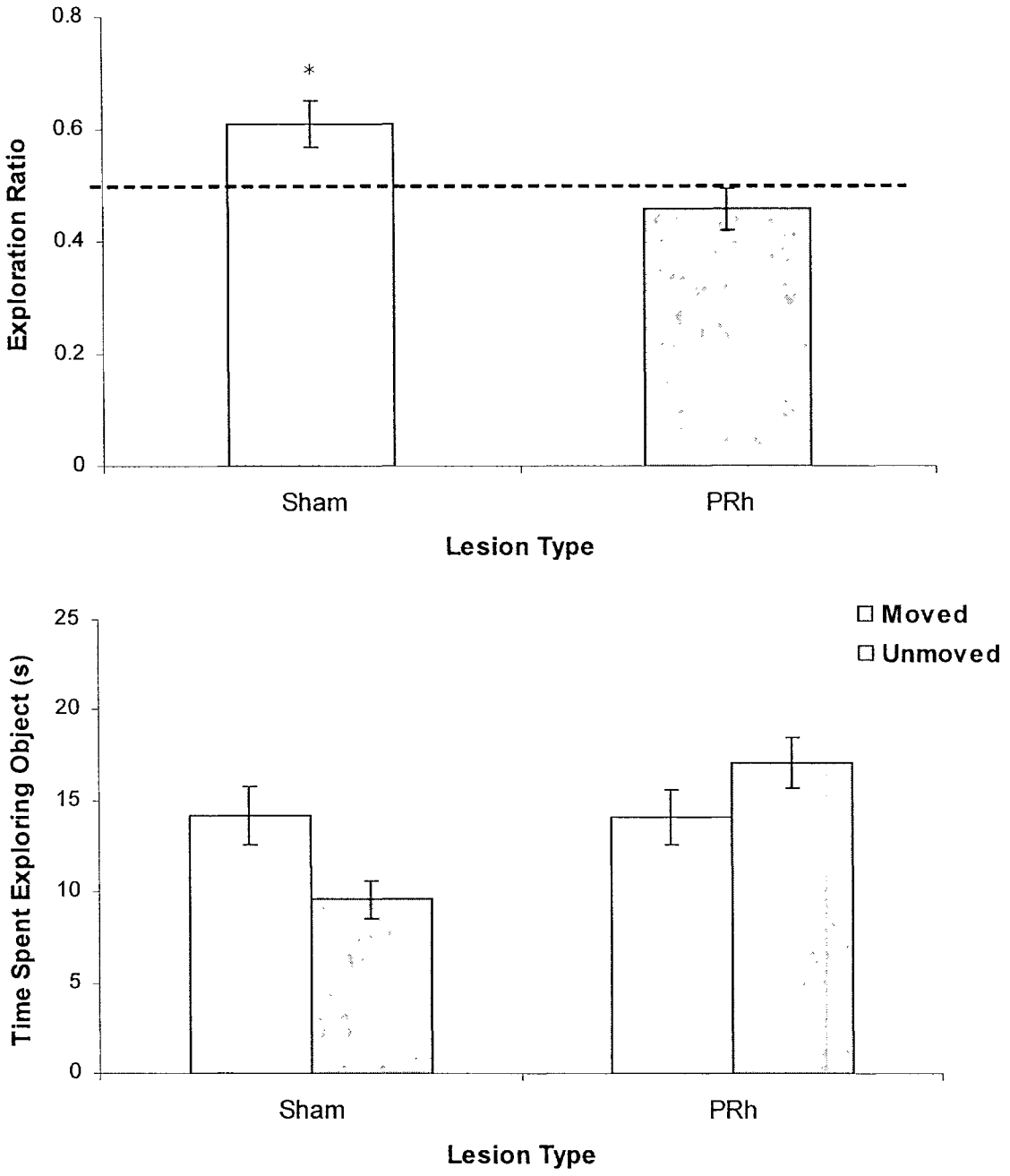


Figure 47. Mean exploration ratios of Sham and PRh rats (top panel) and the mean time each group spent exploring the moved and unmoved objects (bottom panel) during the first minute of the test session. The error bars represent S.E.M. and the dashed line indicates chance performance (no preference for either object). Asterisks denote mean ratios that are significantly higher than chance level (one-sample t-test, $p < .05$).

unmoved object ($t[26] = -3.639$, $p = .001$), but there were no significant differences in the amount of time Sham and PRh rats spent exploring the moved object ($t[26] = 0.05$, $p = .480$). In addition, Sham rats spent significantly more time exploring the moved object than the unmoved object ($t[13] = 2.347$, $p = .018$), whereas PRh rats tended to spend more time with the unmoved object ($t[13] = -1.37$, $p = .097$). The main effect of Object was not statistically significant ($F < 1$).

4.3.3 Summary

Aspiration PRh lesions impaired the ability of rats to remember the locations of objects encountered during the week of surgery. PRh rats, unlike Sham rats, did not show a preference for the object that was moved to a novel location. Sham rats spent more time exploring the moved object than the unmoved object during the first 2 minutes of the retention test and displayed exploration ratios that were significantly higher than chance performance. PRh rats explored the moved object as much as Sham rats, but also as much as they explored the unmoved object. Additionally, the exploration ratios of PRh rats did not differ from chance, further indicating that they did not prefer the moved object.

The retrograde amnesia for object location following PRh lesions observed in the present experiment is consistent with the finding from Experiment 4 that aspiration PRh lesions impaired the ability of rats to remember the location of a platform that was taught during the week of surgery. In the present experiment we only examined the ability of rats to remember the locations of objects encountered during the week of surgery and were therefore unable to determine whether retrograde amnesia was temporally graded. In Experiment 4, PRh rats that learned a water-maze problem 4 weeks before surgery performed as well as Sham rats on the retention test conducted after surgery. Thus, the focus in the present experiment was on the 1-week time point to determine whether there would

be concordance in the findings using a different place memory task. In Experiment 2, electrolytic PRh lesions impaired retention of water-maze problems learned 4 weeks before and during the week of surgery— retrograde amnesia without a temporal gradient. However, as previously discussed, the contribution of the lesion method has not been ruled out. Yet, the combined findings from these three experiments provide compelling evidence that the PRh contributes to retrograde place memory.

4.4 Discussion

In Experiment 9 of the present chapter, PRh lesions failed to produce anterograde amnesia for place information. Rats with PRh lesions displayed a preference for the object that was moved to a new location in the arena. The results of Experiment 10 suggest the PRh is important for the retention of place information acquired before surgery; PRh rats, unlike Sham rats, did not show a preference for an object that was moved from the location they had encountered it in before surgery. This is in contrast to the findings from Experiments 7 and 8 in which PRh rats were able to discriminate between novel objects and objects they had encountered 4 weeks before and during the week of surgery. Taken together, these findings suggest that retrograde amnesia following PRh lesions is specific to the type of information that is required for successful recognition: place, but not object.

It is possible that the ability of animals to remember sufficient details about a place so that they may recognize that objects are not in their original positions is a more complex process than remembering one or more features of an object so that they may recognize that a new object is not the same as the one they previously encountered. The ability of rats to recall sufficient information about a place so that they may quickly swim to the location of a hidden platform would also require a similarly complex representation. Thus, in the retrograde experiments, PRh lesions may impair retention of place information because place memories are not fully formed due to the brain damage in a region that participates in their formation, or the place representation may be altered by the brain damage thereby requiring rats to reacquire some information about the place. This latter hypothesis would not necessarily require the formation of a completely new place representation, as portions of the original may still be present. Rather, it may be necessary for those portions of the representation that are changed or lost to be reintegrated into the existing representation. In

both cases, retrograde amnesia is likely to be mild and transient. This was the case for the deficits in retention of the water maze problem learned before surgery. Unfortunately, it is not possible to make a similar determination using NPT. Rats either show a preference or they do not. It is difficult to argue that a greater preference for novelty is indicative of better memory for the sample objects, relative to a preference that is significant, but not as strong.

The water maze task and the place version of NPT both differ in a number of important ways. The water maze task is aversively motivated, the maze itself is a much larger arena than the one used in NPT (137 cm diameter versus 60 x 70 cm), and many remote cues are available outside of the maze. In addition to being smaller, the NPT arena has high walls (70 cm) and a video camera and tripod obscure the view from inside. NPT is based on the motivation of rats to explore novelty. However, in both tasks, the rat is required to learn about the location of objects in its environment.

In the water maze, rats must navigate to a target location, and this presumably occurs through the recall of a previously stored cognitive map of the environment, which includes objects that serve as remote cues. In NPT, rats are not required to navigate to any particular location, but their preference for moved objects will only be revealed if they have learned where objects were previously located within the environment. Thus, despite many differences, the water maze and NPT tasks may ultimately rely on the same types of place memory abilities.

Further evidence that these two tasks require similar abilities of rats to acquire and remember place information is indirectly derived from studies in which lesions of the HPC impaired performance on both tasks (Morris et al., 1982; Mumby et al., 2002; Gaskin et al., 2003). The HPC is thought to be critical for the acquisition and retention of place information (see O'Keefe and Nadel, 1978), but it is less clear to what extent the adjacent

PRh supports this function. If the HPC is dependent on the input of cortical information through the rhinal cortices, it follows that damage to this region should produce deficits in place memory. However, to fully test the validity of this supposition it is necessary to make complete lesions of the rhinal cortex thereby greatly limiting the input of cortical information to the HPC (see Eichenbaum et al., 1994). The tendency of PRh lesions in the present thesis to spare anterograde place memory (Experiments 1 and 9) is consistent with the notion that the HPC is still able to receive sufficient cortical information via the spared portions of rhinal cortex. The retrograde amnesia for places observed in the present thesis (Experiment 2, 4 and 10) is consistent with the notions that the PRh either participates in the formation or the retrieval of place representations.

Chapter 5

On Lesion Method

In the experiments on place memory and object recognition, the patterns of memory loss following PRh lesions depended on the lesion method. Rats with electrolytic lesions displayed poor retention of place and object problems learned before surgery, whereas rats with aspiration lesions showed good retention of place and object problems learned before surgery. The differences in behaviour could not be attributed to the extent of PRh damage or damage to adjacent cortical or subcortical areas. These findings suggest that there are different consequences of the electrolytic and aspiration methods for the functions of brain regions outside of the PRh. The experiment described in this chapter specifically addressed this hypothesis by comparing the expression of the immediate early gene, *c-fos*, throughout the forebrain in rats that received either electrolytic or aspiration lesions of the PRh. Transcription of *c-fos* occurs following depolarization of a neuron and expression of its protein products occurs shortly after. Therefore, the expression of these products is a useful tool for assessing neural activity.

5.1 Experiment 11: Fos expression following electrolytic and aspiration lesions of the PRh

Both electrolytic and aspiration lesion techniques produce widespread damage to cell bodies and fibres in the target region. The results of lesion studies that employ these methods are difficult to interpret because it is possible that damage to the fibres passing through the lesion site may be responsible for behavioural deficits, if observed. However, in the present thesis, electrolytic lesions appeared to have a more profound effect on learning and memory than aspiration lesions. Thus, despite the gross tissue damage produced by both methods, it is hypothesized here that the strong, electrical current used during the electrolytic surgery (1.5 mA for 10 s at 5 sites per hemisphere) may produce an abnormal cascade of activation in structures efferent to the PRh.

Many researchers have recently adopted the use of excitotoxins to make selective lesions to cell bodies in specific brain regions. By sparing fibres of passage, researchers are able to more confidently attribute deficits to the loss of cells in the target area. This experiment also included a group of rats that received excitotoxic (NMDA) lesions of the PRh. The hypothesis that the electrolytic current may lead to abnormal activation in other brain regions may, in fact, apply to excitotoxic lesions as well. The primary manner in which excitotoxins produce cell death is through over-excitation of the cell. It is not known whether there are consequences to this in structures efferent to the lesion site. Furthermore, Liu and Bilkey (1998b) compared the effects of electrolytic and excitotoxic lesions of the PRh on place memory and found that both produced deficits of comparable magnitude.

The aim of the present experiment was to identify the locus and extent of differential neuronal activation by comparing the expression of the protein products of the immediate early gene, *c-fos*, as a marker of neuronal activation in rats with electrolytic, aspiration, or excitotoxic lesions of the PRh. The primary efferent target of the PRh is the entorhinal cortex, which projects almost exclusively to the HPC. Thus, due to the close anatomical relation between the PRh and the HPC/entorhinal cortex, and the importance of the HPC/entorhinal cortex to place memory (Glenn & Mumby, 1998; Jarrard, 1983; O'Keefe & Nadel, 1978; Morris et al., 1982), these were key sites for assessing differential neuronal activation; however other areas were examined as well.

5.1.1 Method

5.1.1.1 Subjects

Twenty-one experimentally naïve rats, weighing between 300 and 350 g, served as subjects in this experiment. Housing and colony conditions were as described in Chapter 2.

5.1.1.2 Procedure

5.1.1.2.1 Surgery. Rats were assigned to one of five surgical groups: 1) bilateral aspiration lesions of the PRh (PRh-Asp, n=5), 2) bilateral electrolytic lesions of the PRh (PRh-Elec, n=5), 3) bilateral excitotoxic lesions of the PRh (PRh-NMDA, n=3), 4) bilateral electrolytic lesions of the internal medullary lamina (IML-Elec Con, n=3, this group served as an electrolytic control group), or 5) sham surgery (Sham, n=5).

The surgical procedures for the aspiration and electrolytic lesions were as described in Experiments 1 and 2, respectively. The excitotoxic lesions of the PRh were made by infusing 0.4 μl of NMDA (N-methyl-D-aspartate; Sigma Chem. Co., St. Louis, MO; 5.1 M in 0.1 M phosphate buffered saline, PBS pH 7.4) at 5 sites through the PRh in each hemisphere and the infusion cannula was angled at 10° to the vertical plane. Infusions were made at a flow

rate of 0.15 μl/minute and the coordinates for each of the 5 injections sites are shown in Table 2. The electrolytic lesions of the IML were made using the same materials as described in Experiment 2 for the electrolytic PRh surgery. Current was applied at 5 sites per hemisphere, but the electrode was not angled. The coordinates for this surgery are shown in Table 3. Sham surgery was as described in Experiment 1.

5.1.1.2.2 Immunocytochemistry. One hour after the completion of surgery, rats were given a supplemental dose of sodium pentobarbitol and were transcardiallly perfused with ice-cold saline, followed by 4% paraformaldehyde. Brains were extracted and immersed in a 30% sucrose-paraformaldehyde solution for 48 hours. They were sectioned using a cryostat and every fifth, 30 μm 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 hours later were processed for Fos-like immunoreactivity (Fos-lir). Each treatment group was represented in all assays to eliminate the confounding effects of inter-assay variability. The sections were first incubated for 30 minutes 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 minutes in blocking serum consisting of 0.3% Triton X (TTX, Sigma) and 3% normal goat serum (NGS, Vector Laboratories) in TBS. Following further washing with TBS, the sections were incubated in a primary antibody solution for 48 hours. This solution consisted of a polyclonal antibody (Ab-5, PC-38, Oncogene Research Products, MA) 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 hour. The sections were then washed again in TBS and incubated in a tertiary, avidin-biotin complex solution (ABC,

Table 2

Coordinates in mm relative to bregma for each of the 5 sites at which NMDA was infused during the excitotoxic lesions of the PRh

Anterior-Posterior	*Medial-Lateral*	*Dorsal-Ventral*
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

Table 3

Coordinates in mm relative to bregma for each of the 5 sites at which current was delivered during the electrolytic lesions of the IML

Anterior-Posterior	*Medial-Lateral*	*Dorsal-Ventral*
2.0	1.1	6.8
2.8	1.1	7.2
2.8	1.1	7.3
3.6	1.1	7.3
3.0	1.1	7.4

Vector Laboratories). Twenty-four hours later, DAB (diaminobenzidine, nickel intensified) was used for visualization of Fos-lir. Stained sections were mounted on gelatin-coated slides and coverslipped for microscopic analysis.

5.1.1.2.3 Image analysis. Due to 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 analyses, 7 regions were selected for closer examination. According to our hypothesis we were primarily interested in the HPC and entorhinal cortex. However, our initial examination of tissue revealed marginal Fos-lir within the hippocampus. There were detectable Fos-lir cells in the dentate gyrus, but little to no Fos-lir cells were observed in the CA cell fields of the hippocampus. For this reason, only the dentate gyrus of the HPC was selected for further analysis. The preliminary examination also revealed a substantial number of Fos-lir cells in cortical regions. Thus, the remaining 6 areas examined were the entorhinal, temporal, parietal, retrosplenial agranular and granular, and frontal cortices.

To obtain an estimate of the number of Fos-lir cells, a rectangular selection (150 x 300 pixels) was made within each region and the numbers of cells expressing Fos-lir within that selection were counted using a density slice technique (Scion Image Beta 3b, imaging software). Three sections through each region were analyzed in this way 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.

5.1.1.2.4 Statistical analyses. The final averages were used to calculate the mean and S.E.M. for each of the 5 lesion groups. These values are displayed in Figures. One-way,

between-subjects 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 HSD tests. When ANOVAs were not statistically significant, planned comparisons between each condition and the Sham group were made using independent samples t-tests. Significance level for all statistical tests was set at 0.05, but probability values falling between 0.05 and 0.10 were noted.

5.1.2 Results

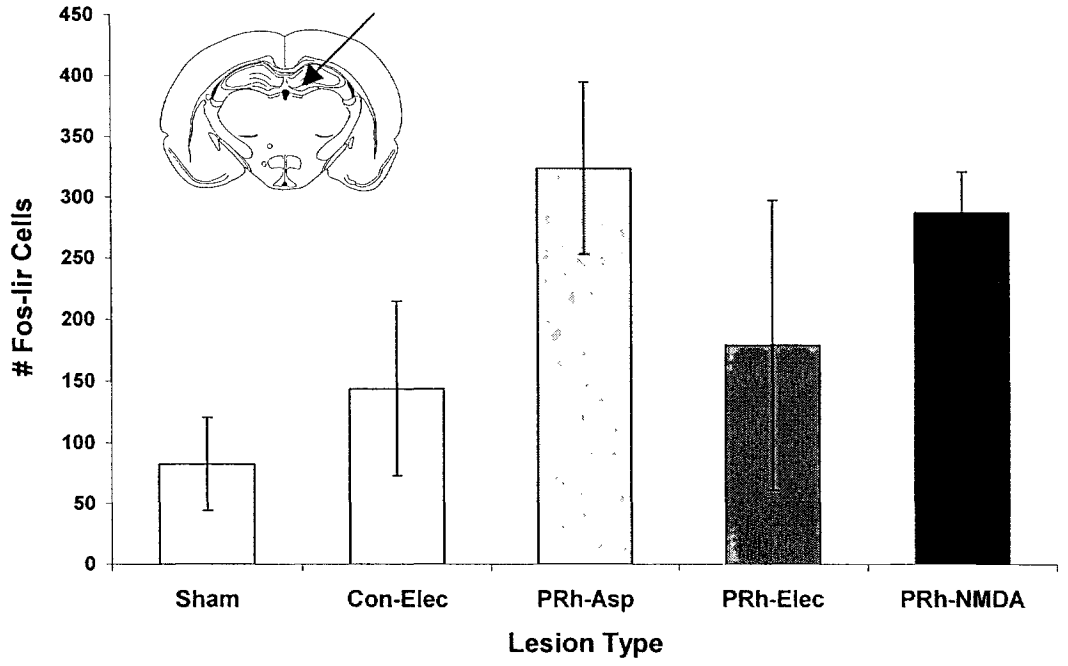
5.1.2.1 Dentate gyrus

Figure 48 shows the average number of Fos-lir cells detected in the dentate gyrus of each group and a representative section of this region from each of the lesion conditions. 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 t-tests were used to compare each experimental group to the Sham-lesion condition. These tests revealed that rats with PRh aspiration lesions ($t[8] = -3.147$, $p = .014$) and rats with PRh NMDA lesions ($t[8] = 4.422$, $p = .004$) displayed significantly more Fos-lir cells in the dentate gyrus than Sham rats. The other lesion groups were not significantly different from Sham rats (all $ps > .10$).

5.1.2.2 Entorhinal cortex

Figure 49 shows the average number of Fos-lir cells detected in the entorhinal cortex of each group and a representative section of this region from each of the lesion conditions. The sections used in this analysis were between 5.5 and 6.0 mm posterior to bregma.



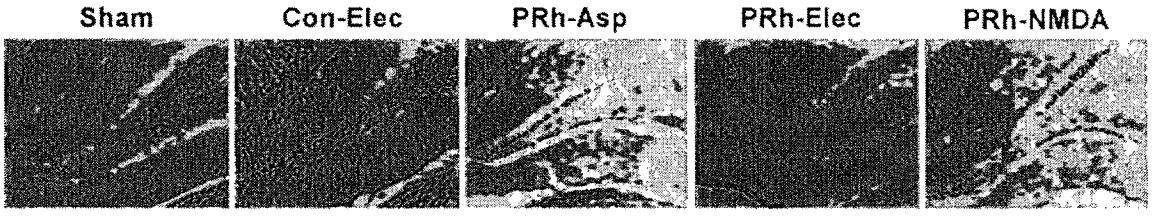
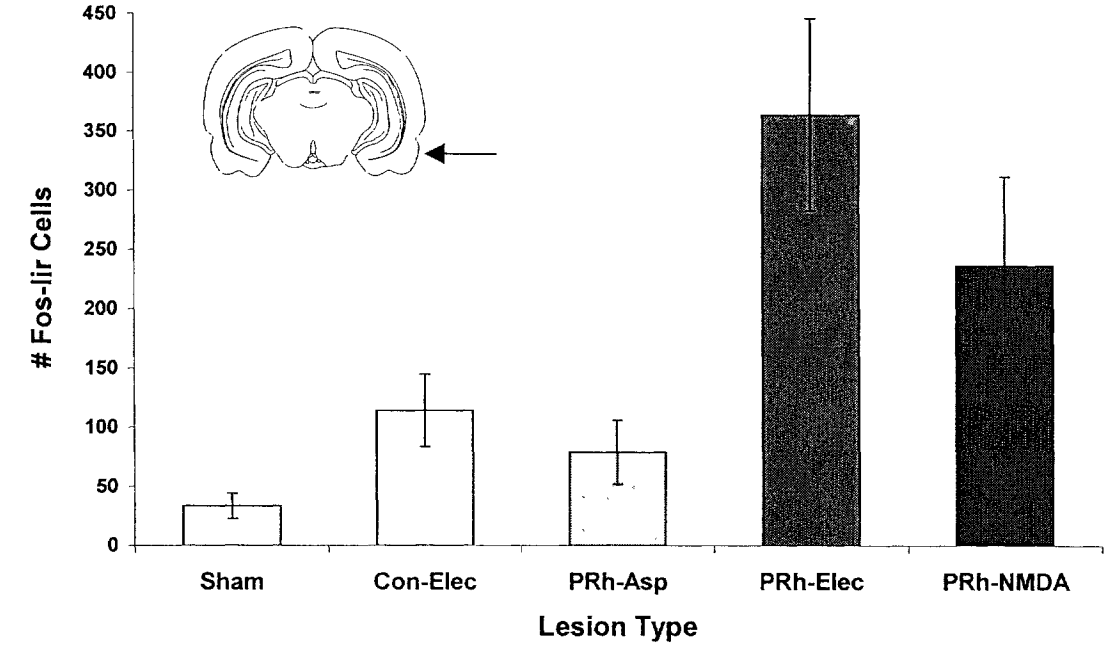


Figure 48. The top panel shows the mean number of Fos-stained cells in the dentate gyrus following each of 5 surgical procedures. Error bars represent S.E.M. The inset in the top left portion of the graph shows the approximate location of the area examined. The bottom panel shows a representative section from each condition.



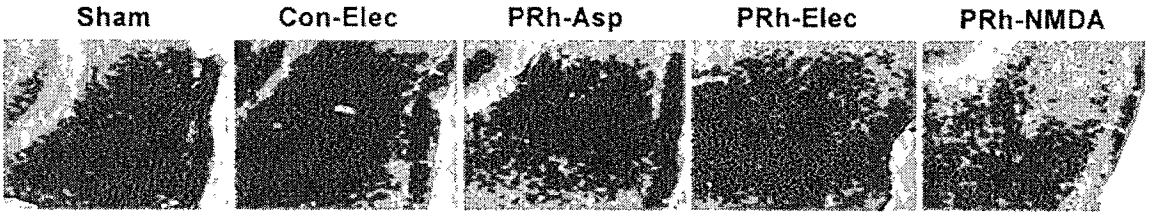


Figure 49. The top panel shows the mean number of Fos-stained cells in the entorhinal cortex following each of 5 surgical procedures. Error bars represent S.E.M. The inset in the top left portion of the graph shows the approximate location of the area examined. The bottom panel shows a representative section from each condition.

The results from the ANOVA indicated that there were statistically significant differences among the groups ($F[4,16] = 7.298$, $p = .002$). Posthoc Tukey 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 ($ps > .10$). In addition, there were significantly more Fos-lir cells in the entorhinal cortex following electrolytic PRh lesions than aspiration PRh lesions and electrolytic control lesions ($p = .006$ and $p = .043$, respectively). No other pairwise comparisons were statistically significant ($ps > .10$).

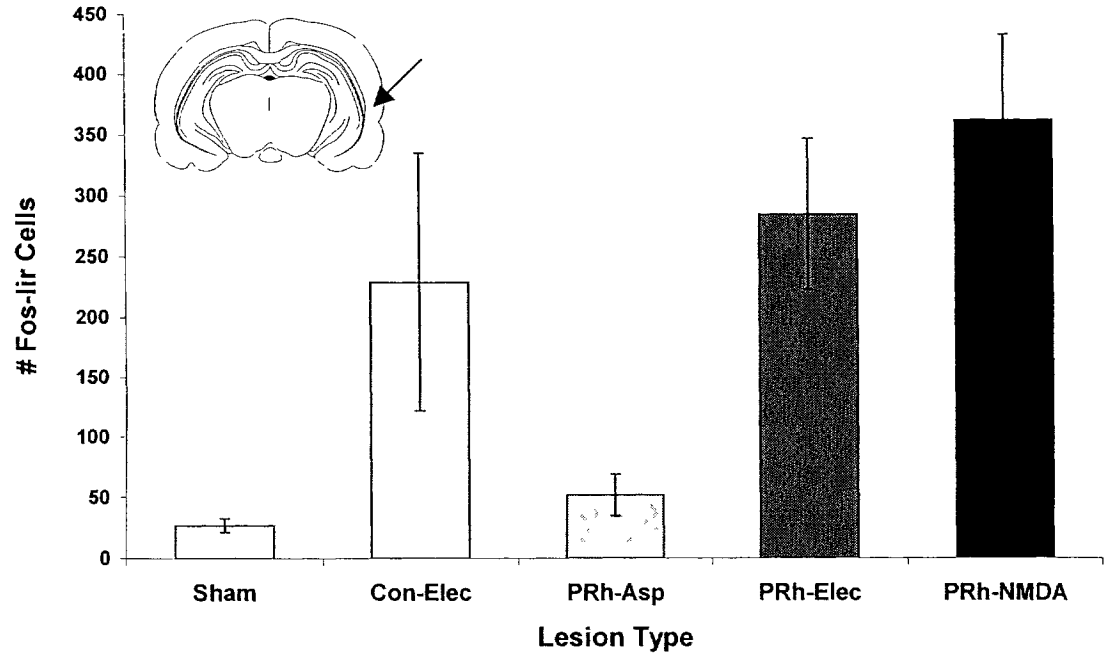
5.1.2.3 Temporal cortex

Figure 50 shows the average number of Fos-stained cells detected in the temporal association cortex of each group and a representative section of this region from each of the lesion conditions. 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 ($F[4,16] = 7.857$, $p = .001$). Tukey tests revealed that electrolytic and NMDA PRh lesions led to more Fos-lir cells in the temporal association cortex relative to Sham lesions ($p = .011$ and $p = .003$, respectively). Additionally, both the electrolytic PRh lesion and the NMDA PRh lesion resulted in more Fos-lir cells than the aspiration PRh lesion ($p = .024$ and $p = .008$, respectively). No other pairwise comparisons were statistically significant ($ps > .10$).

5.1.2.4 Parietal cortex

Figure 51 shows the average number of Fos-lir cells detected in the parietal cortex of each group and a representative section of this region from each of the lesion conditions. The sections used in this analysis were between 4.0 and 4.5 mm posterior to bregma.



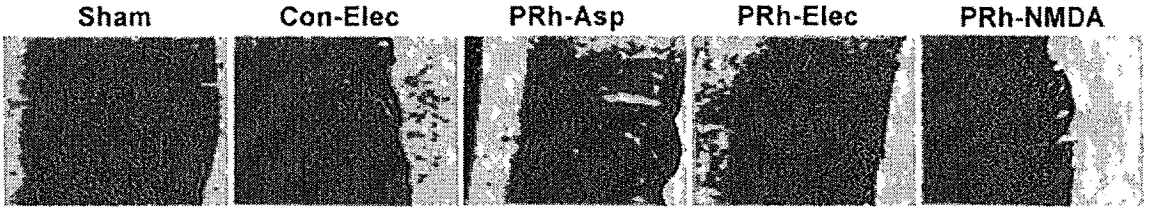
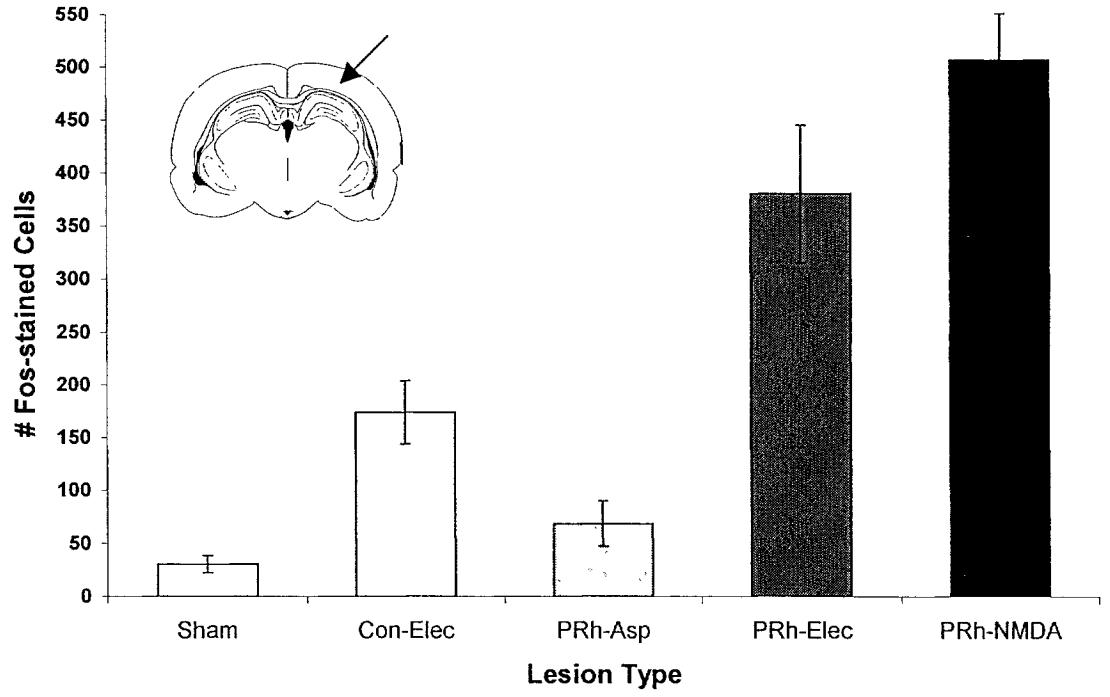


Figure 50. The top panel shows the mean number of Fos-stained cells in the temporal cortex following each of 5 surgical procedures. Error bars represent S.E.M. The inset in the top left portion of the graph shows the approximate location of the area examined. The bottom panel shows a representative section from each condition.



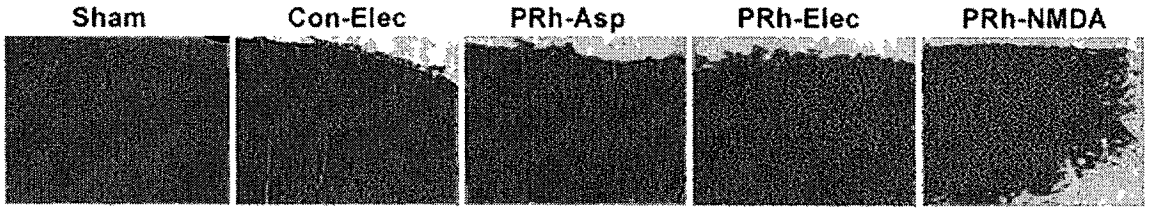


Figure 51. The top panel shows the mean number of Fos-stained cells in the parietal cortex following each of 5 surgical procedures. Error bars represent S.E.M. The inset in the top left portion of the graph shows the approximate location of the area examined. The bottom panel shows a representative section from each condition.

The results from the ANOVA indicated that there were statistically significant differences among the groups ($F[4,16] = 24.189$, $p = .001$). Tukey tests revealed that electrolytic and NMDA PRh lesions led to more Fos-lir cells in the parietal cortex relative to Sham lesions ($p = .001$ and $p = .001$, respectively). Additionally, both the electrolytic and NMDA PRh lesions resulted in more Fos-lir cells than the aspiration PRh lesion ($p = .001$ and $p = .001$, respectively) and electrolytic control lesions ($p = .027$ and $p = .001$, respectively). No other pairwise comparisons were statistically significant ($ps > .10$).

5.1.2.5 Frontal cortex

Figure 52 shows the average number of Fos-lir cells detected in the frontal cortex of each group and a representative section of this region from each of the lesion conditions. 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 tests revealed that electrolytic and NMDA PRh lesions led to more Fos-lir cells in the frontal cortex relative to Sham lesions ($p = .001$ and $p = .016$, respectively). Additionally, both the electrolytic and NMDA PRh lesions resulted in more Fos-lir cells than the aspiration PRh lesion ($p = .001$ and $p = .021$, respectively). Furthermore, the electrolytic PRh lesion resulted in more Fos-lir cells in this region than the electrolytic control lesions ($p = .048$). No other pairwise comparisons were statistically significant ($ps > .10$).

5.1.2.6 Retrospenial agranular cortex

Figure 53 shows the average number of Fos-lir cells detected in the retrosplenial agranular cortex of each group and a representative section of this region from each of the lesion conditions. The sections used in this analysis were between 3.5 and 4.0 mm posterior to bregma.

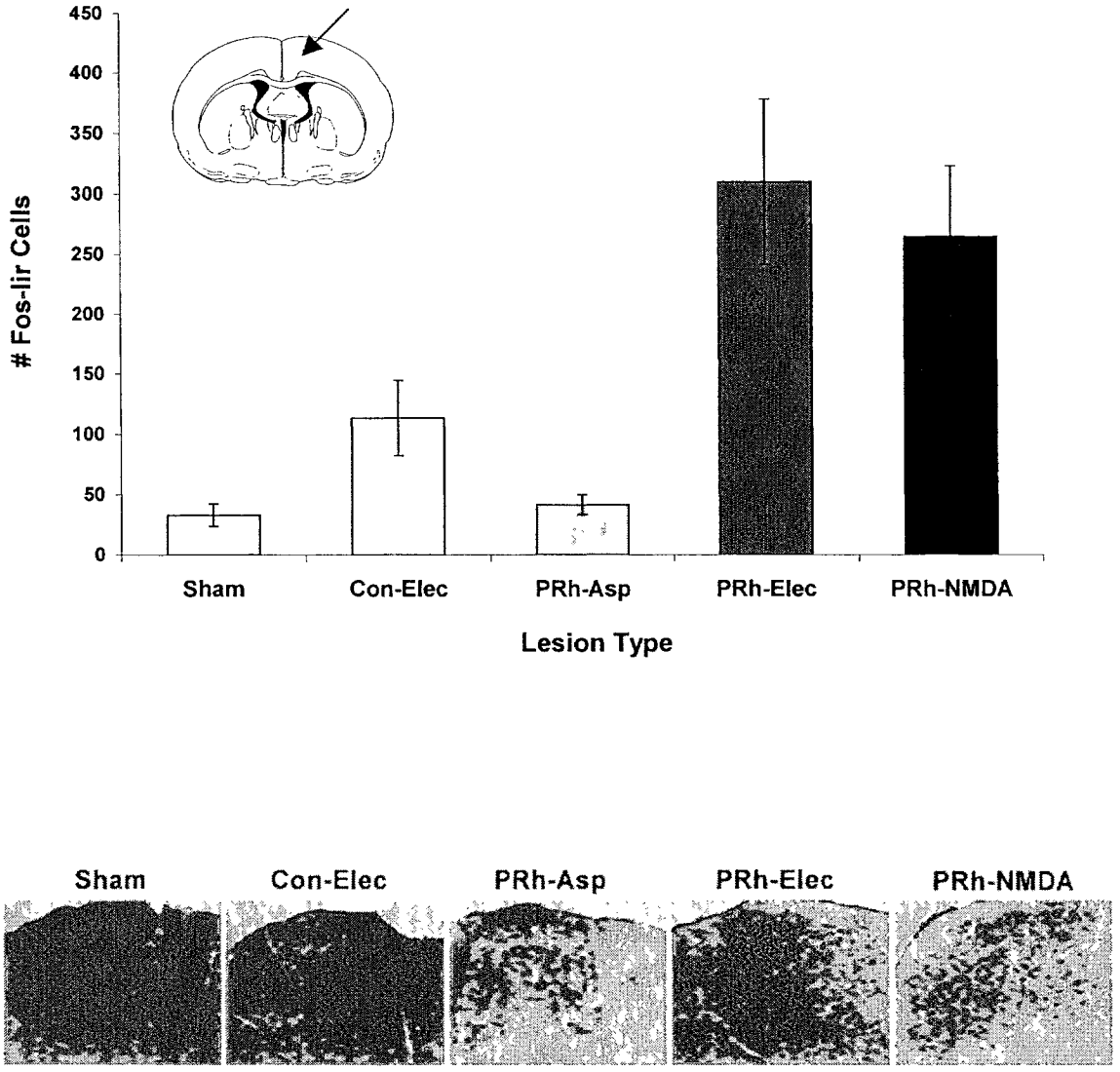
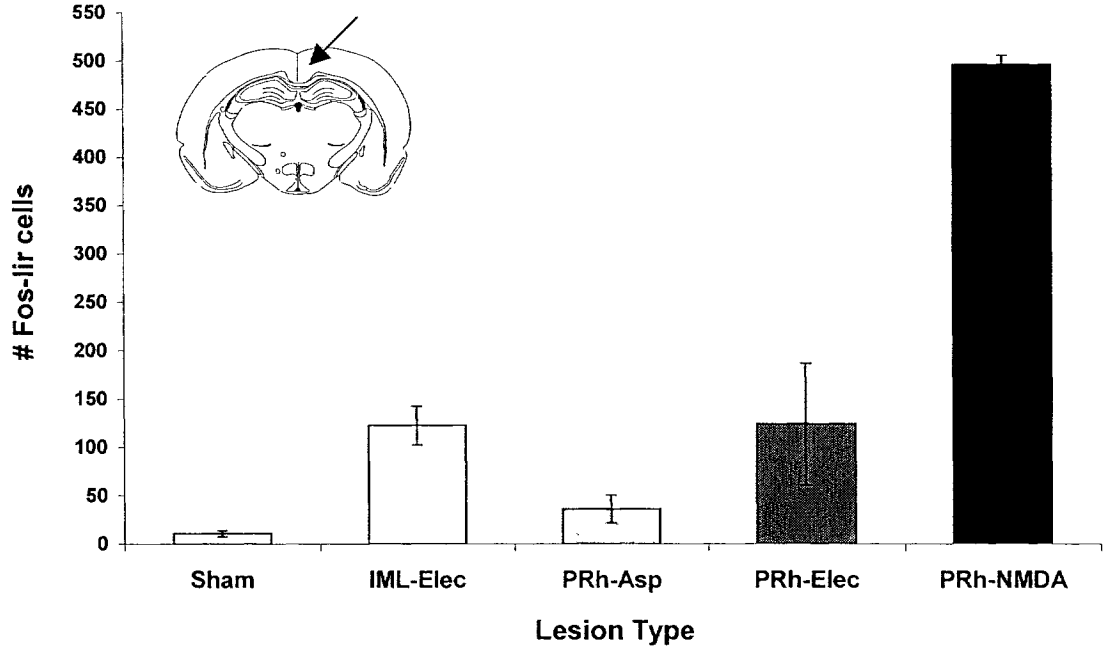


Figure 52. The top panel shows the mean number of Fos-stained cells in the frontal cortex following each of 5 surgical procedures. Error bars represent S.E.M. The inset in the top left portion of the graph shows the approximate location of the area examined. The bottom panel shows a representative section from each condition.



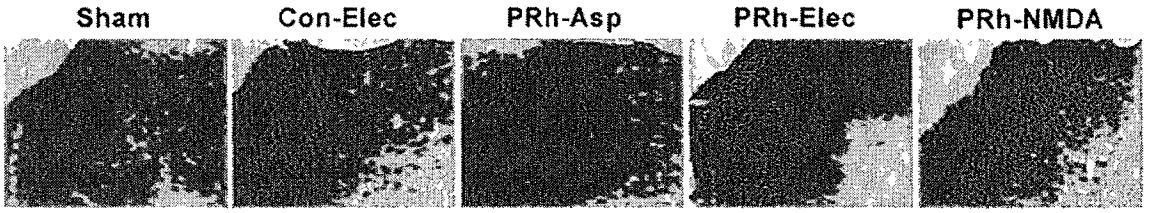


Figure 53. The top panel shows the mean number of Fos-stained cells in the retrosplenial agranular cortex following each of 5 surgical procedures. Error bars represent S.E.M. The inset in the top left portion of the graph shows the approximate location of the area examined. The bottom panel shows a representative section from each condition.

The results from the ANOVA indicated that there were statistically significant differences among the groups ($F[4,16] = 24.046$, $p = .001$). Tukey 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 ($ps > .10$).

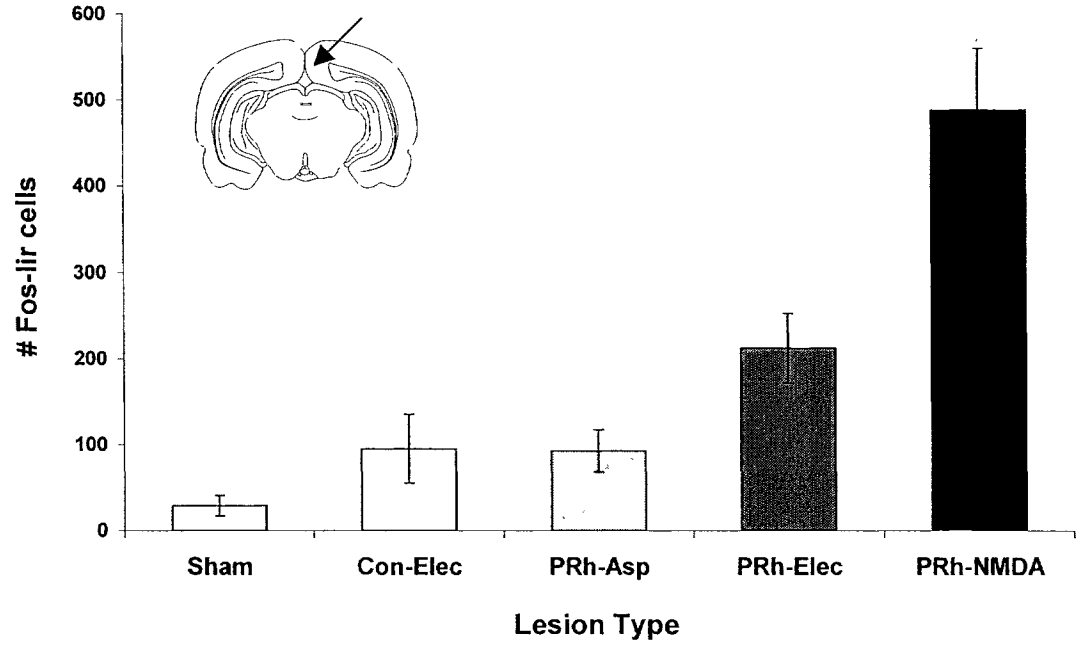
5.1.2.7 Restrospenial granular cortex

Figure 54 shows the average number of Fos-lir cells detected in the retrosplenial granular cortex of each group and a representative section of this region from each of the lesion conditions. The sections used in this analysis were between 5.5 and 6.0 mm posterior to bregma. In this case, both retrosplenial granular a and b cortex were examined. The same pattern of results were obtained for both regions, therefore, cell counts from all 6 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 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 than Sham lesions ($p = .004$). No other pairwise comparisons were statistically significant ($ps > .10$).

5.2 Summary and Discussion

The main findings of this experiment were that electrolytic and NMDA 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 the Sham group. NMDA PRh lesions, in particular, increased Fos expression, relative to Sham lesions, in every area examined. Electrolytic PRh lesions increased Fos expression in the cortex, but not in the hippocampus. Conversely, aspiration PRh lesions increased Fos expression in the dentate gyrus, but not in the cortex.



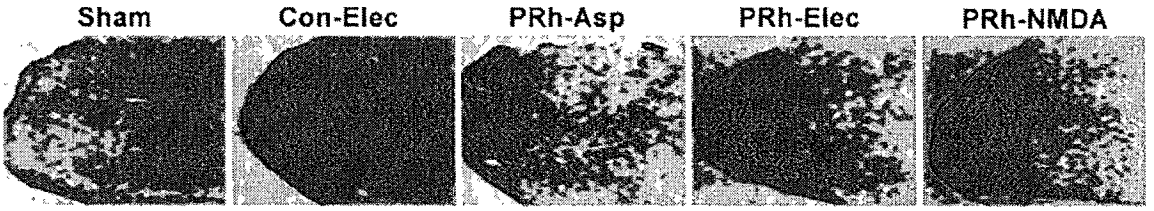


Figure 54. The top panel shows the mean number of Fos-stained cells in the retrosplenial granular cortex following each of 5 surgical procedures. Error bars represent S.E.M. The inset in the top left portion of the graph shows the approximate location of the area examined. The bottom panel shows a representative section from each experimental condition.

In the present thesis, electrolytic PRh lesions were found to produce greater deficits on place-memory and object-recognition tasks than aspiration PRh lesions. In Experiment 1, aspiration PRh lesions did not impair anterograde place memory, whereas in previous studies electrolytic (Wiig & Bilkey, 1994; Liu & Bilkey, 1998a; 1998c) and excitotoxic (Liu & Bilkey, 1998b; 1999; 2001) PRh lesions impaired the acquisition of place information. In Experiment 2, electrolytic PRh lesions impaired retrograde place memory, whereas in Experiment 3, aspiration PRh lesions did not. In Experiment 5, electrolytic, but not aspiration, PRh lesions impaired anterograde object recognition. In previous studies, excitotoxic PRh lesions impaired the acquisition of object information (Bussey et al., 1999; 2000). Finally, in Experiment 6, electrolytic PRh lesions impaired retrograde object recognition, whereas in Experiment 7 and 8, aspiration PRh lesions did not.

The amount of damage to the PRh was comparable in the experiments described above. Both lesion methods resulted in gross tissue damage, destroying both cell bodies and fibres of passage. There were differences in the extent of damage to structures outside of and adjacent to the PRh following the two lesion methods; specifically, aspiration PRh lesions tended to include more damage to the postrhinal and entorhinal cortices, the hippocampus, and the amygdala than electrolytic PRh lesions. It seems unlikely, however, that larger, less selective lesions would produce fewer deficits in learning and memory. Instead, it was hypothesized that the electrolytic technique itself may have adverse consequences for the function of brain regions outside of the PRh. The electrolytic current applied through the extent of the PRh during surgery may lead to an abnormal cascade of activation in efferent structures, such as the HPC or entorhinal cortex. Consistent with this hypothesis, the pattern of memory loss observed after electrolytic PRh lesions tended to resemble that which occurs after HPC lesions.

The results of the present experiment are consistent with the hypothesis that electrolytic PRh lesions increase activation in other brain regions. However, contrary to our expectations, increased activation was not observed in the HPC. Instead, the activation was present throughout the cortex. NMDA PRh lesions also increased activation throughout the cortex as well as in the HPC. 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. Unexpectedly, however, aspiration PRh lesions did lead to increased activation in the dentate gyrus.

Of the regions selected for analysis in the present experiment, the retrosplenial and parietal cortex 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, Stefanacci, Squire, & Zola, 1998; Buffalo et al., 1999). The entorhinal cortex is the primary source of neocortical input to the HPC. Additionally, these polymodal association cortices are also thought to be 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.

The primary limitation of the present research is the use of Fos as a marker for neuronal activation. This technique provides some clue as to what regions become activated following a manipulation, but does not indicate the underlying cause or significance of that activation, nor can the suppression of neuronal responses be detected. Increased Fos activation

following electrolytic and excitotoxic PRh lesions supports the hypothesis that these techniques have adverse neurological and behavioural consequences that extend beyond the PRh damage, but further research is required to determine if that is indeed the case. Additionally, the absence of Fos activation does not necessarily indicate that the region is unaffected by the manipulation. It is possible that changes that may be occurring in some regions are not detected by this technique. The use of markers of the activation of other immediate-early genes would provide more information about the patterns of differential activation that may occur following these lesions.

Though the use of Fos to assess neuronal activation is limited in its ability to provide information about the nature of differences between regions where it is expressed and regions where it is not, it is a versatile tool for evaluating discrepant responses to a manipulation. In the present experiment, the aim was to determine whether the different lesion methods would lead to differential activation of Fos. This is a first and necessary step, and with its use we were able to determine that the lesion methods differentially activated other brain regions. Thus, based solely on this information, it can be concluded that electrolytic and NMDA PRh lesions do differ from aspiration PRh lesions. However, other types of assays would be required to identify the consequences of the differential activation following these lesions.

In the present experiment, rats were killed 1 hour after surgery. Therefore, the analysis of neuronal activation was limited in the extent to which it could provide information about long-lasting changes that may adversely affect performance on anterograde tests that are conducted 2 weeks after surgery. For this reason, it would be beneficial to examine other markers of neuronal function that can be used to study the time-course of changes following the different lesions. 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 responses in other brain regions. Finally, neurogenesis in the adult HPC has been detected in response to learning and experience (for review see Hastings, Tanapat, & Gould, 2000). Therefore, it is possible that cell birth, differentiation, or proliferation in the HPC is differentially affected when animals with PRh lesions made in different ways learn new information.

Chapter 6

General Discussion

The purpose of this thesis was to examine the pattern of spared and impaired memory abilities in rats with lesions of the PRh. More specifically, it examined the ability of rats with PRh lesions to acquire information about objects and places (anterograde memory), as well as their ability to remember information about objects and places acquired before surgery (retrograde memory). An additional aim of this thesis was to examine the impact of lesion method and experimental design on the patterns of spared and impaired memory abilities in rats with PRh lesions. Table 4 summarizes the main findings from the experiments described in Chapters 2, 3 and 4.

In Chapter 5, immunocytochemistry was used to explore the expression of the protein products of the immediate early gene, *c-fos*, (Fos-lir) as a marker for neuronal activation in rats that received PRh lesions by aspiration, electrolysis, or excitotoxicity. The main findings were that 1) excitotoxic, NMDA lesions of the PRh led to marked increases in the number of cells expressing Fos-lir in the dentate gyrus of the HPC as well as throughout the cortex (including frontal, parietal, temporal, entorhinal, and restroplenial agranular and granular cortices), 2) electrolytic lesions of the PRh led to marked increases in the number of cells expressing Fos-lir throughout the cortex (same areas listed above), but did not increase Fos-lir in the dentate gyrus, and 3) aspiration lesions of the PRh led to increases in the number of cells expressing Fos-lir in the dentate gyrus, but did not increase staining in the cortex.

Table 4

Summary of the main findings from Chapters 2, 3, and 4.

	Methodology		
	Lesion Type	Design	Results
Anterograde Memory			
Memory for Places			
Water Maze	Aspiration	--	Spared
NPT-Standard	Aspiration	--	Spared
NPT-Modified	Aspiration	--	Spared
Memory for Objects			
NPT-Standard	Aspiration	--	Spared
	Electrolytic	--	Impaired
NPT-Modified	Aspiration	--	Impaired
Retrograde Memory			
Memory for Places			
Water Maze	Electrolytic	Within-subjects	Impaired/Ungraded
	Aspiration	Within-subjects	Spared
	Aspiration	Between-subjects	Impaired/Graded
NPT	Aspiration	--	Impaired
Memory for Objects			
NPT	Electrolytic	Between-subjects	Impaired/Ungraded
	Aspiration	Between-subjects	Spared
	Aspiration	Within-subjects	Spared

6.1 PRh ablation and memory

In the following sections the present findings are discussed from two perspectives: Section 6.1.1 discusses anterograde memory for objects and places and Section 6.1.2 discusses retrograde memory for objects and places.

6.1.1 Anterograde amnesia for objects and places

The results of the anterograde tests of object recognition and place memory allow a comparison of the relative contribution of the PRh to the acquisition of these two types of information. This comparison is important to determine whether the PRh is important for both types of memory, consistent with a unitary system view of MTL function and a broader view of PRh function, or whether the PRh is important for only object memory, consistent with a specialized role in object recognition. The following discussion is divided based on the lesion method, as different behavioural outcomes were observed with different methods.

6.2.1.1 The effects of aspiration lesions of the PRh

Overall, rats with aspiration PRh lesions displayed intact object-recognition abilities. Aspiration PRh lesions only produced deficits when exposure to sample objects was limited. Thus, it is possible that the ability of rats to detect that an object is familiar or novel is unaffected by PRh damage, except when tasks demands are high. This tendency of animals with PRh damage to specifically fail difficult object-recognition tasks is consistent with other findings in the literature (e.g. Eacott & Gaffan, 1994).

In contrast to the findings from the anterograde object memory tests, rats with aspiration PRh lesions were not impaired on tests of anterograde place memory. These rats learned the location of a hidden, stationary platform in the water maze as quickly as Sham rats. Additionally, like Sham rats, they remembered the location of objects in an open field, even when exposure to sample objects was limited. This pattern of findings suggests that the

functions of the PRh are specifically important for anterograde object recognition, but not place memory. This dissociation of function has been previously reported (e.g. Glenn & Mumby; 1996; Bussey et al., 2000), and combined with the present findings, has been demonstrated using a wide variety of object and place tasks (reviewed by Aggleton & Brown; 1999).

6.2.1.2 The effects of electrolytic PRh lesions

Unlike aspiration lesions, electrolytic PRh lesions impaired object recognition on the standard version of NPT. This finding suggests that the electrolytic lesion method may exacerbate the effects of a PRh lesion, possibly by producing abnormal function in other brain regions that may normally compensate for the PRh damage when it occurs by aspiration. This interpretation is supported by evidence obtained in Chapter 5, that cells in a number of cortical areas displayed increased activation following electrolytic, but not aspiration PRh lesions.

In the present thesis, the effects of electrolytic PRh lesions on anterograde place memory were not directly evaluated. However, rats with this lesion displayed rapid recovery from their retrograde place memory deficits in Experiment 2. This finding suggests that place memory was intact; however, it would be useful to conduct an anterograde place memory test in naïve rats with electrolytic PRh lesions to confirm that this is the case.

6.2.1.3 PRh ablation and anterograde memory

The general conclusions based on the findings from the present thesis and previous reports are that the functions of the PRh are specialized to support the acquisition of object, but not place information. Furthermore, electrolytic lesions appear to exacerbate the effects of PRh damage.

6.2.2 Retrograde amnesia for objects and places

The results of the retrograde tests of object and place memory described in this thesis allow a comparison of the relative contribution of the PRh to the retention of these two types of information when acquired prior to surgery. As with the anterograde perspective, the following discussion is divided based on the lesion method as different behavioural outcomes were observed with different methods.

6.2.2.1 The effects of aspiration lesions of the PRh

Rats with aspiration PRh lesions displayed retrograde memory deficits for places, but not objects. These findings are in contrast to the opposite pattern of memory loss observed on the anterograde tests and suggest that the PRh may have functions that are important for the retrieval of information learned prior to surgery that are dissociable from its role in the acquisition or short-term recall of similar types of information. Even if just the place and object versions of NPT are considered, the ability of rats to detect that an object has moved to a new location may rely on their ability to recall many features of the presurgery learning event, whereas the ability of rats to detect that a novel object is present could be supported by their ability to remember any one feature of the sample object, an ability not dependent on the integrity of the PRh.

6.2.2.2 The effects of electrolytic lesions of the PRh

Rats with electrolytic PRh lesions displayed retrograde memory deficits for object and place information at all time points evaluated. Thus, this type of lesion interfered with the ability of rats to retrieve sufficient information from learning events that occurred between 4 weeks and 2 days before surgery. These findings suggest that, as with the anterograde memory tests, electrolytic lesions exacerbate the effects of PRh damage. The findings from Chapter 5 are consistent with this notion. It is possible that the increased activation observed

in cortex following electrolytic PRh lesions interferes with normal memory formation. Alternatively, it may be that electrolytic lesions disrupt normal function in brain regions outside of the PRh where representations of object features may exist. These representations are likely the basis for novelty preference observed in rats with aspiration PRh lesions on retrograde retention tests for object memory.

6.2.2.3 PRh ablation and retrograde memory

Retrograde place memory appears to be more vulnerable to PRh damage than retrograde object memory. Both rats with aspiration and electrolytic PRh lesions displayed retrograde amnesia for places. On the contrary, only electrolytic PRh lesions produced retrograde amnesia for objects. Furthermore, 12 of the 18 rats with PRh aspiration lesions that failed to display retrograde amnesia for objects in Experiment 7 were the same rats that displayed retrograde amnesia for places in Experiment 4. Thus, the effects of PRh lesions on retrograde memory for places and objects are dissociable.

6.2 Theoretical Implications

In the introductory chapter of this thesis several models of MTL memory function were described. In the following sections the findings are discussed in relation to each of these models.

6.2.1 The PRh as a component in a MTL memory system

The MTL memory system model proposed by Squire and Zola-Morgan (1991) contends that the structures in this region, including the HPC, the PRh, and the entorhinal and parahippocampal (postrhinal) cortices, are components in a unitary system that is necessary for the formation of long-term declarative memories. They further contend (Alvarez & Squire, 1994; Squire, 1991) that this system is critical for consolidation processes by which memories becoming permanently established over time. Therefore, the integrity of this

system is necessary at the time of learning and for some, as yet unidentified, period of time after learning.

The role of the PRh in this system is not precisely delineated, but it is implied that the HPC would be unable to communicate effectively with other brain regions without the integrity of the adjacent cortices. Yet, the proponents of this model also maintain that the rhinal cortices make an important contribution to memory. The nature of this contribution is not described. Accordingly, the presence or absence of memory deficits in animals with PRh lesions can always be integrated into this model; when memory deficits are observed following PRh ablation, it can be argued that the functions of the system have been disrupted and when memory deficits are not observed it can be argued that there was not sufficient damage to fully disrupt the system and the remaining tissue is able to adequately compensate. Ultimately, this model is overly simplistic and fails to capture the complex nature of memory and the neural systems that support it.

6.2.2 The PRh as a component in an object-processing system

An emerging view of MTL function is that structures in this region can be separated into multiple, functionally distinct systems that support different types of memory abilities. Some proponents of this view dissociate individual systems based on information attributes. In particular, it has been proposed that the functions of the PRh are important for the processing of object, but not place, information, while the functions of the HPC are important for the processing of place, but not object, information. Very specific predictions can be derived from this view, and thus far, there are many studies that obtained results that support it (Bussey et al., 2000; Gaffan, 1994; Glenn & Mumby, 1996; but see Liu & Bilkey, 2001).

The experiments described in this thesis test the notion that the functions of the PRh are important for the processing of object, and not place, information and were designed to use tasks with those specific information attributes. Overall, however, the findings from these experiments do not clearly follow the hypothesized pattern. Aspiration PRh lesions clearly produced retrograde amnesia for places, but not objects, electrolytic PRh lesions produced retrograde amnesia for places and objects, and both lesion types led to anterograde amnesia for objects, though not under the same conditions. These data are not consistent with a specialized role for the PRh in only object memory. The dissociation in function is evident when anterograde memory is considered. However, the findings from the present retrograde memory experiments suggest that the dissociation between PRh and HPC function may not exist when assessing retention of presurgically acquired information.

6.2.3 The PRh as a component in a recognition system

The model proposed by Aggleton and Brown (1999) also attempts to dissociate the functions of the PRh and HPC. According to their model, HPC inputs to the anterior thalamic nuclei via the fimbria-fornix system are important for episodic memory, or more specifically, the ability to explicitly recall details of past events. The PRh, on the other hand, is thought to be part of a system that includes the medial dorsal nucleus of the thalamus and this circuit is proposed to be involved in familiarity-based recognition, or more specifically, the ability to recognize that something has been previously encountered, without explicitly recalling the event itself.

The present findings are not entirely consistent with Aggleton and Brown's model. Aspiration PRh lesions largely spared the ability of rats to prefer a novel object over a familiar object. Thus, recognition memory is not always dependent on the integrity of the PRh. The dissociation between retrograde place and object memory following PRh lesions is

also inconsistent with the model. Rather, the retrograde place memory deficits in PRh rats suggest that there may be some interdependence in the functions of the PRh and HPC.

6.2.4 PRh function and memory

The aspect of the present findings that is most difficult to reconcile with the preceding models of MTL memory function is the dissociation between the effects of PRh lesions on anterograde and retrograde memory. However, as previously discussed, NPT as it was used to assess retrograde memory for objects may have been unsuitable to reveal the contribution of the PRh to the formation of representations for this type of information. Therefore, it is possible that the PRh was integral in forming these representations, but retrieval of them was able to proceed in its absence. The finding of impaired object recognition on the modified version of NPT is consistent with this notion.

When NPT was used to assess retrograde memory for object location, PRh rats did not show a preference for the moved object. By contrast, PRh rats did show a preference for the moved object when they acquired information about object location after surgery. Thus, the formation of place representations was possible after PRh damage, but the retrieval of a place representation that included the placement of the objects in the open field appeared to require the integrity of the PRh. The findings that PRh rats acquired a water maze problem normally, but showed impaired retention when the problem was learned before surgery are consistent with this notion.

The best way to integrate these findings is to incorporate features of the two most prominent views of MTL function: structures in the MTL have specialized functions but there are circumstances under which their cooperative function is required. Thus, in terms of anterograde memory, the functions of the PRh are specialized to form representations about objects that aid recognition, whereas the functions of the HPC are specialized to process

place information. In terms of retrograde memory, the rhinal cortices and the HPC may function interdependently to support recall of place representations. In Experiment 1, PRh lesions did not impair long-term retention of place information. This further suggests that when place representations are formed after brain damage, retrieval of them can be supported by intact structures (that may have participated in their formation).

Nadel and Moscovitch (1997) proposed a *multiple trace theory* of memory formation that more adeptly accounts for the present findings. According to Nadel and Moscovitch, multiple memory traces are formed in the MTL when new information is acquired and each time that information is recalled. In terms of information acquisition then, traces are stored in a distributed manner throughout the MTL. However, they contend that individual MTL structures may participate in specific types of learning events. This accounts for the dissociation in function between the PRh and HPC.

Similar to the views of Alvarez and Squire (1994), Gaffan (1991), and Aggleton and Brown (1999), Nadel and Moscovitch maintain that the HPC has a unique function for episodic memory. Each time the temporal or spatial aspects of a memory are to be retrieved the integrity of the HPC is always required to direct the recall of the appropriate traces that together constitute that specific memory. It is argued that the HPC would rely on the adjacent rhinal cortices to perform this function. This accounts for the perturbations in retrograde place memory observed following PRh lesions. Additionally, it is possible that when the place representation is formed after PRh damage, memory traces are stored in a distributed manner throughout the spared portions of the MTL and are, therefore, retrievable.

6.3 On Methodology

This thesis examined the effects of different lesion methods on the ability of rats to learn and remember place and object information and the impact of experimental design. The results following these variations in methodology revealed notable differences in behaviour. These are discussed in the following sections.

6.3.1 Lesion Method

A lesion-based approach to studying learning and memory processes must always be tempered with the fact that the brain of the subject is not normal. The subject is attempting to solve a problem with a damaged brain. If the subject is unable to solve a particular problem then it can be concluded that the brain damage is responsible. However, it is necessary to bear in mind that attributing particular functions to a brain region based solely on this approach may lead to erroneous conclusions. For this reason, it is also important to obtain converging evidence from other types of research. Two other major sources of information are electrophysiological and pharmacological manipulations and overall the findings from the present thesis are consistent with these types of studies.

The present findings that different lesion methods lead to different behavioural outcomes warrant careful consideration, especially when different laboratories make use of varied techniques. This limits the validity in comparing the findings from different reports. Not only are the brains of subjects with lesions abnormal due to damage or surgical removal of tissue, it is also possible that the manner in which brain damage occurs could adversely affect functioning in regions outside of the target area. Thus, it is even more difficult to attribute functional deficits to the damaged region. However, despite the differences in memory loss following electrolytic and aspiration PRh lesions in the present thesis, it was evident that the both lesion types were affecting performance on the same tasks, but to

different degrees. At least in the case of these lesion methods, there appears to be a worsening of deficits with electrolytic lesions, rather than a completely different profile of deficits.

6.3.2 Experimental Design

The study of retrograde memory is typically plagued with numerous limitations. It is rarely possible to confirm the nature, extent, and time of acquisition of knowledge possessed by human subjects before the onset of amnesia. Some standardized tests exist, but they rest on the assumption that the human patient had certain prior knowledge. Also, brain damage in humans is rarely localized and may occur through a number of mechanisms: including surgical ablation, disease, stroke, or trauma. It is furthermore extremely unlikely that a group of patients with similar and verifiable knowledge bases *and* comparable brain damage exists. Therefore, the study of retrograde memory in human subjects is limited to descriptive accounts of memory loss following indiscriminate brain damage.

Animal experiments allow researchers to overcome the limitations associated with studying human subjects. It is possible to exert control over what is learned, how well it is learned, and when it is learned. It is also possible to make relatively discrete lesions of specific brain areas. Therefore, the effects of localized brain damage on the retention of specific types of information acquired at different time points prior to surgery can be easier to identify. Animal experiments are not without disadvantages, however. For example, when the experimental design is within-subjects, such that animals learn multiple problems at different time points prior to surgery, researchers have to decide whether to equate exposure to a problem or facility with a problem. In the former case, animals receive the same number of trials on each problem. Since animals tend to develop learning sets when presented with similar problems, they will inevitably acquire subsequent problems at a faster rate than

preceding problems. Thus, they are likely to 'overlearn' the later problems. In the latter case, animals receive as many trials as it takes to reach a learning criterion. Due to the development of learning sets, animals will receive fewer trials, and thus less exposure, with later problems.

One way to overcome the above dilemma is to utilize a between-subjects experimental design in which different groups of animals learn a single problem at different time points prior to surgery. Statistical power is lost with this solution, however, and a potentially serious problem is that the learning experiences of the animals are more difficult to generalize to those of human patients. Animals in these studies encounter only one problem before surgery and the remainder of their experience is limited to standard laboratory conditions. Human subjects, on the other hand, experience many events up to the time of brain damage. It may be argued, however, that animal experiments are always limited in the extent to which they can address human memory, but what they can provide is crucial information about the neural bases of some learning and memory processes. Therefore, exerting the control necessary to do this is beneficial.

In the present thesis, rats with aspiration lesions of the PRh displayed good retention of water-maze problems with a within-subjects design, whereas with a between-subjects design they displayed impaired retention of a water maze problem when it was learned during the week before surgery, but not when it was learned 4 weeks before surgery. This finding suggests that animals that learn multiple problems may do so differently than animals that learn a single problem, and consequently different brain regions may be recruited in the former than in the latter situation. For example, when Time of Learning was a within-subjects variable in Experiments 2 and 3, rats learned each of the place problems in different testing rooms. Thus, the nature of the second problem may have changed such that the

training context was correlated with a different goal location and the two problems together could be viewed as a conditional discrimination.

In Chapter 3, aspiration PRh lesions did not impair retention when either between- or within-subjects designs were used. However, the possibility that the nature of this variable may also lead to different patterns of memory loss on a task that is sensitive to PRh lesions remains. Therefore, it will be important to evaluate retrograde memory using both designs in future studies.

6.4 Future Research Considerations

The neural bases of the ability of animals to learn new information and later remember it is not well understood. There are many ways in which a specific brain area might contribute to learning and memory. Information is encoded, processed, stored, and retrieved. Learning may be impaired because information is not encoded, mechanisms of representation formation are disrupted, or storage does not occur. Memory loss may come about because previously stored representations are lost or the mechanisms by which they are retrieved are disrupted. Based on the present findings, formation of new place memories can still occur without the PRh. The processing and utilization of information required to form this representation can still occur through other brain areas. Retrograde memory loss was subtle and transient, suggesting that the PRh may normally participate in the formation of place memories, but the consolidation and long-term storage of this information occurs elsewhere, most likely in the HPC. It is also possible that useful, but non-essential, features of the place representation where represented by the PRh and after surgery this information was lost, resulting in the retrograde amnesia that we observed. Alternatively, the PRh may be aid in the retrieval of aspects of place memories stored elsewhere in the brain.

The use of reversible lesions would be one way to help isolate the contribution of the PRh to these processes. The place version of NPT would be particularly well suited for this type of study. NPT is also well suited for an electrophysiological approach. A place or object test following the same sample session might reveal whether neurons in the PRh respond preferentially to a novel or a moved object. Similarly, markers of neuronal activation could be used to determine whether differential responses are observed in different MTL structures to object and place tests. Additionally, using NPT, both anterograde and retrograde tests could be conducted and compared.

PRh lesions in the present thesis impaired the acquisition of object information under some circumstances. However, the ability of rats with PRh lesions to form long-term memories about objects was not evaluated. It is possible that PRh rats that displayed intact object-recognition after delays of minutes may not recall object information as well as Sham rats when tested several weeks after the postsurgery sample sessions.

A major limitation of the studies presented in this thesis is the use of only rats with PRh lesions. Comparing the effects of this type of damage with the effects of damage to other MTL structures would aid in identifying dissociations or similarities in functions. In particular, the contribution of the postrhinal cortex to the types of memory abilities assessed in this thesis is not known. It has been argued that the visual and visuospatial input to the postrhinal cortex may be indicative of its involvement in place memory (Burwell, 2001). Preliminary data indicate that lesions of the postrhinal cortex do not impair object or place recognition using NPT (Poirier, 2002), but may affect water-maze learning (Poirier, Glenn, & Mumby, unpublished data). It is possible that a combined lesion of the PRh and postrhinal cortex would produce greater deficits in retrograde place memory than those observed here, reflecting the cooperative functions of these two structures in the retrieval of

place representations. Accordingly, it is also possible that lesions restricted to the postrhinal cortex may produce retrograde place memory deficits resembling those following PRh lesions.

6.5 Conclusions

The findings from the experiments in this thesis clearly indicate that the PRh is important for normal learning and memory. The present attempt to characterize the effects of PRh lesions on the acquisition and retention of place and object information demonstrates the complexity of its role in these types of memories and the limitations associated with categorizing memory abilities based on stages of processing or information attributes.

PRh lesions have little effect on the ability of rats to acquire new place representations. Conversely, PRh lesions disrupt efficient retrieval of place representations. Thus, it seems that in the absence of the PRh, the HPC is able to access sufficient sensory information, perhaps via the postrhinal and/or entorhinal cortices. However, it seems that, when intact, the PRh participates in the formation of place representations and subsequently their retrieval.

PRh lesions disrupt the acquisition of object information. However, this is only revealed under specific conditions (DNMS and modified NPT), suggesting that object recognition can sometimes proceed in its absence. It is possible that the rat versions of DNMS and NPT do not require a whole-object representation (stimulus identification, see Murray and Richmond, 2001), but may instead be solvable based on the retention of certain features of the object. Damage to the PRh may interfere with the ability of animals to collect and process object information. Therefore, when there is limited exposure to an object, they are less able to assemble sufficient information to support recall. This interpretation is consistent

with normal DNMS performance at very short delays (4 and 15 seconds) and with normal NPT performance when they have explored sample objects for about 80 seconds. Thus, when time with the sample object is limited (DNMS) they are able to either collect sufficient information to support recall over very brief delays, and when they have more time with the sample object (NPT) they show adequate recall over longer delays. But when time with the sample object is limited and the delay period is long (DNMS and NPT) they are impaired.

PRh lesions tended to spare retrograde memory for objects. The present method of providing ample exploration time of sample objects may have contributed to this result. It is possible that this afforded the formation of multiple representations of the objects and their features, and these representations were fully formed by the time of surgery. Thus, sufficient representations outside of the PRh may have been spared and retrievable following PRh damage.

The present thesis provides new evidence regarding the role of the PRh in anterograde and retrograde memory for objects and places. The findings indicate that the possible functions of the PRh are varied and complex, the neural substrates of anterograde and retrograde memory may not be the same, and careful attention to methodology is crucial in neurobiological research aimed at correlating brain damage with changes in behaviour.

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Appendix A

Additional Behavioural Indices of Water Maze Performance for Chapter 2

A-2.1 Aim of Appendix A

The following sections describe the statistical analysis and interpretation of additional behavioural measures that were collected during the experiments described in Chapter 2. Analysis of swim speed on regular trials, during which the platform was present, and latency to the first platform crossing and number of platform crossings on probe trials, during which the platform was absent, enables the detection of more subtle differences between groups. It is sometimes the case that lesioned rats may be swimming slower or faster than Sham rats resulting in escape latencies that are either overly long or short, respectively. As described in the main text of the thesis, there were no statistically significant differences between the groups on our primary measures. As described below, there was no evidence from our additional measures that could account for the lack of an effect in the PRh rats.

A-2.2 Experiment 1: Anterograde reference memory in the water maze following aspiration lesions of the PRh

A-2.2.1 Water-maze training

A-2.2.1.1 Swim speed. Figure A-1 shows the average swim speed of each group of rats on the 28 acquisition trials. A 2 x 3 x 8 (Lesion x Day x Trial) mixed-factorial ANOVA revealed significant main effects of Day ($F[2,18] = 9.138$, $p = .002$) and Trial ($F[7,63] = 4.062$, $p = .001$). Overall, rats tended to swim faster on Days 1 and 2 and on initial trials. The interaction between Lesion and Trial ($F[7,63] = 4.055$, $p = .001$) and Lesion, Day and Trial ($F[14,126] = 1.861$, $p = .037$) were also statistically significant. Sham rats tended to swim faster on initial trials than on later trials, particularly on Days 1 and 2. PRh rats only showed this pattern on Day 1, and otherwise tended to maintain a constant swim speed across trials. The main effect of Lesion and the remaining interactions were not statistically significant (all $ps > .10$).

A-2.2.1.2 Probe trials. The latency to the first platform crossing and the number of platform crossings on the early and late probe trials on Day 2 are shown in Figure A-2. A 2 x 2 (Lesion x Probe) mixed-factorial ANOVA conducted on the latencies to the first platform crossing revealed a significant main effect of Probe ($F[1,9] = 7.657$, $p = .022$). Overall, rats took longer to make their first platform crossing on the early probe trial than on the late probe trial. The main effect of Lesion and the interaction between Lesion and Probe were not statistically significant ($ps > .10$, see Table 4 in Appendix A). The same ANOVA conducted on the number of platform crossings also revealed a significant main effect of Probe ($F[1,9] = 9.252$, $p = .014$). Overall, rats made more platform crossings on the late probe trial than on the early probe trial. The main effect of Lesion and the interaction between Lesion and Probe were not statistically significant ($ps > .10$).

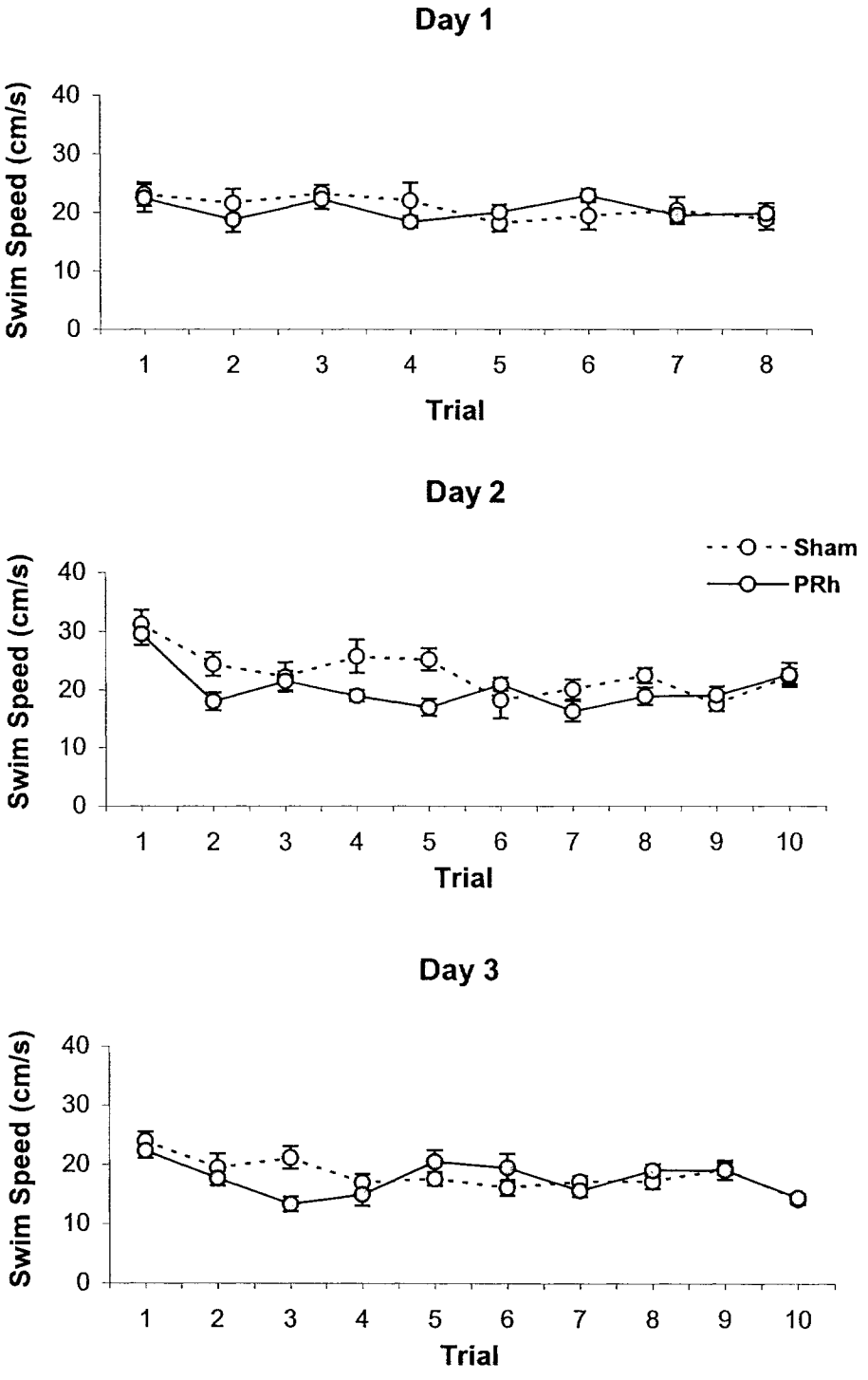

Figure A-1. Mean swim speed on the 28 water-maze acquisition trials, shown by day. The error bars represent S.E.M.

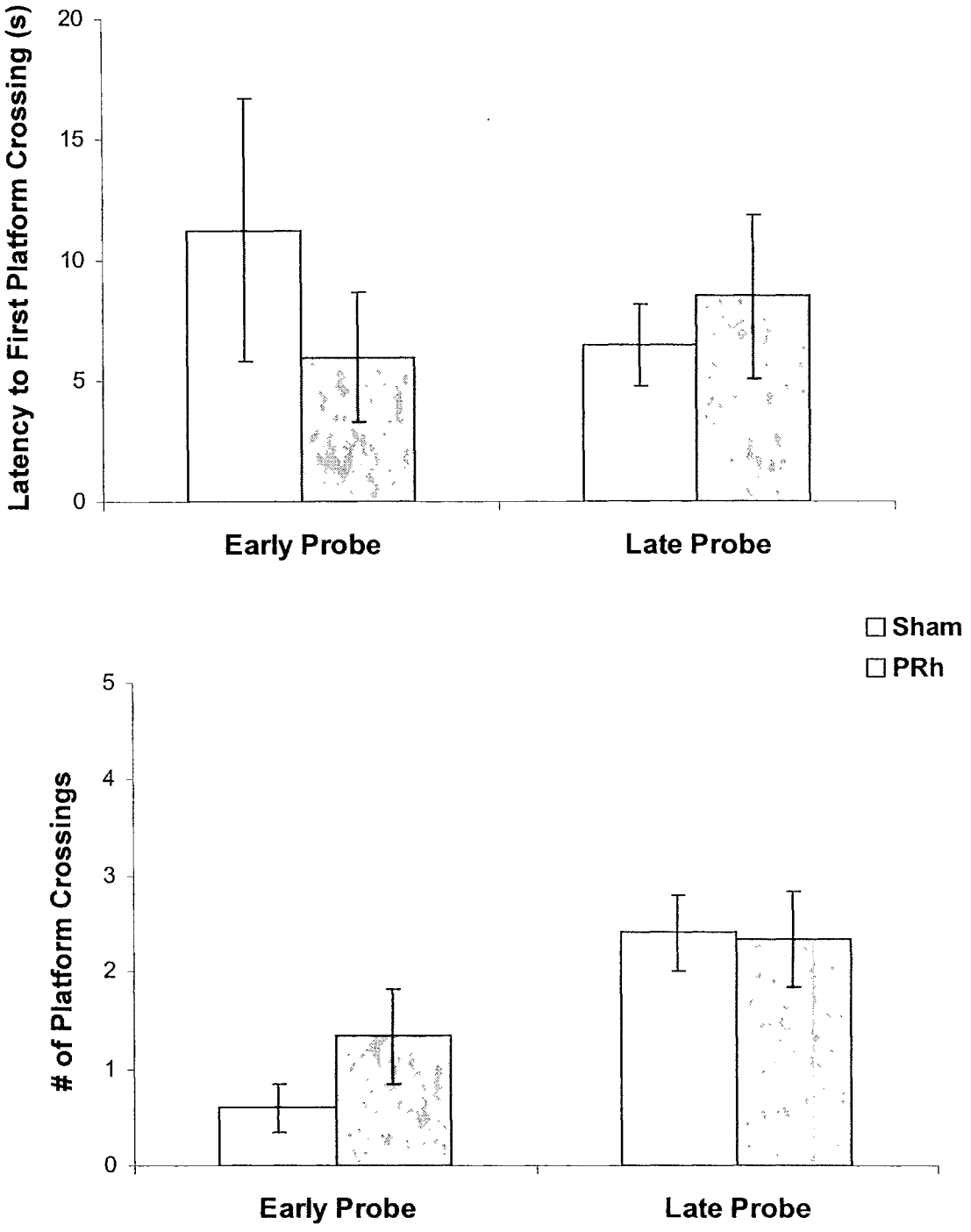

Figure A-2. The top panel shows the mean latency to the first platform crossing of Sham and PRh rats on the early and late probe trials conducted on Day 2. The bottom panel shows the mean number of platform crossings made by Sham and PRh rats on the Day 2 probe trials. The error bars represent S.E.M.

The latency to the first platform crossing and the number of platform crossings on the early and late probe trials on Day 3 are shown in Figure A-3. A 2 x 2 (Lesion x Probe) mixed-factorial ANOVA conducted on the latencies to the first platform crossing did not reveal any significant effects (ps $>.01$, see Table 7 in Appendix A). The same ANOVA conducted on the number of platform crossings showed that the main effect of Probe approached statistical significance ($F[1,9] = 3.824$, $p = .082$), indicating that there were more platform crossings, overall, on the late probe trial than on the early probe trial. The main effect of Lesion and the interaction between Lesion and Probe were not statistically significant (ps $>.10$).

A-2.2.2 Retention testing

A-2.2.2.1 Swim speed. The mean swim speeds of Sham and PRh rats on each trial of the retention test conducted three weeks after training are shown in Figure A-4. A 2 x 10 (Lesion x Trial) mixed-factorial ANOVA conducted on swim speed revealed a significant main effect of Trial ($F[9,81] = 4.19$, $p = .001$). Overall, rats tended to swim faster on initial trials. The main effect of Lesion and the interaction between Lesion and Trial were not statistically significant (ps $>.10$).

A-2.2.2.2 Probe trials. The latency to the first platform crossing and the number of platform crossings on the early and late probe trials of the retention test are shown in Figure A-5. A 2 x 2 (Lesion x Probe) mixed-factorial ANOVA conducted on the latencies to the first platform crossing did not reveal any significant effects (ps $>.01$). The same ANOVA conducted on the number of platform crossings revealed a significant main effect of Probe ($F[1,9] = 18.994$, $p = .002$), indicating that there were more platform crossings on the late probe trial than on the early probe trial. The main effect of Lesion and the interaction between Lesion and Probe were not statistically significant (ps $>.10$).

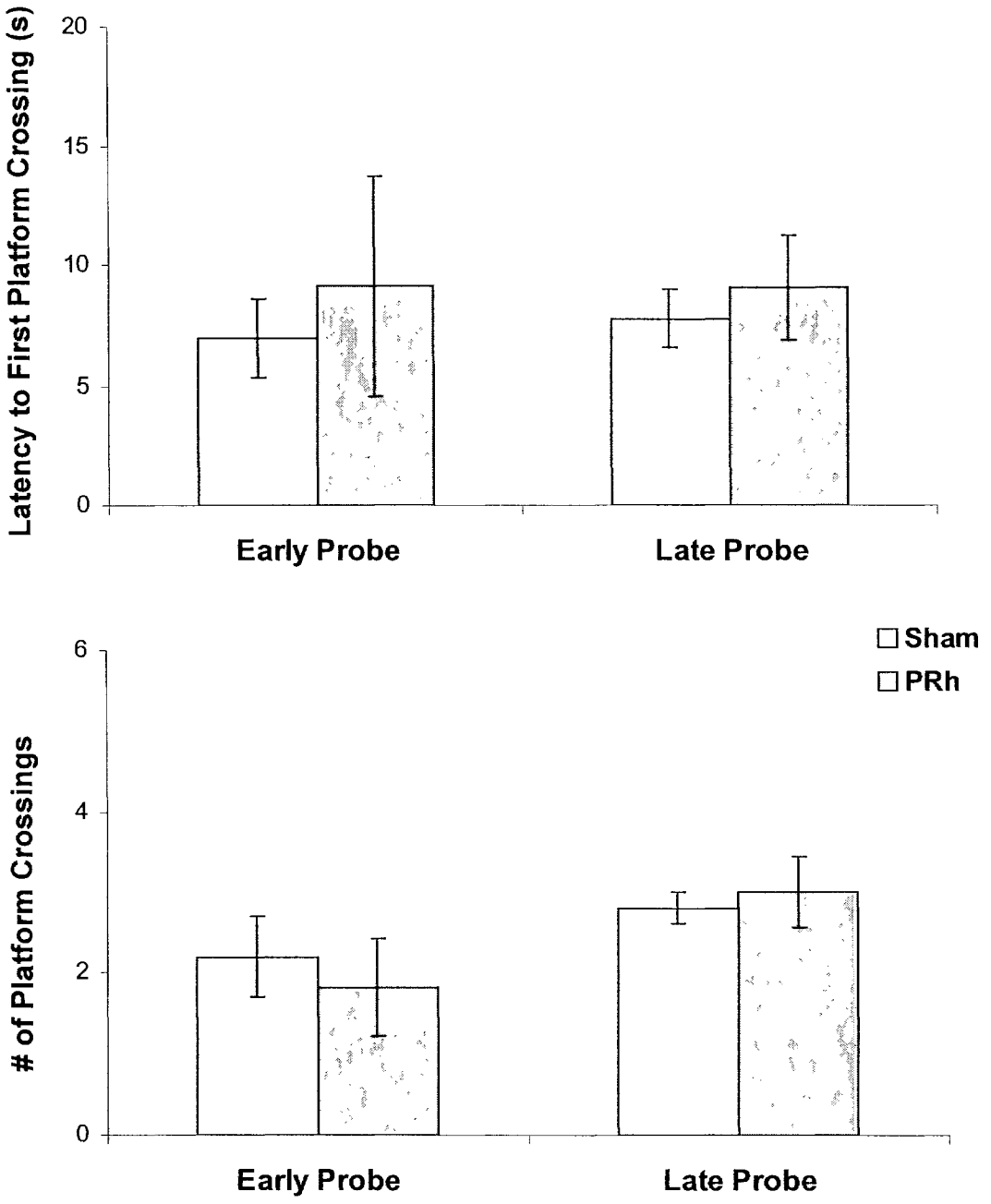

Figure A-3. The top panel shows the mean latency to the first platform crossing of Sham and PRh rats on the early and late probe trials conducted on Day 3. The bottom panel shows the mean number of platform crossings made by Sham and PRh rats on the Day 3 probe trials. The error bars represent S.E.M.

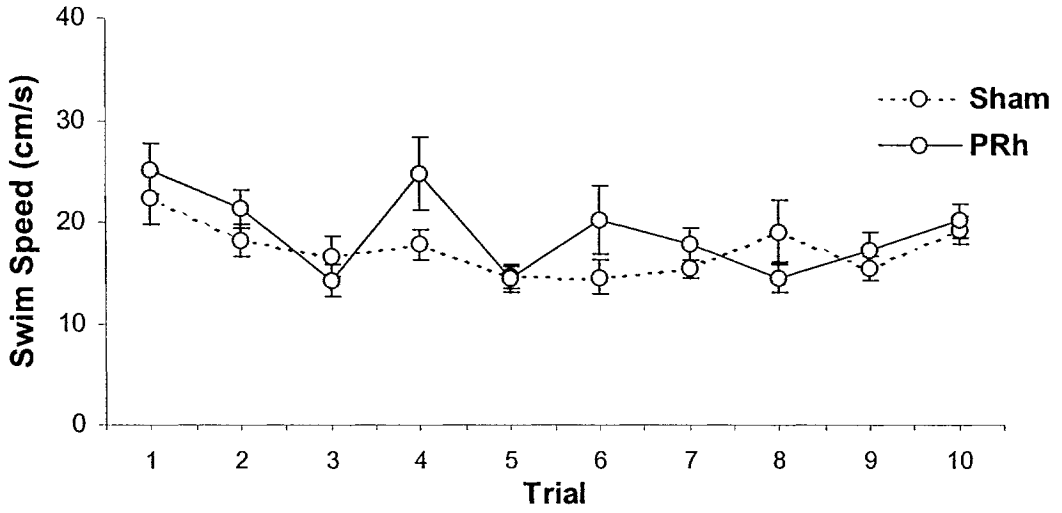

Figure A-4. Mean swim speed of Sham and PRh rats on the 10 trials of the retention test. The error bars represent S.E.M.

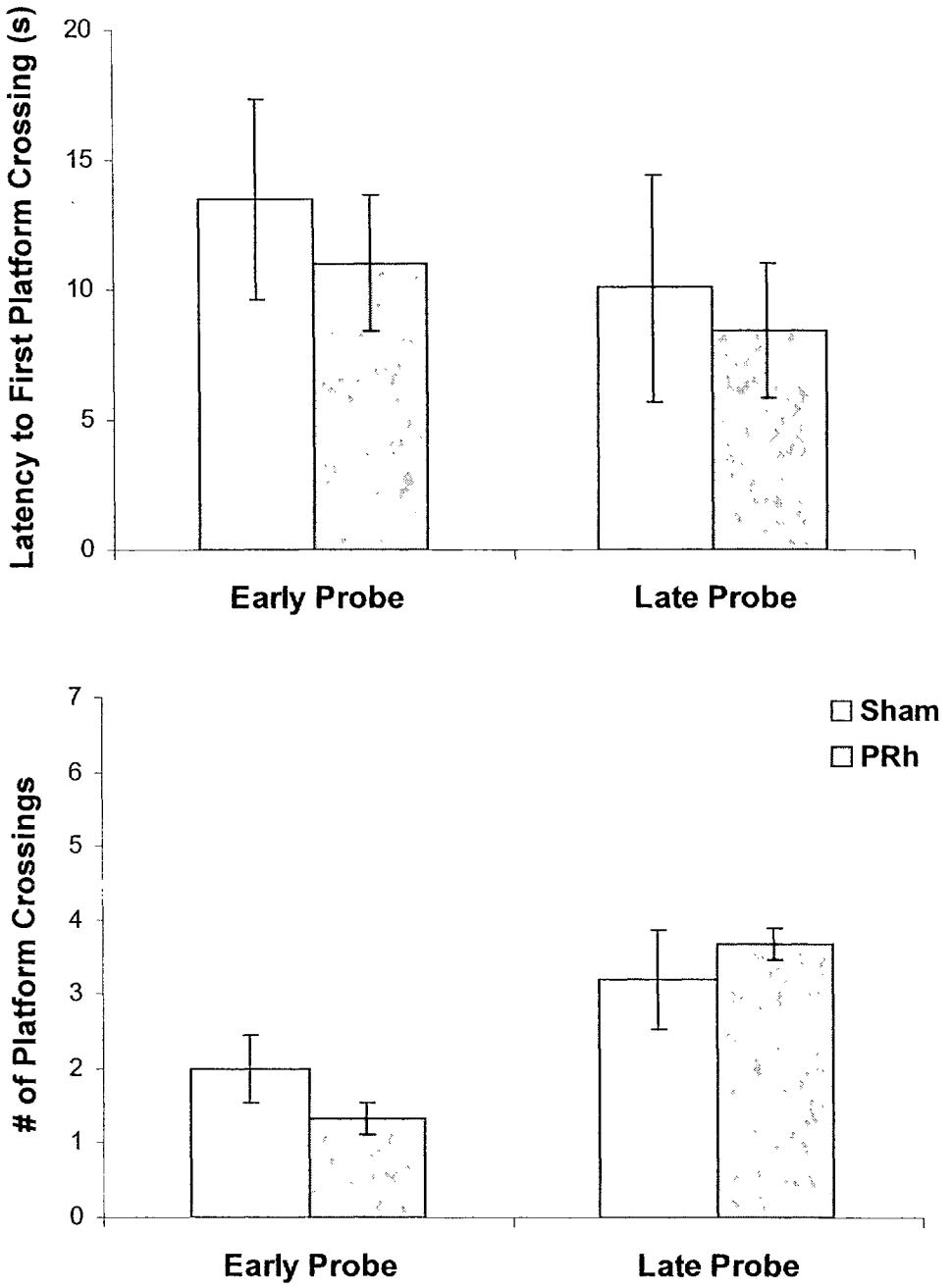

Figure A-5. The top panel shows the mean latency to the first platform crossing of Sham and PRh rats on the early and late probe trials conducted on the retention test. The bottom panel shows the mean number of platform crossings made by Sham and PRh rats on the retention test probe trials. The error bars represent S.E.M.

A-2.2.3 Summary of additional measures from Experiment 1

The main finding of water-maze training, as described in the main text, was that Sham and PRh rats did not differ in their ability to learn the location of a hidden and stationary platform in a water maze. Both groups of rats displayed similar rates of acquisition as determined by their decreasing latencies to find and escape onto the hidden platform on the 24 training trials conducted during the 3 days of training. The additional analysis of swim speed shown here indicates that Sham and PRh rats also swam at similar speeds throughout training. Therefore, it is unlikely that a deficit in the PRh group is being masked by their inability to swim at speeds comparable to that of Sham rats.

On Days 2 and 3 of water-maze training, probe trials, in which the platform was removed from the pool, were conducted at the beginning and end of the session (Trials 1 and 10 and Trials 1 and 8, respectively). The primary dependent measure for probe trials, described in the main text, was the percent time Sham and PRh rats spent in the target quadrant of the maze. There were no statistically significant differences between the groups on this measure for any of the probe trials conducted during training. The present analyses of the additional measures of latency to the first platform crossing and number of platform crossings also indicated that Sham and PRh rats displayed similar behaviour on probe trials.

Analysis of latency on regular trials of the retention test conducted 3 weeks after training, as described in the main text, revealed that Sham and PRh rats did not differ significantly in their ability to remember the platform's location. The additional analysis of swim speed described here also failed to reveal any significant differences between the groups. Also as described in the main text, analysis of time spent in the target quadrants during the early and late probe trials conducted on trials 2 and 10 of the retention test failed to reveal any significant group differences. The present analyses of the additional measures

from probe trials also failed to reveal group differences: Sham and PRh rats did not differ significantly in their latencies to the first platform crossing or in the total number of platform crossings on the early and late probe trials.

The additional measures described here augment the main findings from Experiment 1. Overall, they failed to elucidate subtle group differences that may not have been revealed by examining only latency on regular trials and time spent in the target quadrant on probe trials. Taken together, all the findings from Experiment 1 indicate that PRh rats do not display anterograde amnesia for place information.

A-2.3 Experiment 2: Retrograde place memory following electrolytic lesions of the PRh: Within-subjects design

A-2.3.1 Swim speed during retention testing

The average swim speeds of both groups during the REMOTE and RECENT retention tests are shown in Figure A-6. A 2 x 2 x 16 (Lesion x Time of Learning x Trial) mixed-factorial ANOVA revealed a significant interaction between Time of Learning and Trial ($F[15,210] = 2.663$, $p = .001$). This effect was due to both Sham and PRh rats swimming faster during the middle portion (Trials 8-10) of the RECENT retention test. This pattern was also evident in Sham rats on the REMOTE problem, but only on Trial 6; PRh rats displayed consistent swim speeds during the REMOTE retention test. The main effect of Lesion approached statistical significance ($F[1,14] = 13.293$ $p = .091$), consistent with a tendency for PRh rats to swim faster than Sham rats, overall. No other main effects or interactions were statistically significant ($ps > .10$).

A-2.3.2 Probe trials

Figure A-7 shows the average latency of Sham and PRh rats to make their first platform crossing on the early and late probe trials during the REMOTE and RECENT retention tests. A 2 x 2 x 2 (Lesion x Time of Learning x Probe) mixed-factorial ANOVA revealed only a significant main effect of Probe ($F[1,14] = 15.379$, $p = .002$). Overall, rats took longer to make their first platform crossing on the early probe trials, compared to the late probe trials. The remaining main effects and all interactions were not statistically significant ($ps > .10$), however, PRh rats took significantly longer than Sham rats to make their first platform crossing on the early probe trial of the RECENT retention test ($t[14] = -2.806$, $p = .007$).

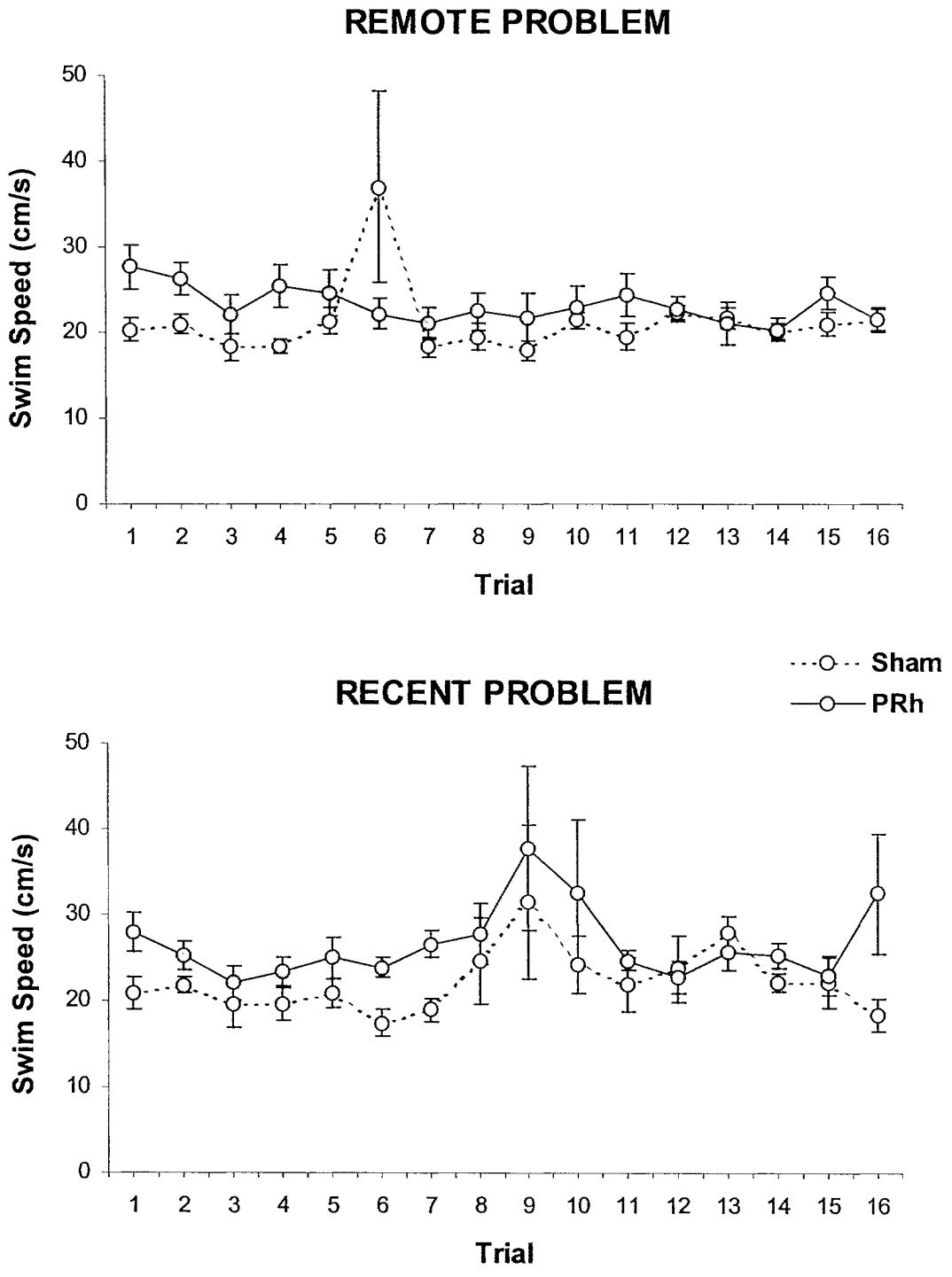

Figure A-6. Mean swim speed of Sham and PRh rats during retention and reacquisition testing on the REMOTE and RECENT place problems. The error bars represent S.E.M.

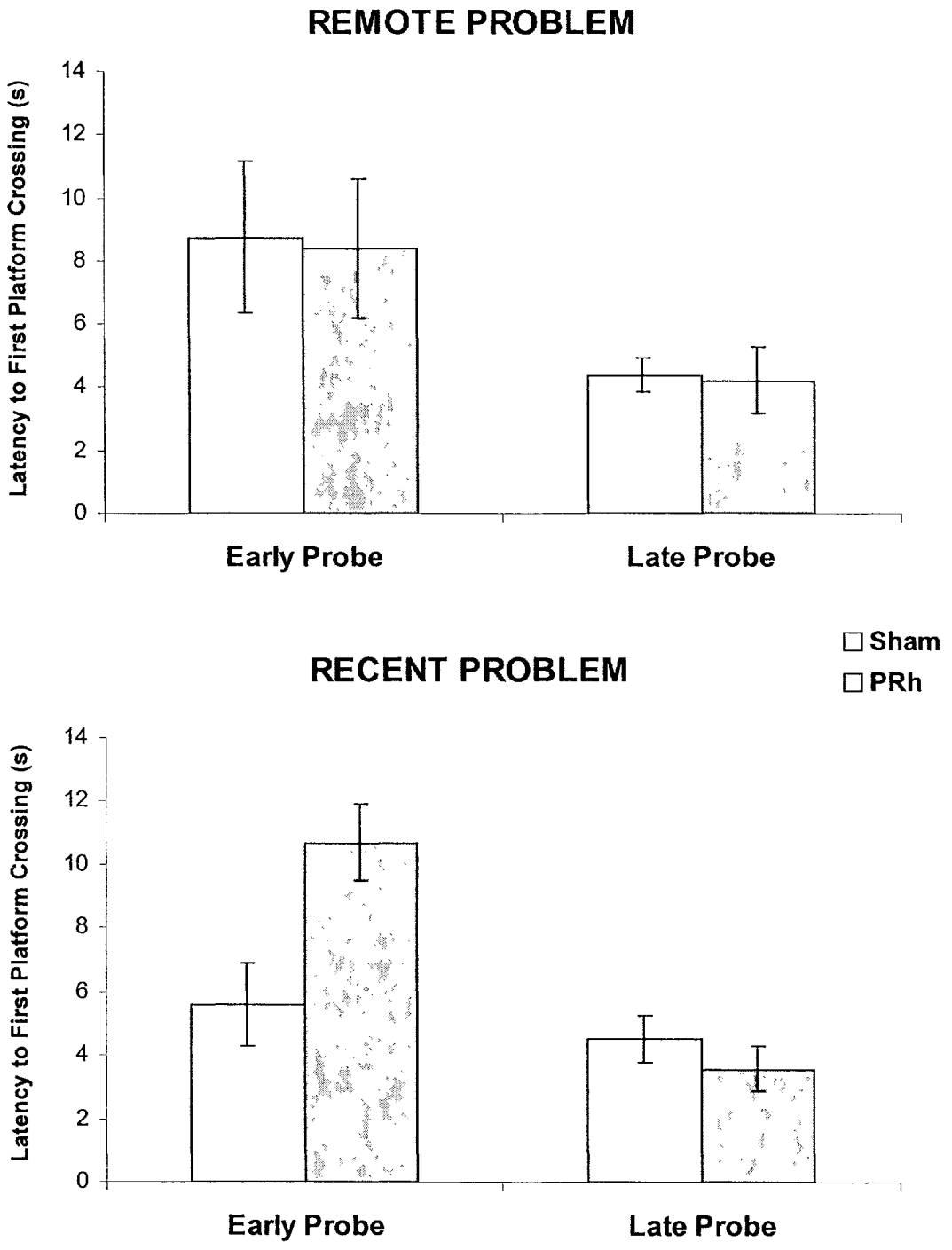

Figure A-7. Mean latency of Sham and PRh rats to make their first platform crossing on the early and late probe trials of the REMOTE and RECENT retention tests. The error bars represent S.E.M.

Figure A-8 shows the average number of platform crossings made by Sham and PRh rats on the early and late probe trials during the REMOTE and RECENT retention tests. A 2 x 2 x 2 (Lesion x Time of Learning x Probe) mixed-factorial ANOVA revealed a significant main effect of Probe ($F[1,14] = 31.263$, $p = .001$) and a significant interaction between Lesion and Probe ($F[1,14] = 8.77$, $p = .010$). Overall, rats made more platform crossings on the late probe trials compared to the early probe trials. Sham and PRh rats did not differ significantly in the number of platform crossings they made on the early probe trial of the REMOTE retention test. However, on the early probe trial of the RECENT retention test, PRh rats made significantly fewer platform crossings than Sham rats ($t[14] = 2.012$, $p = .032$). Conversely, on both late probe trials, PRh rats tended to make more platform crossings than Sham rats (REMOTE: $t[14] = -1.623$, $p = .064$; RECENT: $t[14] = -1.729$, $p = .053$). No other main effects or interactions were statistically significant ($ps > .10$).

A-2.3.3 Summary of additional measures from Experiment 2

The primary analysis of the escape latencies of Sham and PRh rats during the retention tests for the water maze problems learned 4 weeks (REMOTE) and during the week of (RECENT) surgery revealed that they were, overall, not statistically different. However, the present analysis of swim speed during the two retention tests revealed that PRh rats tended to swim faster than Sham rats. Thus, the faster swim speed of PRh rats may enable them to reach the platform nearly as quickly as Sham rats when in fact they are unable to pinpoint its location as well as Sham rats. Additionally, during the RECENT retention test, PRh rats had slightly longer latencies on initial trials. As is evident in Figure A-6, PRh rats were swimming faster on those trials and it can therefore be argued that if swim speeds were comparable PRh rats may have taken much longer to find the hidden platform relative to Sham rats. Alternatively, it is possible that nonspecific lesion effects such as increased activity or

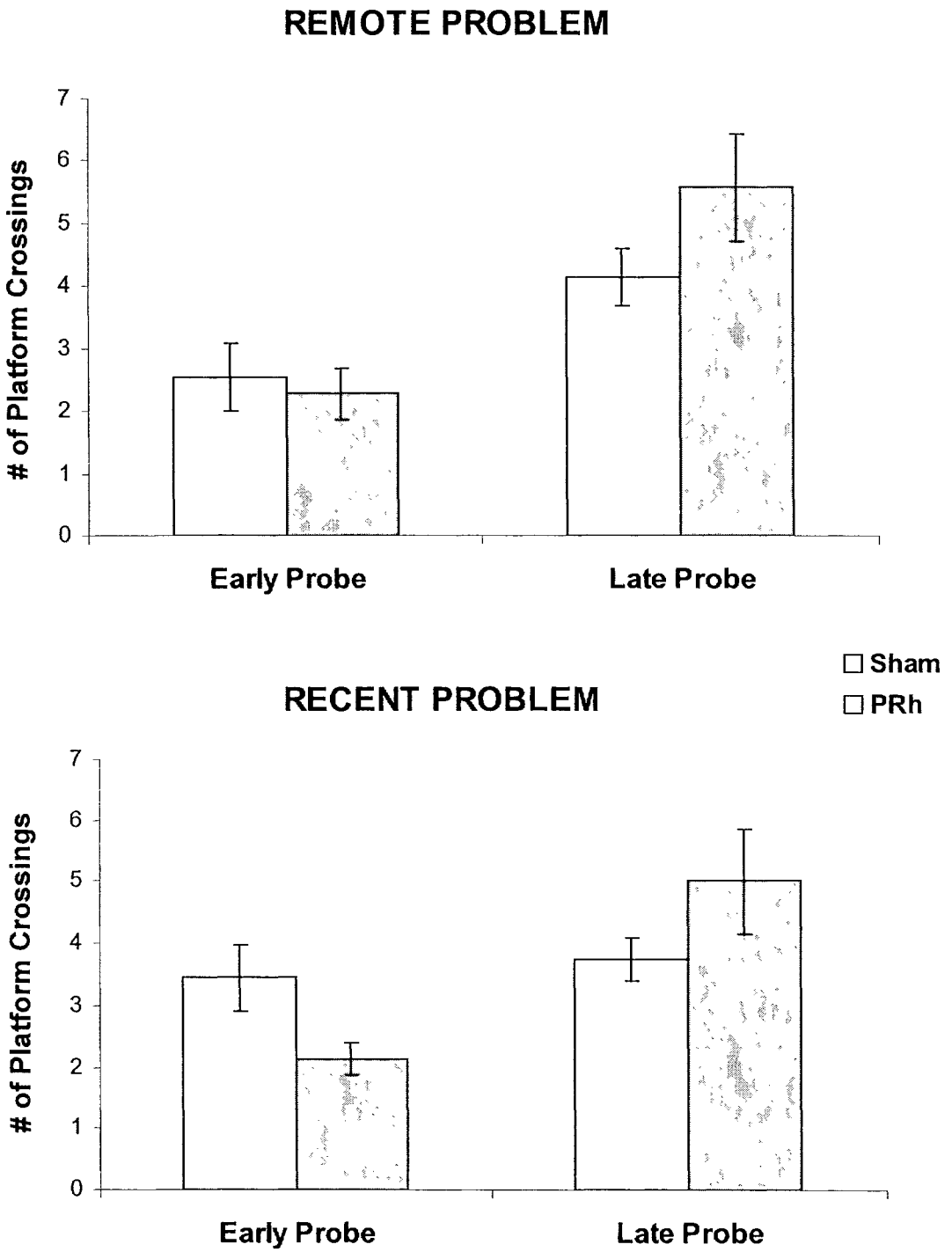

Figure A-8. Mean number of platform crossings made by Sham and PRh ratson the early and late probe trials of the REMOTE and RECENT retention tests. The error bars represent S.E.M.

excitability underlies their increased swimming speed and also interferes with their ability to locate the platform, independent of their knowledge of its location.

The main finding from Experiment 2, as described in the main text, was that PRh rats did not show a preference for the target quadrant on the early probe trial of both the REMOTE and RECENT retention tests. The analyses of the additional dependent measures from the early and late probe trials conducted during each of the retention tests indicated that PRh rats took longer to make their first platform location and made fewer platform crossings than Sham rats during the early probe trial of the RECENT retention test. Thus, when only percent time spent in the target quadrant on probe trials was considered PRh rats appeared to have retrograde amnesia, without a temporal gradient, for the water maze problems learned before surgery. However, when the present analyses are considered it appears that PRh rats are impaired on more of the measures pertaining to the RECENT problem. Thus, the retrograde amnesia may be temporally graded.

When all the analyses conducted on data from the REMOTE and RECENT retention tests are considered together they provide a more comprehensive view of the pattern of memory loss following PRh lesions: 1) PRh rats tend to take longer than Sham rats to locate the hidden platform on initial trials of the RECENT problem, despite swimming faster than Sham rats, 2) they take significantly longer than Sham rats to make their first platform crossing on the early probe of the RECENT, but not the REMOTE retention test, 3) they make fewer platform crossings during the early probe of the RECENT, but not the REMOTE retention test, and 4) they do not show a preference for the target quadrant on the early probe of the RECENT retention test, though this is also the case during the early probe of the REMOTE retention test. Thus, PRh rats displayed retrograde amnesia and there is evidence of a temporal gradient.

A-2.4 Experiment 3: Retrograde memory for places following aspiration lesions of the PRh: Within-subjects design

A-2.4.1 Swim speed during retention testing

Figure A-9 shows the average swim speed of both groups on each trial of the REMOTE and RECENT retention tests. A 2 x 2 x 16 (Lesion x Time of Learning x Trial) mixed factorial ANOVA revealed only a significant main effect of Trial ($F[15,225] = 5.619$, $p = .001$); all other main effects and all interactions were not statistically significant ($ps > .10$). Swim speed tended to fluctuate over trials, but did so similarly for both groups.

A-2.4.2 Probe trials

Figure A-10 shows the latency for Sham and PRh to make their first platform crossing on the early and late probe trials of the REMOTE and RECENT retention tests. A 2 x 2 x 2 (Lesion x Time of Learning x Probe) mixed factorial ANOVA revealed a significant main effect of Probe ($F[1,15] = 20.668$, $p = .001$) and a significant interaction between Time of Learning and Probe ($F[1,15] = 4.396$, $p = .053$). Overall, rats took longer to make their first platform crossing on the early probe trial compared to the late probe trial. Additionally, rats took longer to make their first platform crossing on the early probe trial, relative to the late probe trial, during the REMOTE retention test; this was less true during the RECENT retention test. All other main effects and interactions were not statistically significant ($ps > .10$).

Figure A-11 shows the number of platform crossings made by Sham and PRh rats on the early and late probe trials of the REMOTE and RECENT retention tests. A 2 x 2 x 2 (Lesion x Time of Learning x Probe) mixed factorial ANOVA revealed a significant main effect of Lesion ($F[1,15] = 11.243$, $p = .004$), Probe ($F[1,15] = 5.370$, $p = .035$), and a significant interaction between Lesion and Probe ($F[1,15] = 4.355$, $p = .054$). Overall, PRh

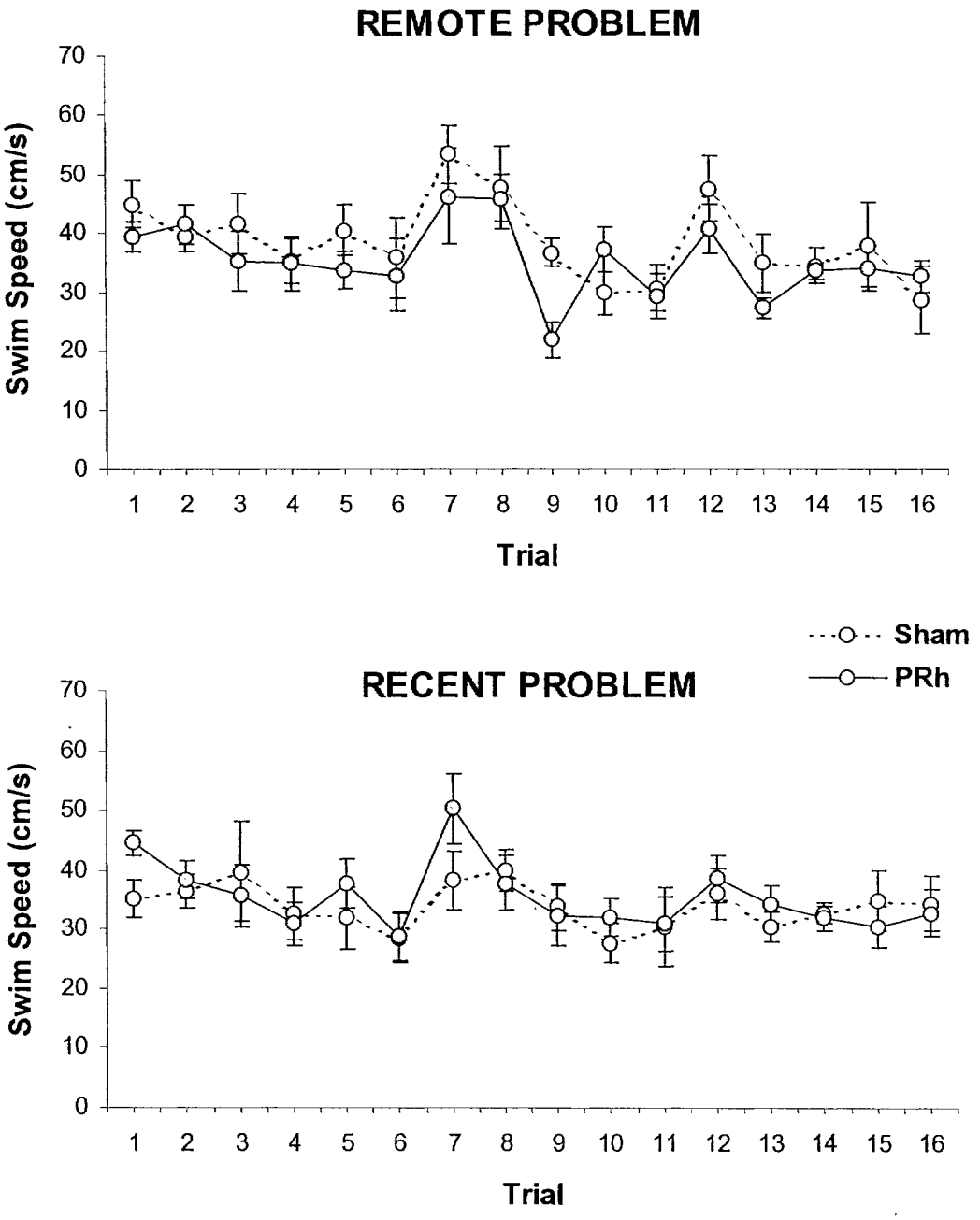

Figure A-9. Mean swim speed of Sham and PRh rats during retention and reacquisition testing on the REMOTE and RECENT place problems. The error bars represent S.E.M.

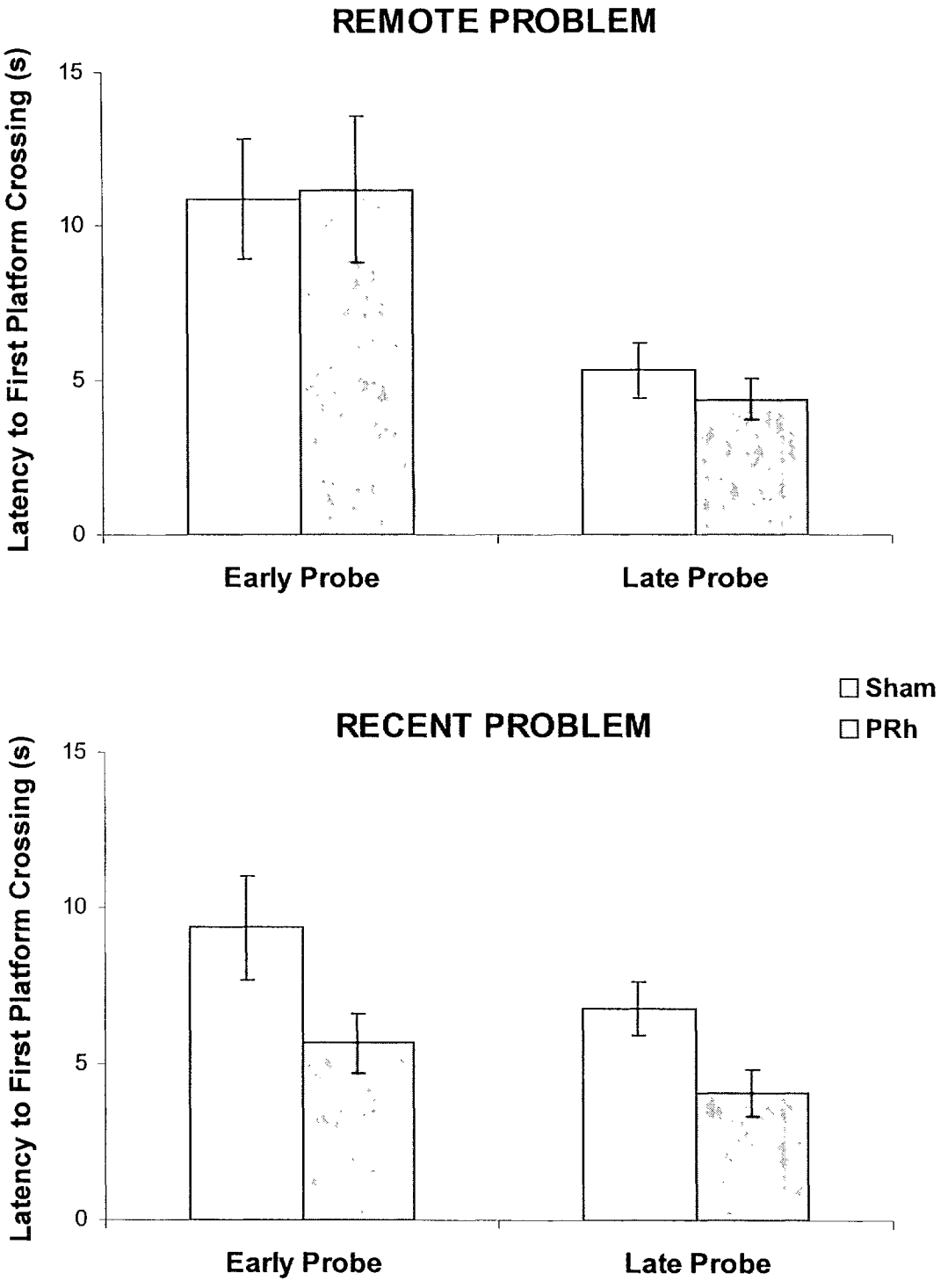

Figure A-10. Mean latency of Sham and PRh rats to make their first platform crossing on the early and late probe trials of the REMOTE and RECENT retention tests. The error bars represent S.E.M.

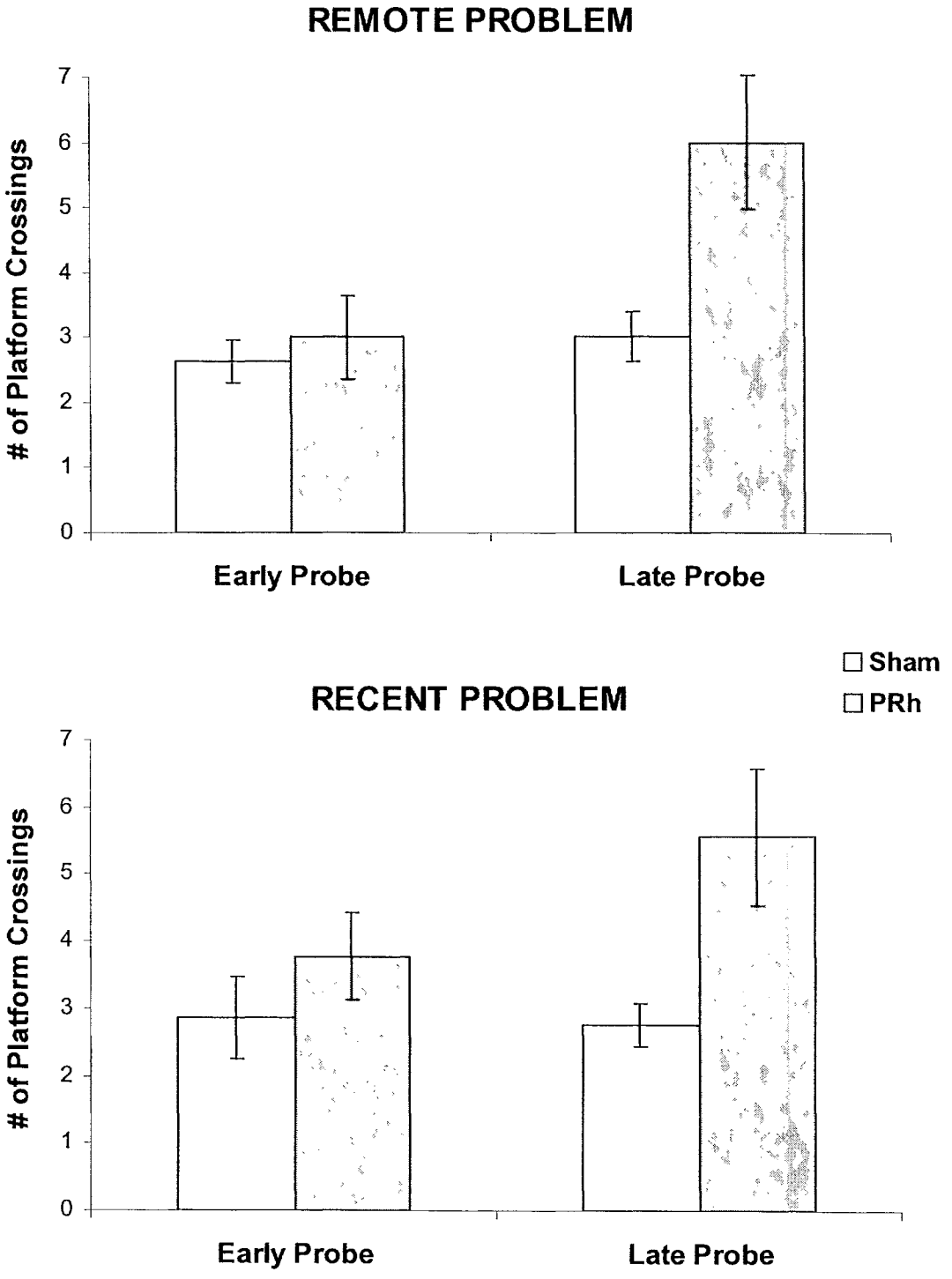

Figure A-11. Mean number of platform crossings made by Sham and PRh ratson the early and late probe trials of the REMOTE and RECENT retention tests. The error bars represent S.E.M.

rats made more platform crossings than Sham rats; more specifically, PRh rats made more platform crossings than Sham rats on late probe trials (REMOTE: $t[15] = -2.609$, $p = .020$; RECENT: $t[15] = -2.504$, $p = .024$), but the groups were not significantly different on early probe trials. Consequently, there were more platform crossings, overall, on late probe trials than on early probe trials. All other main effects and interactions were not statistically significant.

A-2.43 Summary of additional measures from Experiment 3

In this Experiment, PRh lesions failed to produce retrograde amnesia for places. No group differences in escape latency during regular trials of the REMOTE and RECENT retention test were observed, nor did the groups differ in the percent time spent in the target quadrant on probe trials. The present analysis revealed that swim speed was comparable for Sham and PRh rats and this variable is therefore unable to account for the lack of group differences in latencies. In addition, the additional measures from the probe trials showed that PRh rats performed better than Sham rats. PRh rats made their first platform crossing quicker than Sham rats on the early and late probe trials of the RECENT retention test and also made more platform crossings on the late probe trial of that test. Thus, these additional analyses augment the findings from the primary dependent measures and clearly indicate that aspiration lesions of the PRh did not produce retrograde amnesia for the water maze problems learned before surgery.

A-2.5 Experiment 4: Retrograde memory for places following aspiration lesions of the PRh: Between-subjects design

A-2.5.1 Swim speed during retention testing

Figure A-12 shows the average swim speed of each of the 4 groups. A 2 x 2 x 15 (Lesion x Time of Learning x Trial) revealed only a significant interaction between Lesion and Trial ($F[14,238] = 1.962$, $p = .021$). As is evident from the figure, this was due to differences between the groups that learned the REMOTE problem: Sham rats tended to swim faster than PRh rats during the middle portion of that retention test. None of the main effects and no other interactions were statistically significant (ps $>.10$).

A-2.5.2 Probe trials

Figure A-13 shows the average time it took each group to make their first platform crossing on the early and late probe trials during retention testing. A 2 x 2 x 2 (Lesion x Time of Learning x Probe) mixed factorial ANOVA revealed only a significant main effect of Probe ($F[1,17] = 7.281$, $p = .015$). Overall, rats took longer to make their first platform crossing on the early probe trial than on the late probe trial. No other main effects and none of the interactions were statistically significant (ps $>.10$).

Figure A-14 shows the average number of platform crossings made by each group on the early and late probe trials. A 2 x 2 x 2 (Lesion x Time of Learning x Probe) mixed factorial ANOVA also revealed a significant main effect Probe ($F[1,17] = 13.024$, $p = .002$). Overall, rats made more platform crossings on the late probe trial than on the early probe trial. No other main effects and none of the interactions were statistically significant (ps $>$ .10).

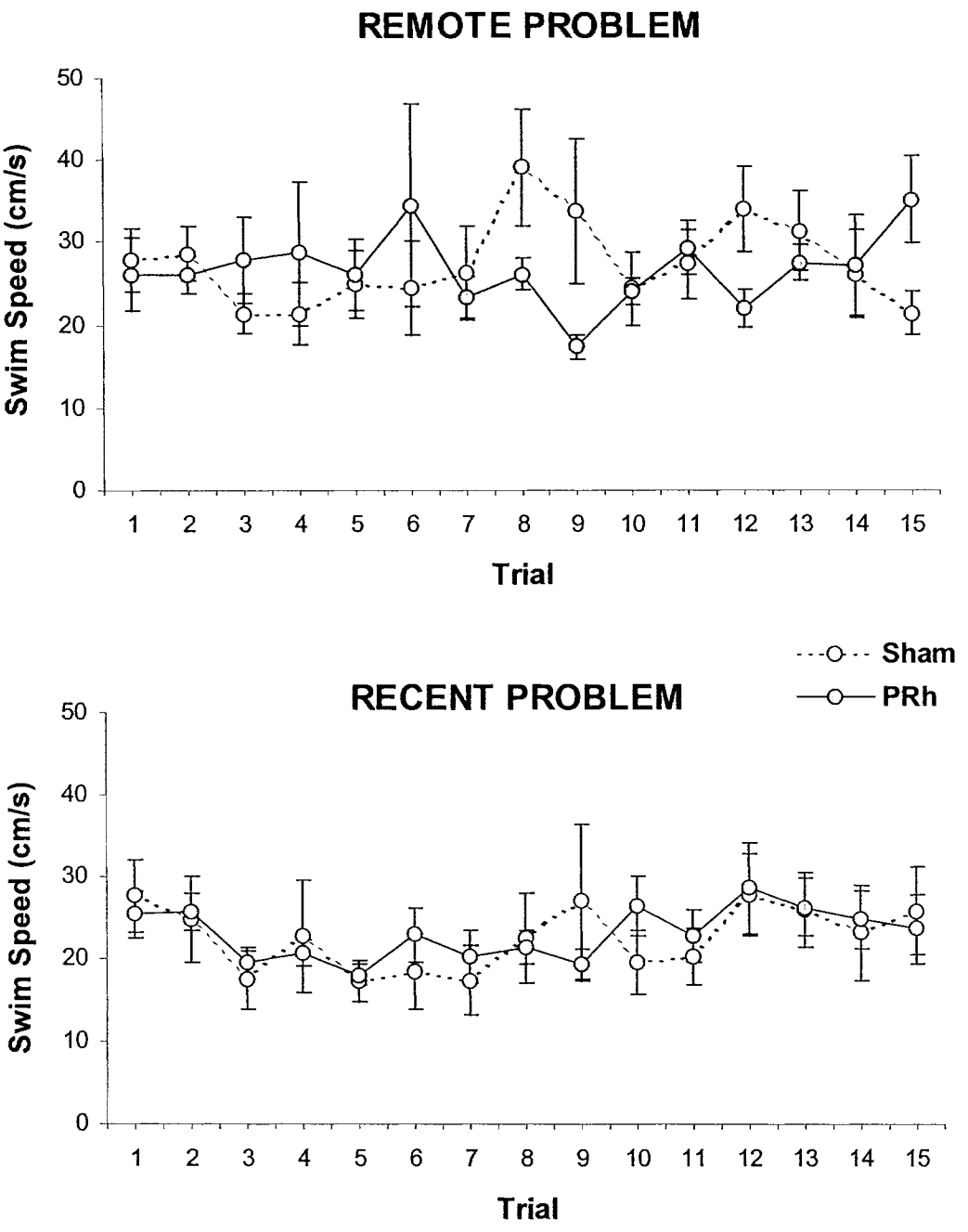

Figure A-12. Mean swim speed during the retention tests for the Sham and PRh rats that learned either the REMOTE or RECENT problem. The error bars represent S.E.M.

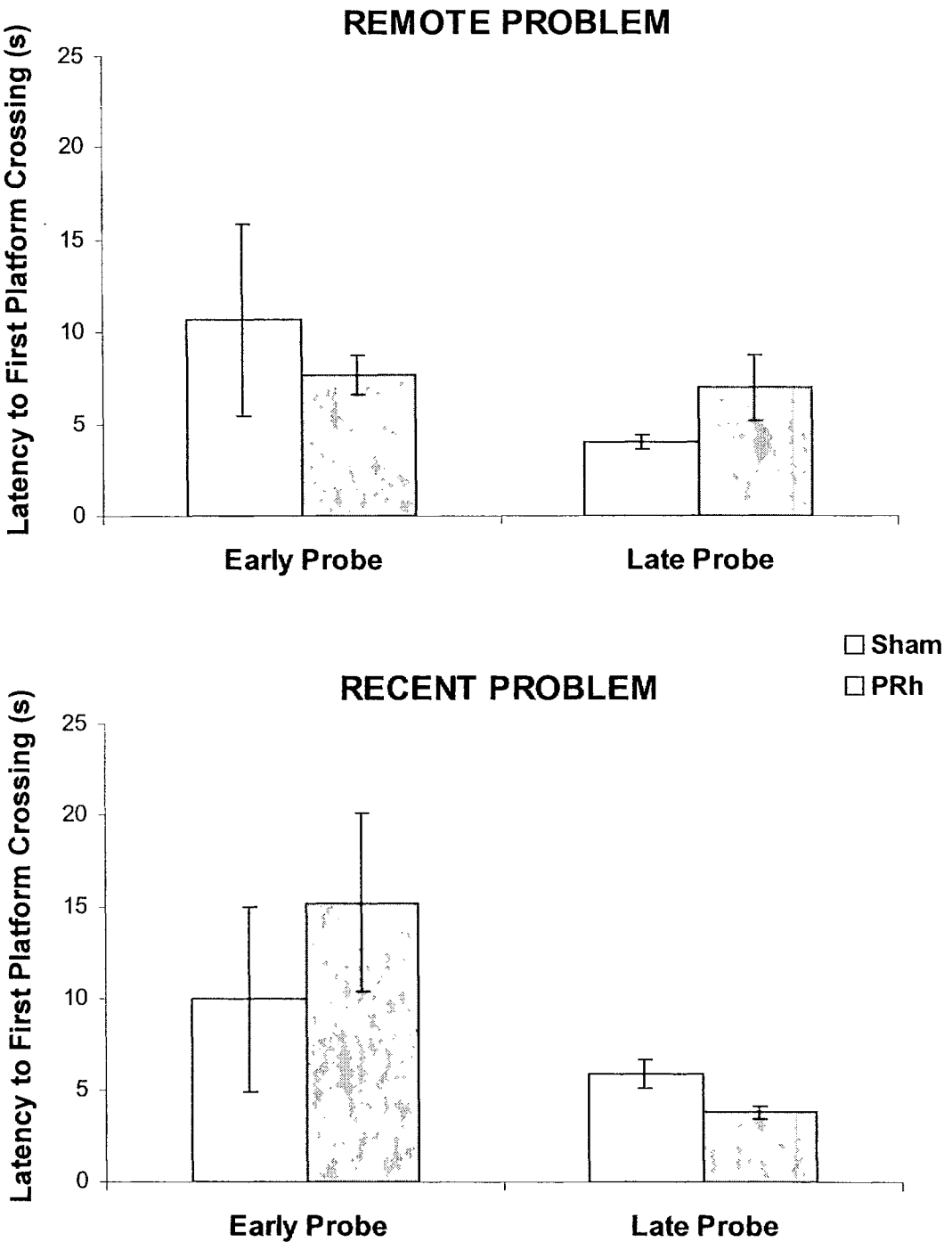

Figure A-13. The mean latency to the first platform crossing of Sham and PRh rats that learned the REMOTE or RECENT problem during the early and late probe trials of the retention test. The error bars represent S.E.M.

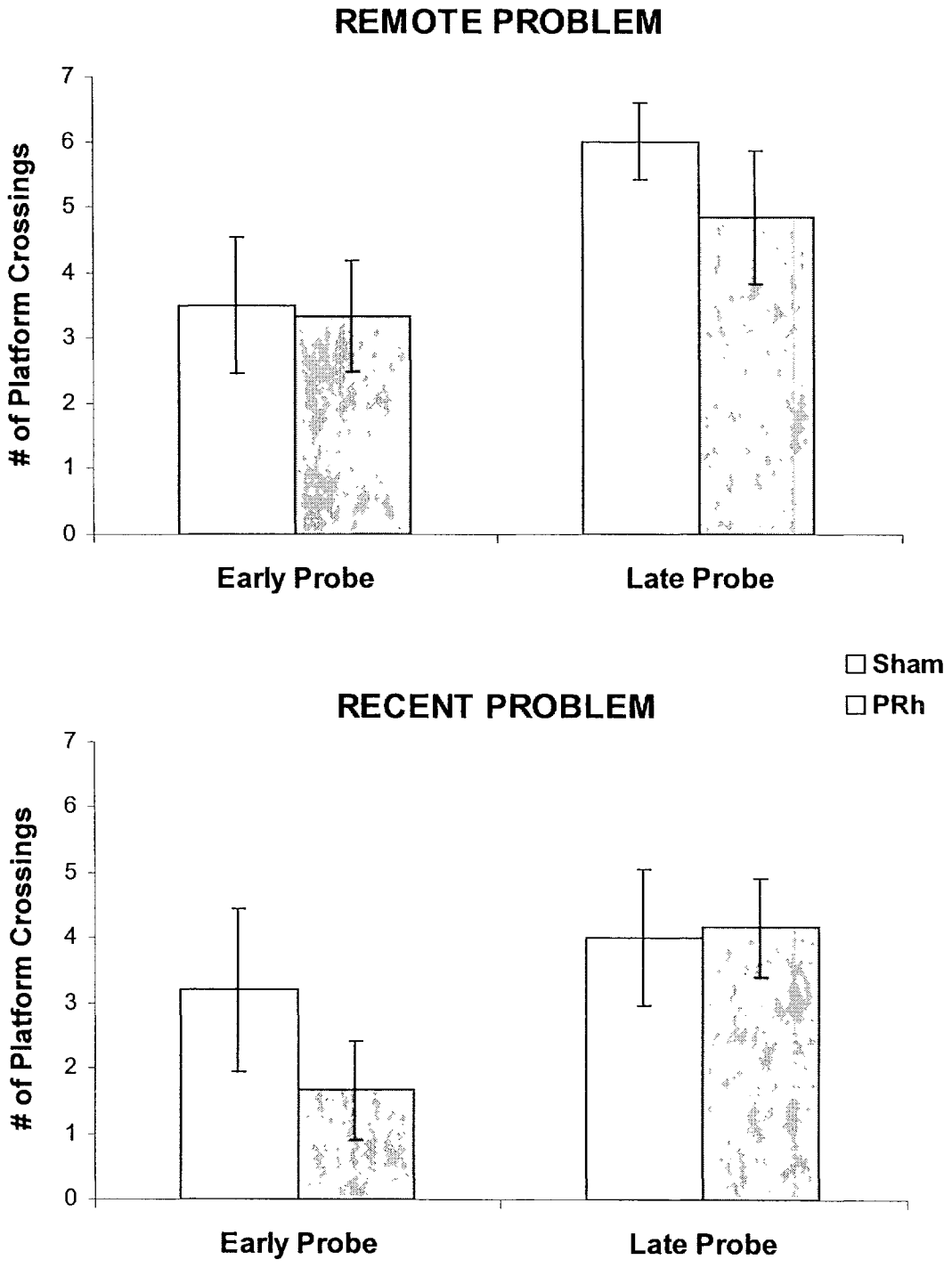

Figure A-14. The mean number of platform crossings made by Sham and PRh rats that learned the REMOTE or RECENT problem during the early and late probe trials of the retention test. The error bars represent S.E.M.

A-2.5.3 Summary of additional measures from Experiment 4

Based on the primary dependent measures of this experiment, PRh rats that learned a water maze problem during the week of surgery displayed longer latencies than Sham rats on the first trial of the retention test and, unlike Sham rats, did not show a preference for the target quadrant on the early probe trial. Conversely, PRh rats that learned a water maze problem 4 weeks before surgery did not differ from Sham rats. The present analysis of additional measures did not reveal group differences in swim speed. Therefore, the difference in escape latency between PRh and Sham rats that learned the RECENT problem cannot be accounted for by differences in swim speed. Additionally, analyses of latency to the first platform crossing and number of platform crossings on probe trials revealed that PRh rats that learned the RECENT problem showed a slight and statistically nonsignificant tendency to reach the platform and made significantly fewer platform crossins on the early probe trial, relative to Sham rats that learned the RECENT problem. These latter findings are consistent with results from the primary dependent measures and support the interpretation that aspiration lesions of the PRh produced a temporally graded retrograde amnesia when a between-subjects design was used.

Appendix B

Additional Behavioural Indices of Object Exploration for Chapter 3

B-3.1 Aim of Appendix B

The primary purpose of this Appendix is to summarize the general patterns of object exploration displayed by the Sham and PRh rats from the experiments described in Chapter 3. In that Chapter several versions of the object version of NPT were used to evaluate the ability of rats with PRh lesions to learn and remember information about objects. Our primary dependent measure in all the experiments described in Chapter 3 was the exploration ratio calculated based on the time spent exploring the novel and sample objects during the first 2 minutes of the test session. Described below are analyses of the total amounts of time rats spent exploring objects during both the sample and test sessions.

Time spent exploring objects during the sample session bears importantly on subsequent discrimination, particularly when anterograde memory was assessed. Nonspecific lesion effects may lead to abnormal patterns of exploration that may underlie inadequate discrimination during the test session. If PRh rats spend less time than Sham rats exploring objects during the sample session, they may spend equivalent amounts of time exploring the novel and sample objects during the test session. Similarly, if PRh rats are less or more inclined than Sham rats to explore objects overall, novelty preference may not be revealed. Thus, it is important to determine whether there are significant differences in object exploration. The analyses described below confirm that PRh lesions did not alter exploratory behaviour.

B-3.2 Experiment 5: Anterograde memory for objects following lesions of the PRh

B-3.2.1 Anterograde object memory in rats with aspiration lesions of the PRh

Figure B-1 shows the total amount of time Sham and PRh rats spent exploring the sample objects during the total 5 minutes of the sample session. An independent samples t-test revealed that the groups were not significantly different ($t[32] = .255$, $p = .80$).

Figure B-2 shows the total amount of time Sham and PRh rats spent exploring objects during the first 2 minutes of the test session. This was the portion of the test that was analyzed for object preference. An independent samples t-test revealed that the groups were not significantly different ($t[32] = .302$, $p = .765$).

To identify whether there were differences between Sham and PRh rats in the pattern of exploration through the sample and test sessions, the time spent exploring each sample object during each minute of the sample session and the time spent exploring the novel and sample objects during each minute of the test session were examined. To achieve a symmetrical comparison between the two sessions, only the rats that received a 5-minute test session were included in this analysis (Sham n=9; PRh n=9). Figure B-3 shows the amount of time Sham and PRh rats spent exploring each object during each minute of the sample and test sessions. A 2 x 2 x 5 x 2 (Lesion x Session x Minute x Object) mixed factorial ANOVA failed to reveal any meaningful effects or group differences (all $ps > .05$). However, as is evident from Figure B-3 that there were some subtle, though not statistically significant, differences between Sham and PRh rats during the test session. In the first minute, both groups spend more time with the novel object. In the second minute, Sham rats spend more time with the sample object, whereas PRh rats spend similar amounts of

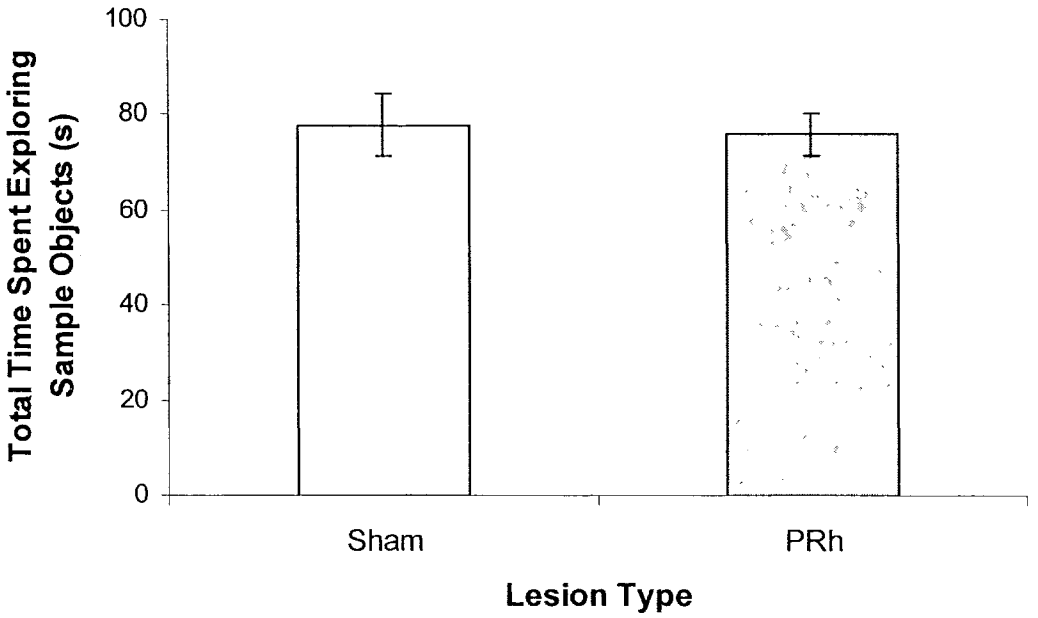

Figure B-1. The mean total time Sham and PRh rats spent exploring objects during the 5-minute sample session. The error bars represent S.E.M.

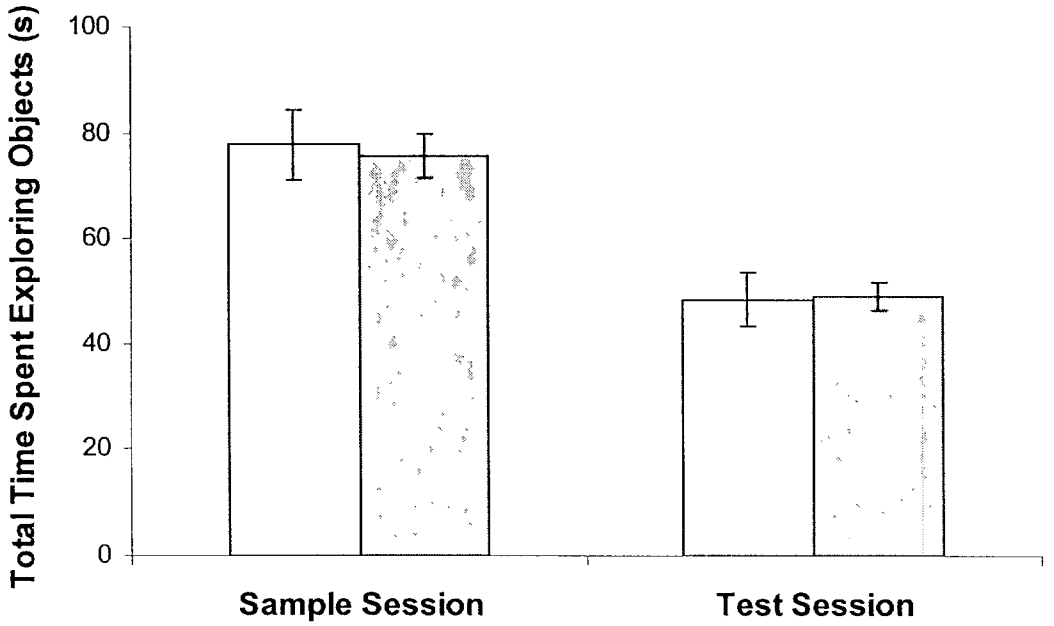

Figure B-2. The mean total time Sham and PRh rats spent exploring objects during the first 2 minutes of the retention test. The error bars represent S.E.M.

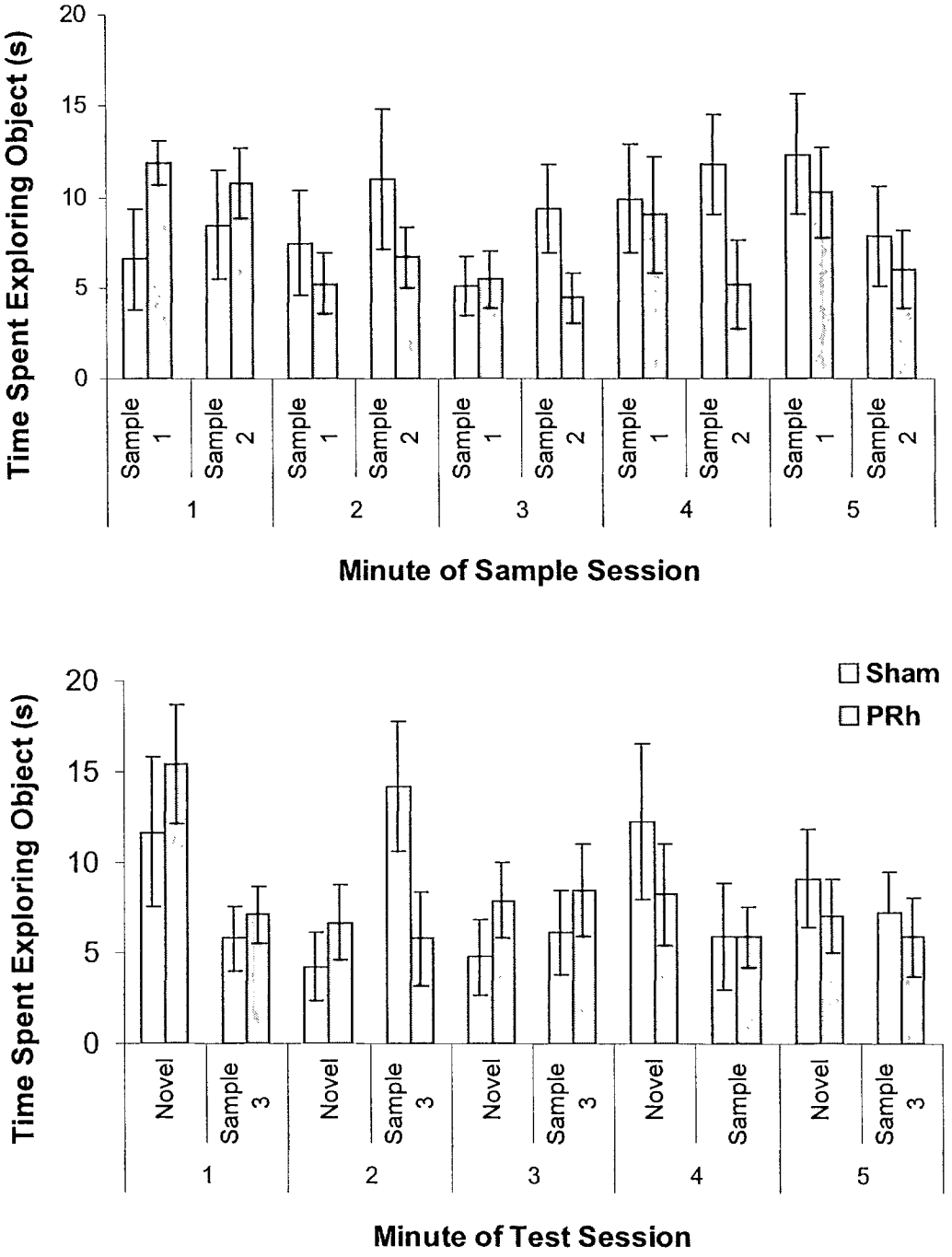

Figure B-3. The mean time Sham and PRh rats spent exploring the sample objects and the sample and novel objects during each minute of the sample and test sessions, respectively. The error bars represent S.E.M.

time with both objects. This analysis also demonstrated that both groups of rats tended to display maximum discrimination during the first minute of the retention test. The slightly lower exploration ratios observed for Sham rats (see Figure 23) reflects their tendency to prefer the novel object in the first minute and the sample object in the second minute; overall, however, they still show a significant preference for the novel object.

B-3.2.2 Anterograde object memory in rats with either electrolytic or aspiration lesions of the PRh

Figure B-4 shows the total amount of time that Sham rats and rats with electrolytic or aspiration PRh lesions spent exploring the sample objects during the 5-minute sample session. This figure separates the Sham group into those rats tested at the same time as the aspiration group (Sham-Asp) and those rats tested at the same time as the electrolytic group (Sham-Elec). As can be seen in the figure, the Sham-Asp and PRh-Asp rats spent more time exploring objects than the Sham-Elec and PRh-Elec rats. However, both PRh groups spent comparable amounts of time exploring objects compared to their respective Sham group.

Figure B-5 shows the total amount of time Sham rats and rats with electrolytic or aspiration PRh lesions spent exploring objects during the first 2 minutes of the retention test. In this case, all rats spent similar amounts of time exploring objects. A one-way, between-subjects ANOVA conducted on these data showed that the groups were not significantly different ($F < 1$).

B-3.2.3 Anterograde object memory in rats with aspiration lesions of the PRh: Limited exposure to the sample objects

In this portion of Experiment 5, the sample session was terminated once rats had accumulated a total of 20 seconds exploring both objects. The amount of time required for

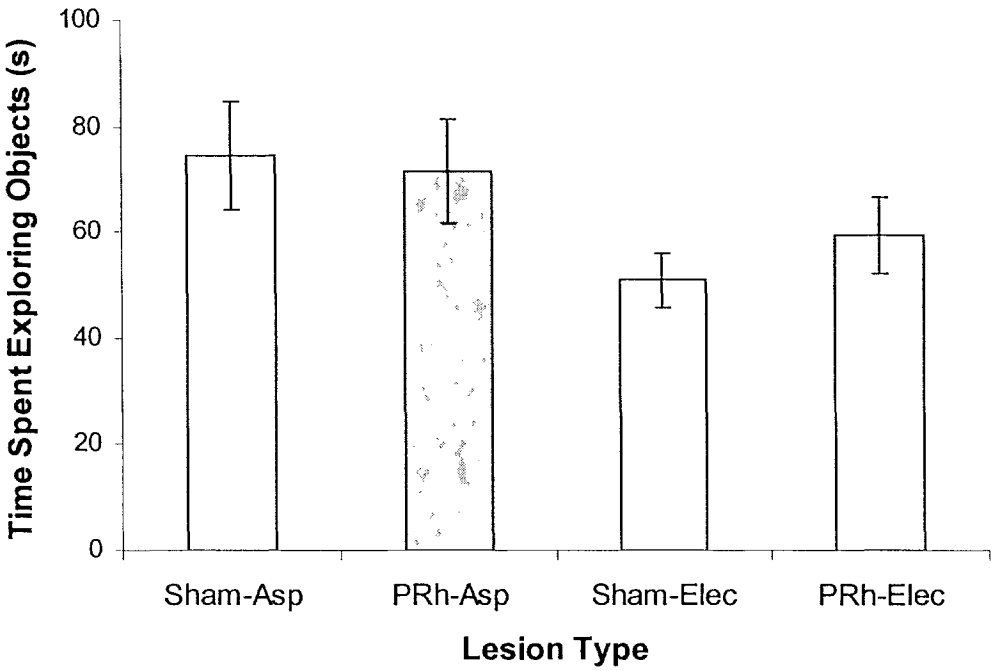

Figure B-4. The mean time Sham and PRh rats spent exploring objects during the total 5 minutes of the sample session. The error bars represent S.E.M.

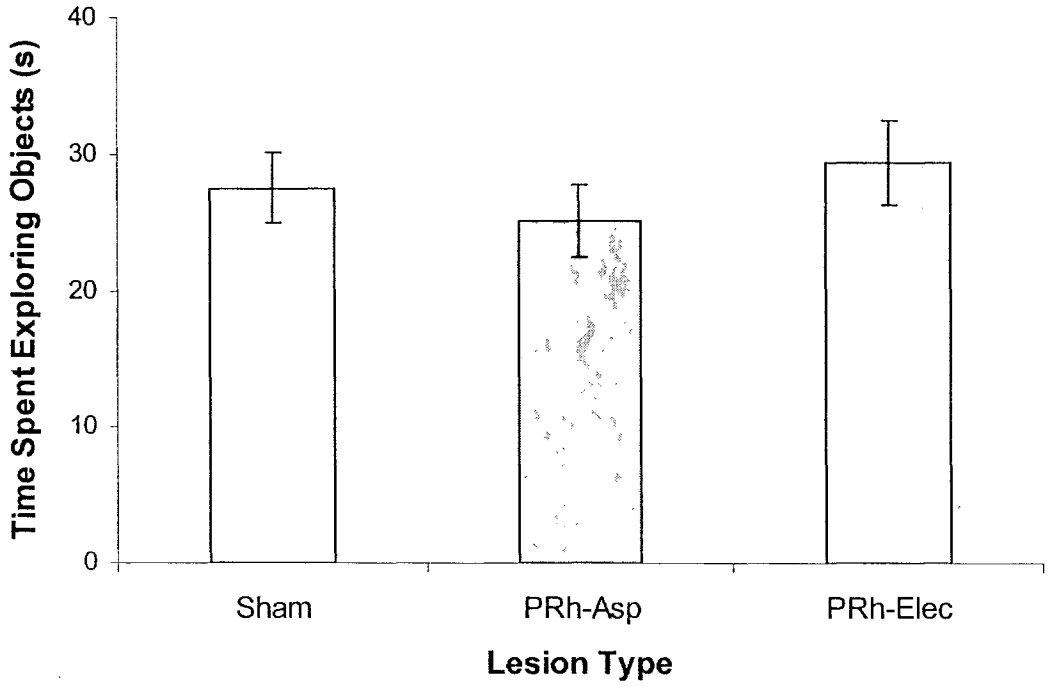

Figure B-5. The mean time Sham and PRh rats spent exploring objects during the first 2 minutes of the test session. The error bars represent S.E.M.

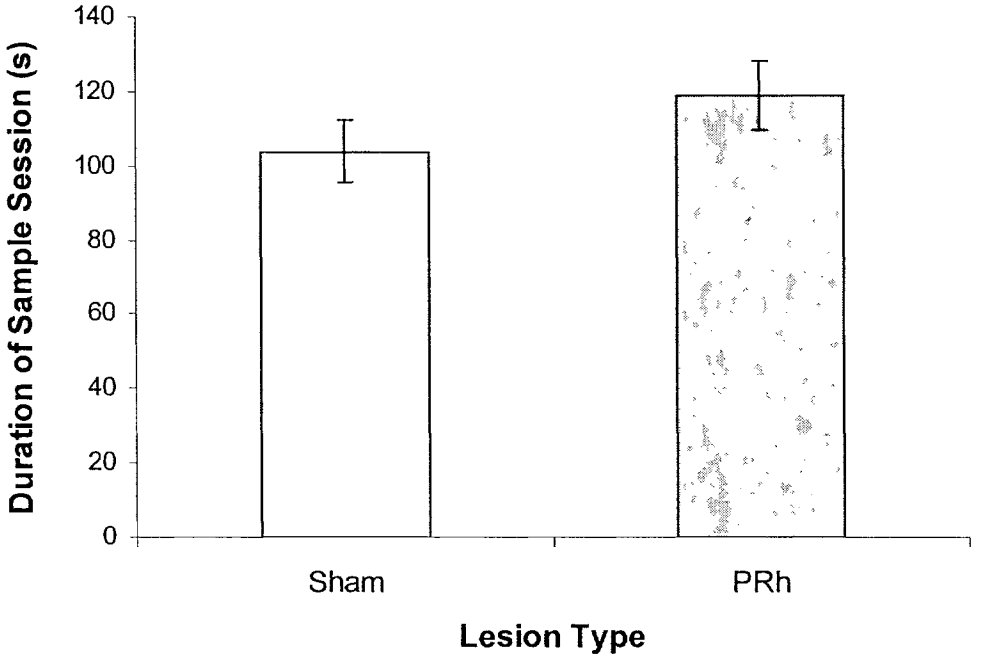

Figure B-6. The mean time required for Sham and PRh rats to accumulate a total of 20 seconds of exploration with the sample objects. The error bars represent S.E.M

Sham and PRh rats to accumulate this amount of exploration is shown in Figure B-6. PRh rats took slightly longer than Sham rats, on average, to accumulate 20 seconds of exploration, but the difference between the groups was not statistically significant ($t[15] = -1.21$, $p = .245$).

Neither Sham nor PRh rats displayed a significant preference for the novel object during the first 2 minutes of the retention test, whereas Sham, but not PRh rats, displayed a significant preference for the novel object during the first minute of the test. Figure B-7 shows the total amount of time Sham and PRh rats spent exploring objects during the first 2 minutes and first minute of the retention test. In each case, PRh rats tended to spend less time than Sham rats exploring objects. Independent samples t-tests revealed that this difference was statistically significant for the first 2 minutes of the retention test ($t[15] = -2.229$, $p = .043$), but not for the first minute ($t[15] = -1.587$, $p = .133$).

B-3.2.4 Summary of object exploration in Experiment 5

In the first part of Experiment 5, rats received a 5-minute sample session, a 5-minute retention delay, and the first 2 minutes of the test session were then examined for object preference. The main finding from this test was that both Sham rats and rats with aspiration PRh lesions displayed a preference for the novel object during this portion of the test. The findings presented here revealed that there were no significant differences between the groups in the amount of time they spent exploring the sample objects during the sample session, nor were there any differences in the amount of time spent exploring objects during the first 2 minutes of the retention test. Therefore, this examination of pattern of object exploration was not able to account for the lack of a deficit in the PRh rats.

In the second part of Experiment 5, rats received a 5-minute sample session, a 15-minute retention delay and the first 2 minutes of the test session were then examined for

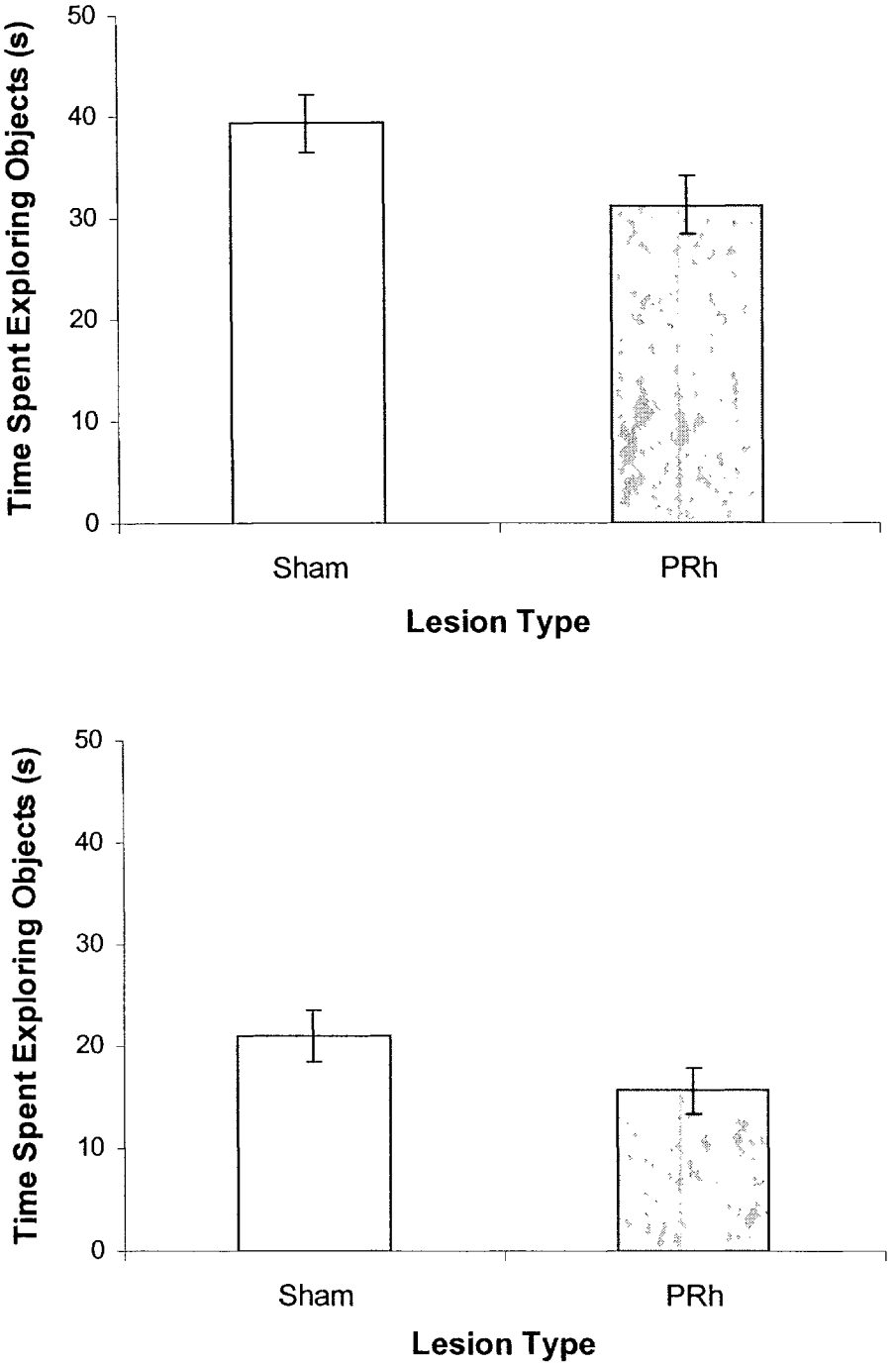

Figure B-7. The mean time Sham and PRh rats spent exploring objects during the first 2 minutes of the test session is shown in the top panel. The bottom panel shows the mean time Sham and PRh rats spent exploring objects during the first minute of the retention test. The error bars represent S.E.M.

object preference. In this case, Sham rats were compared to rats with either aspiration or electrolytic lesions of the PRh. The main finding was that Sham rats and rats with aspiration PRh lesions displayed a preference for the novel object, whereas rats with electrolytic PRh lesions did not. The findings presented here shows that there were no significant differences between the 3 lesion groups in the amount of time they spent exploring sample objects or in the amount of time they spent exploring objects during the first 2 minutes of the test session. Therefore, this examination did not reveal differences in patterns of object exploration that could account for the differences in novelty preference displayed by the groups.

In the third and final part of Experiment 5, the sample session lasted only as long as it took rats to explore both sample objects for a total of 20 seconds. A 5-minute retention delay followed and then the first 2 minutes and the first minute of the test session were examined for object preference. The results from the test session were that neither Sham rats nor rats with aspiration PRh lesions displayed a preference for the novel object during the first 2 minutes, whereas Sham, but not PRh, rats displayed a preference for the novel object during the first minute. In both cases, PRh rats tended to spend less time exploring objects overall, but the difference was only statistically significant during the first 2 minutes and not during the first minute. Therefore, this pattern of object exploration, as in the preceding sections does not account for the deficit in the PRh rats.

B-3.3 Experiment 6: Retrograde memory for objects following electrolytic lesions of the PRh: Between-subjects design

B-3.3.1 Presurgery object exploration

Figure B-8 shows the total time Sham and PRh rats spent exploring the sample objects during the 5 5-minute sample sessions conducted prior to surgery. A 2 x 3 (Lesion x Time of

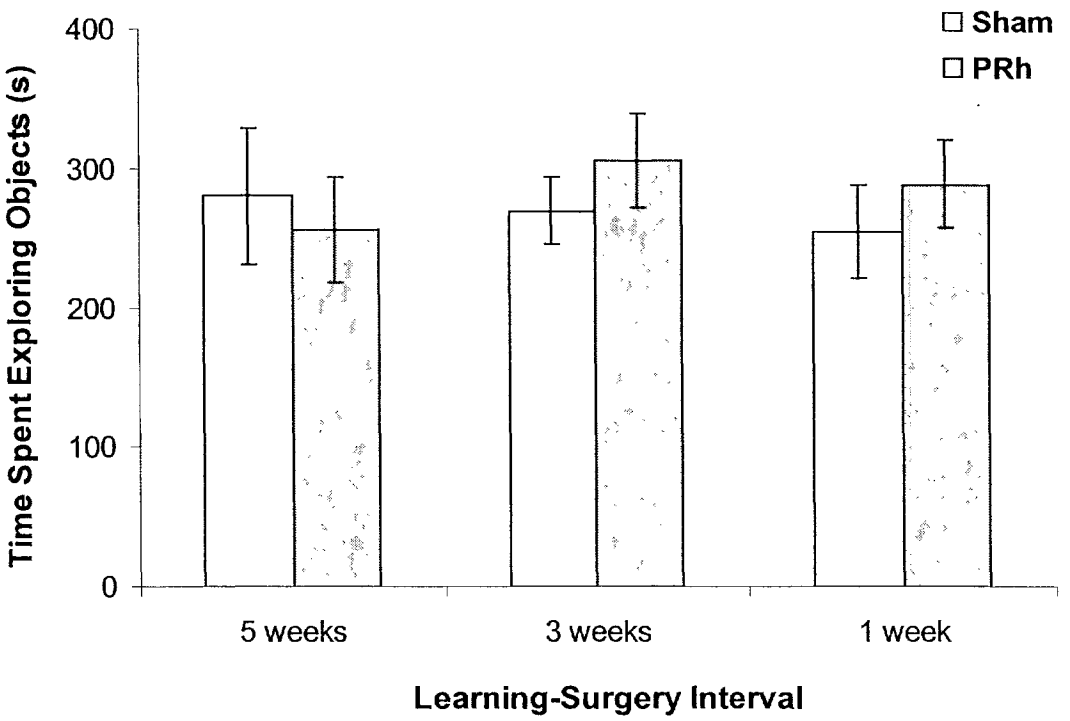

Figure B-8. Mean total times Sham and PRh rats from each time point spent exploring the sample objects during the 5 5-minute sample sessions. The error bars represent S.E.M.

Learning) completely between-subjects ANOVA conducted on these data demonstrated that there were no significant differences between Sham and PRh rats at any time point prior to surgery (all $\underline{F}$s <1).

B-3.3.2 Postsurgery object exploration

Figure B-9 shows the total amount of time Sham and PRh rats spent exploring objects during the first 2 minutes of the retention test. A 2 x 3 (Lesion x Time of Learning) completely between-subjects ANOVA did not reveal any significant differences between the groups ($\underline{F}$s <1). There was a tendency for rats in the 5-week group to spend less time exploring objects than rats in the other groups, but the main effect of Time of Learning was not statistically significant ($\underline{F}[2,33] = 2.276$, $\underline{p} = .119$).

B-3.3.3 Summary of object exploration in Experiment 6

In Experiment 6, rats were divided into three groups of rats that received 5 5-minute sample sessions either 5 or 3 weeks before or during the week of surgery. Approximately half of each group received a sham surgery, and the remaining half received an electrolytic PRh lesion. The main findings were that neither Sham nor PRh rats in the 5-week group displayed a preference for the novel object during the test session administered after surgery, whereas the Sham, but not the PRh, rats in the 3- and 1-week groups displayed a preference for the novel object.

The data presented here showed that the amount of time each group of Sham and PRh rats spent exploring the sample objects before surgery was comparable. Similarly, there were no statistically significant differences in the amount of time spent exploring objects between each group of Sham and PRh rats during the test session. Thus the overall patterns of object exploration were similar for both lesion groups and does not account for differences in object preference observed during the test session.

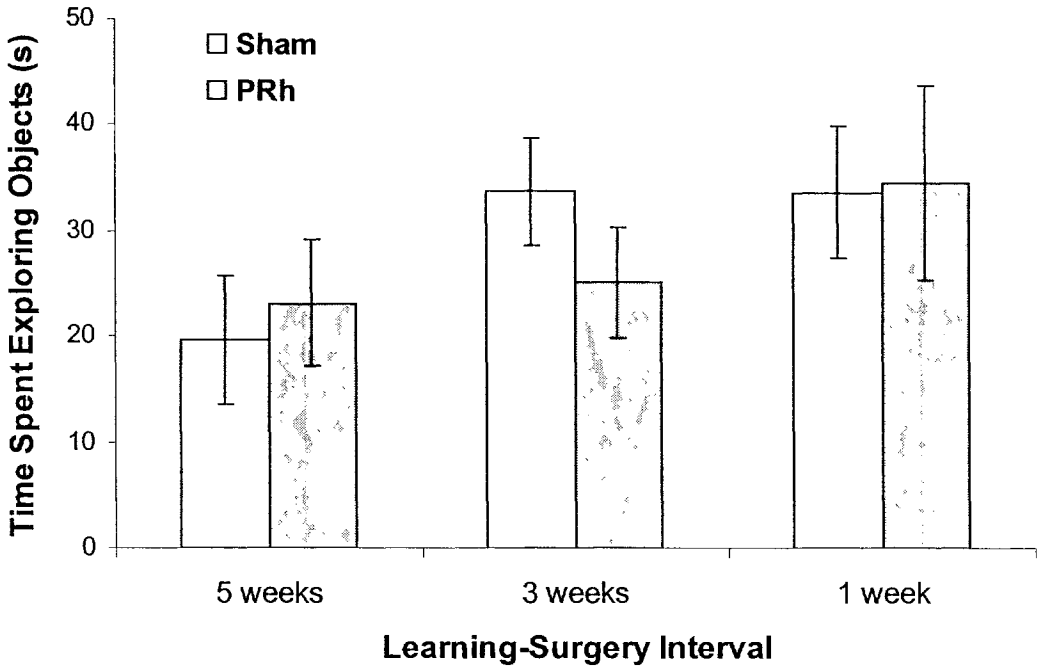

Figure B-9. Mean total times Sham and PRh rats from each time point spent exploring objects during the first 2 minutes of the test session. The error bars represent S.E.M.

B-3.4 Experiment 7: Retrograde memory for objects following aspiration lesions of the PRh: Between-subjects design

B-3.4.1 Presurgery object exploration

Figure B-10 shows the mean total times each of the 4 groups spent exploring the sample objects before surgery. A 2 x 2 (Lesion x Time of Learning) completely between-subjects ANOVA did not reveal any significant differences between the groups (Fs <1). There was a tendency for rats in the REMOTE group to spend more time exploring objects than rats in the RECENT group, but the main effect of Time of Learning was not statistically significant ($F[1,29] = 2.348$, $p = .136$).

B-3.4.2 Postsurgery object exploration

Figure B-11 shows the total amount of time Sham and PRh rats spent exploring objects during the first 2 minutes and first 3 minutes of the test session. It was necessary to analyze both portions of the test to fully capture a tendency for Sham rats to preferentially explore the sample object. For this reason, the total times spent exploring objects during just the first 2 minutes and also during the first 3 minutes are shown here. A 2 x 2 (Lesion x Time of Learning) completely between-subjects ANOVA conducted on the first 2 minutes of the test session did not reveal any significant differences between the groups (ps $>.10$). The same ANOVA conducted on the first 3 minutes of the test session also failed to reveal any significant differences between the groups (all Fs <1).

B-3.4.3 Summary of object exploration in Experiment 7

In Experiment 7, rats were divided into two groups of rats that received 5 5-minute sample sessions either 4 weeks before or during the week of surgery. Approximately half of each group received a sham surgery, and the remaining half received an aspiration PRh

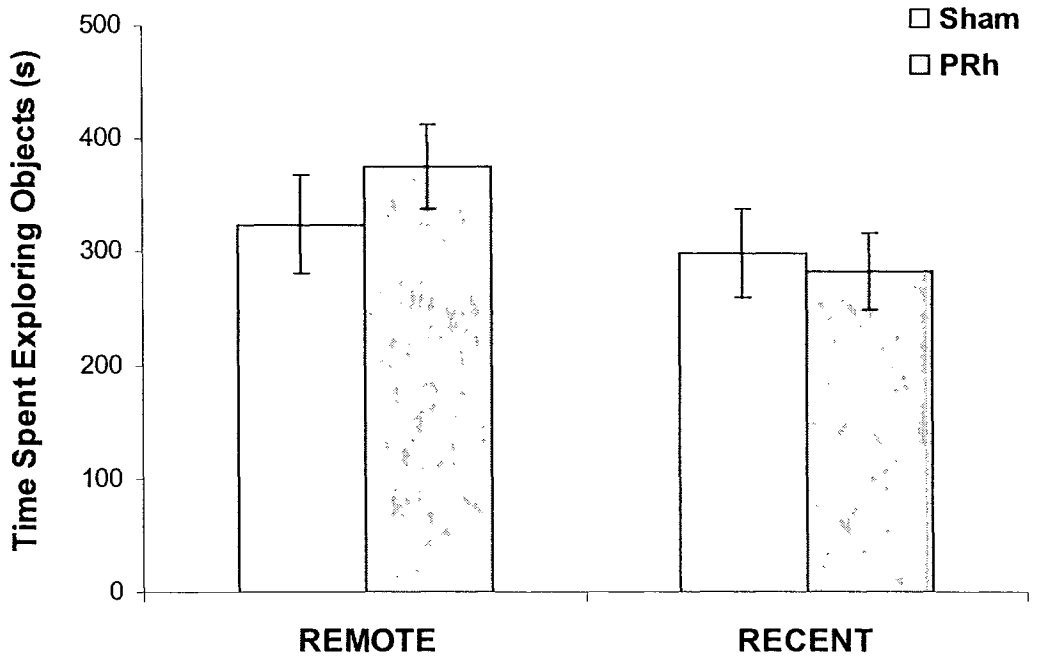

Figure B-10. Mean total times Sham and PRh rats from each time point spent exploring the sample objects during the 5 5-minute sample sessions. The error bars represent S.E.M.

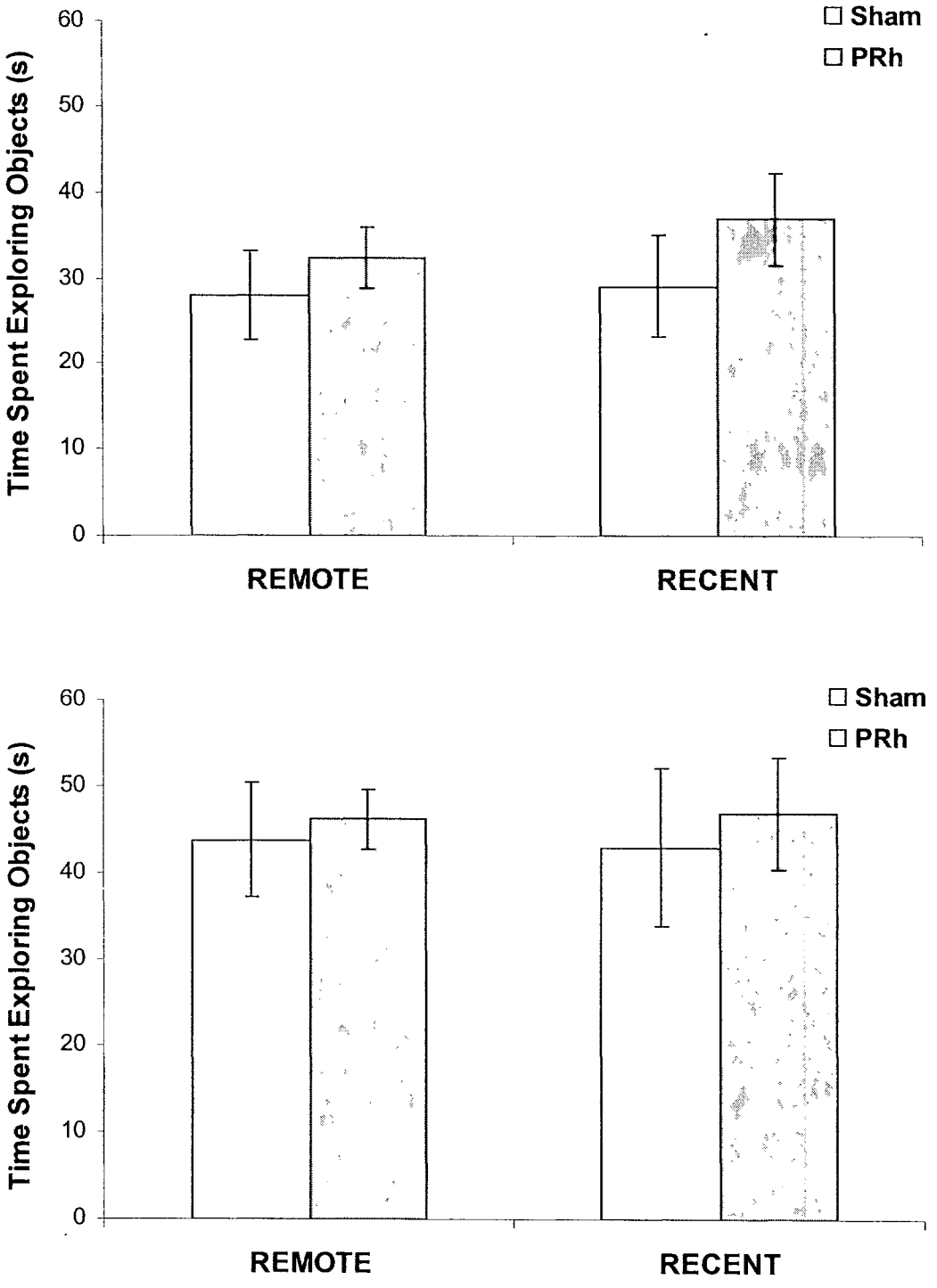

Figure B-11. Mean total times Sham and PRh rats from each time point spent exploring objects during the first 2 minutes of the test session are shown in the top panel. The mean total times Sham and PRh rats spent exploring objects during the first 3 minutes of the test session are shown in the bottom panel. The error bars represent S.E.M.

lesion. The main findings were that both Sham and PRh rats displayed a preference for the novel object during the test session administered after surgery. However, it was necessary to examine both the first 2 minutes and the first 3 minutes of the test session to fully capture the preference for both groups.

The data presented here showed that the amount of time each group of Sham and PRh rats spent exploring the sample objects before surgery was comparable. Similarly, there were no statistically significant differences in the amount of time spent exploring objects betweeneach group of Sham and PRh rats during the test session. Thus, the overall pattern of object exploration was similar for both lesion groups and does not account for differences in object preference observed during the test session.

B-3.5 Experiment 8: Retrograde memory for objects following aspiration lesions of the PRh: Within-subjects design

B-3.5.1 Presurgery object exploration

Figure B-12 shows the mean total times Sham and PRh rats spent exploring the REMOTE and RECENT sample objects. A 2 x 2 (Lesion x Time of Learning) mixed-factorial ANOVA revealed a marginally significant effect of Time of Learning ($F[1,12] = 3.506$, $p = .086$). Overall, rats spent more time exploring the sample objects at the REMOTE time point, relative to the RECENT time point. The main effect of Lesion and the interaction between Lesion and Time of Learning were not statistically significant (ps > .10).

B-3.5.2 Postsurgery object exploration

Figure B-13 shows the total amount of time Sham and PRh rats spent exploring objects during the first 2 minutes and first 3 minutes of the test session. A 2 x 2 (Lesion x Time of

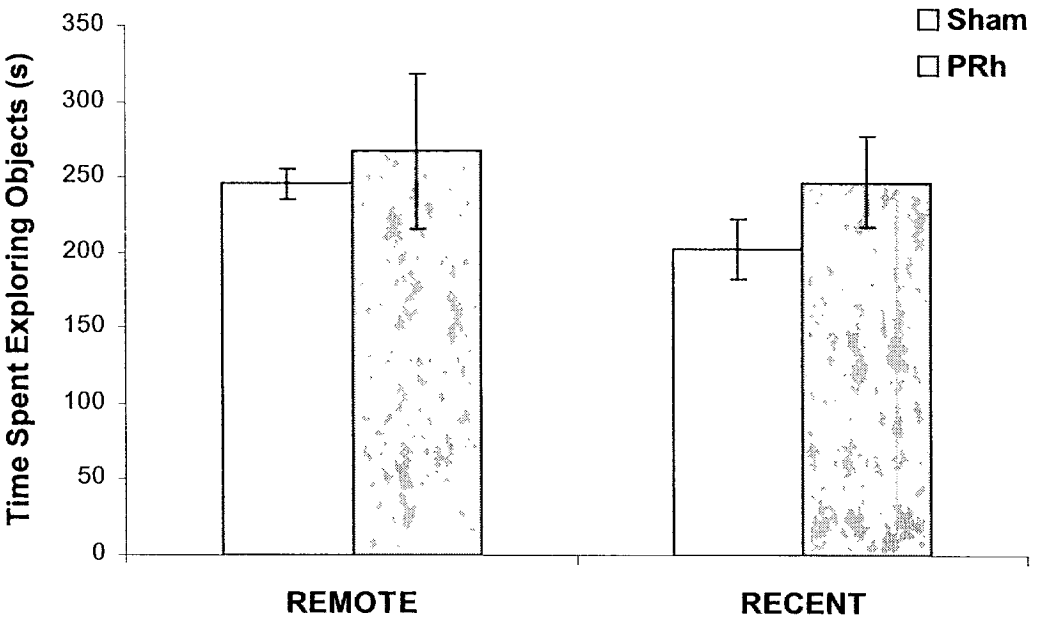

Figure B-12. Mean total times Sham and PRh rats spent exploring the sample objects during the 5 5-minute sample sessions at the REMOTE and RECENT time points. The error bars represent S.E.M.

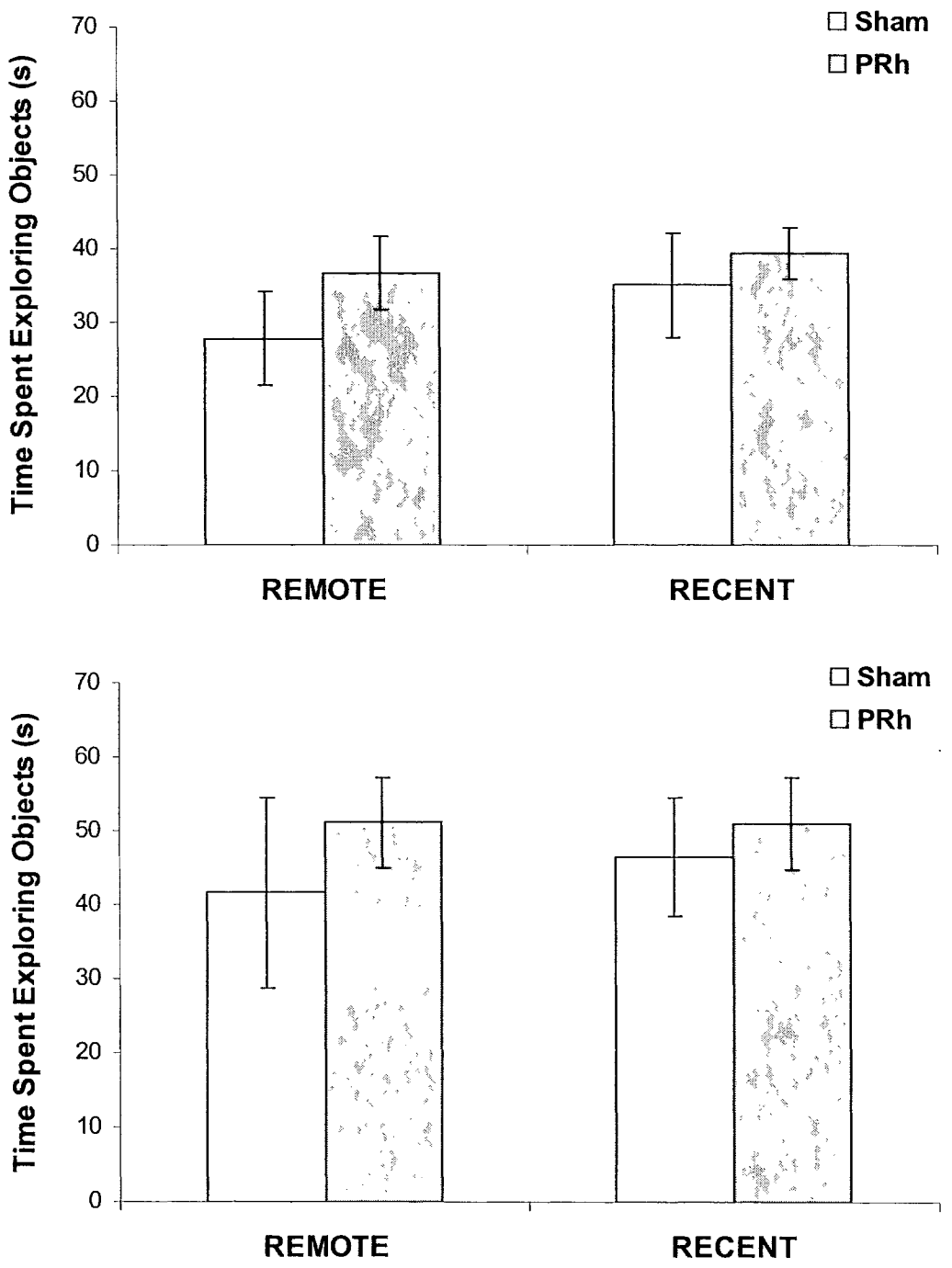

Figure B-13. Mean total times Sham and PRh rats from each time point spent exploring objects during the first 2 minutes of the test session are shown in the top panel. The mean total times Sham and PRh rats spent exploring objects during the first 3 minutes of the test session are shown in the bottom panel. The error bars represent S.E.M.

Learning) mixed-factorial ANOVA conducted on the first 2 minutes of the test session did not reveal any significant differences between the groups (ps $>.10$). The same ANOVA conducted on the first 3 minutes of the test session also failed to reveal any significant differences between the groups (all Fs <1).

B-3.5.3 Summary of object exploration in Experiment 8

In Experiment 8, all rats received 5 5-minute sample sessions 4 weeks before and during the week of surgery. Rats then received either sham surgery or aspiration PRh lesions. The main findings were that both Sham and PRh rats displayed a preference for the novel object during the test session administered after surgery. However, as in Experiment 7, it was necessary to examine both the first 2 minutes and the first 3 minutes of the test session to fully capture the preference for both groups.

The data presented here showed that the amount of time each group of Sham and PRh rats spent exploring the sample objects before surgery was comparable. Similarly, there were no statistically significant differences in the amount of time spent exploring objects between each group of Sham and PRh rats during the test session. Thus, the overall pattern of object exploration was similar for both lesion groups and does not account for differences in object preference observed during the test session.

Appendix C

Additional Behavioural Indices of Object Exploration for Chapter 4

C-4.1 Aim of Appendix C

The primary purpose of this Appendix is to summarize the general patterns of object exploration displayed by the Sham and PRh rats from the experiments described in Chapter 4. In that Chapter several versions of the place version of NPT were used to evaluate the ability of rats with PRh lesions to learn and remember information about the location of objects in the open field. Our primary dependent measure in all the experiments described in Chapter 4 was the exploration ratio calculated based on the time spent exploring the moved and unmoved objects during the first 2 minutes of the test session. Described below are analyses of the total amounts of time rats spent exploring objects during both the sample and test sessions.

As in Appendix B, the primary rationale for the present analyses was to determine whether the patterns of novelty preference that Sham and PRh rats displayed can be accounted for by differences in their overall pattern of object exploration. However, also as in Appendix B, the following analyses confirm that PRh lesions did not alter object exploration.

C-4.2 Experiment 9: Anterograde memory for object location following aspiration lesions of the PRh

C-4.2.1 Standard NPT test

Figure C-1 shows the total amount of time Sham and PRh rats spent exploring objects during the 5-minute sample session. An independent samples t-test revealed that the groups were not significantly different ($t[24] = .449$, $p = .657$).

Figure C-2 shows the total amount of time Sham and PRh rats spent exploring objects during the first 2 minutes of the test session. This was the portion of the test that was analyzed for object preference. An independent samples t-test revealed that the groups were not significantly different ($t[24] = -.844$, $p = .407$).

C-4.2.2 Modified NPT test

In this test the sample session was terminated once rats accumulated a total of 20 seconds of object exploration. Figure C-3 shows the average time required for Sham and PRh rats to obtain this amount of time exploring objects. An independent samples t-test showed that the duration of the sample phase was not significantly different for Sham and PRh rats ($t[26] = .678$, $p = .504$).

Figure C-4 shows the amount of time Sham and PRh rats spent exploring objects during the first 2 minutes and the first minute of the test session. Sham rats showed a preference for the moved object in both analyses, whereas PRh rats did not show a preference for the moved object during the first 2 minutes, but did during the first minute. An independent samples t-test conducted on the first 2 minutes revealed that Sham and PRh rats were not significantly different during this portion of the test ($t[26] = 1.606$, $p = .120$).

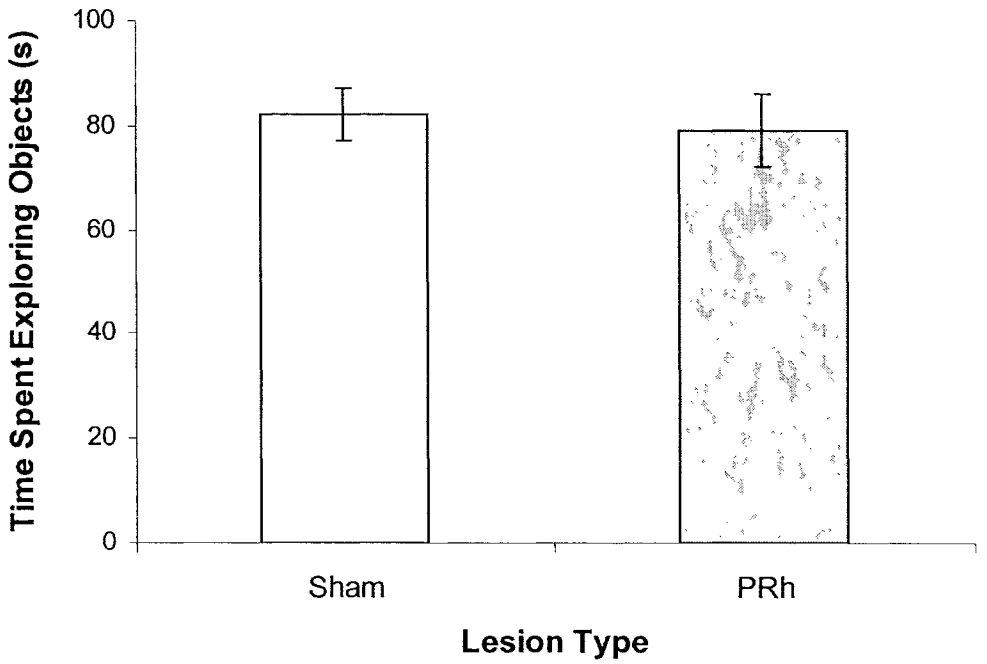

Figure C-1. The mean total time Sham and PRh rats spent exploring objects during the 5-minute sample session. The error bars represent S.E.M.

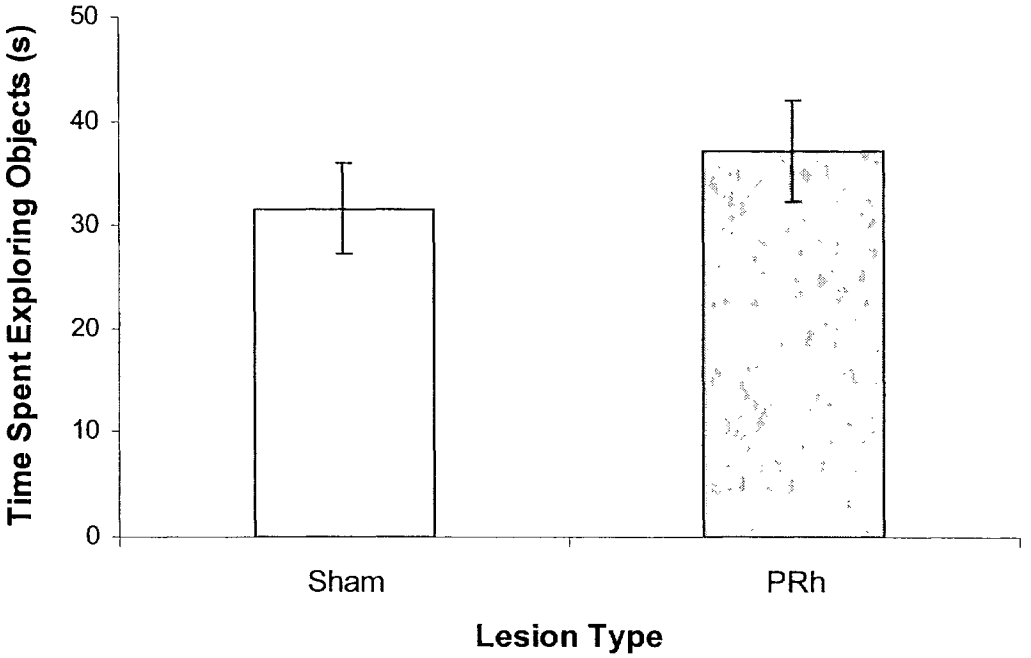

Figure C-2. The mean total time Sham and PRh rats spent exploring objects during the first 2 minutes of the test session. The error bars represent S.E.M.

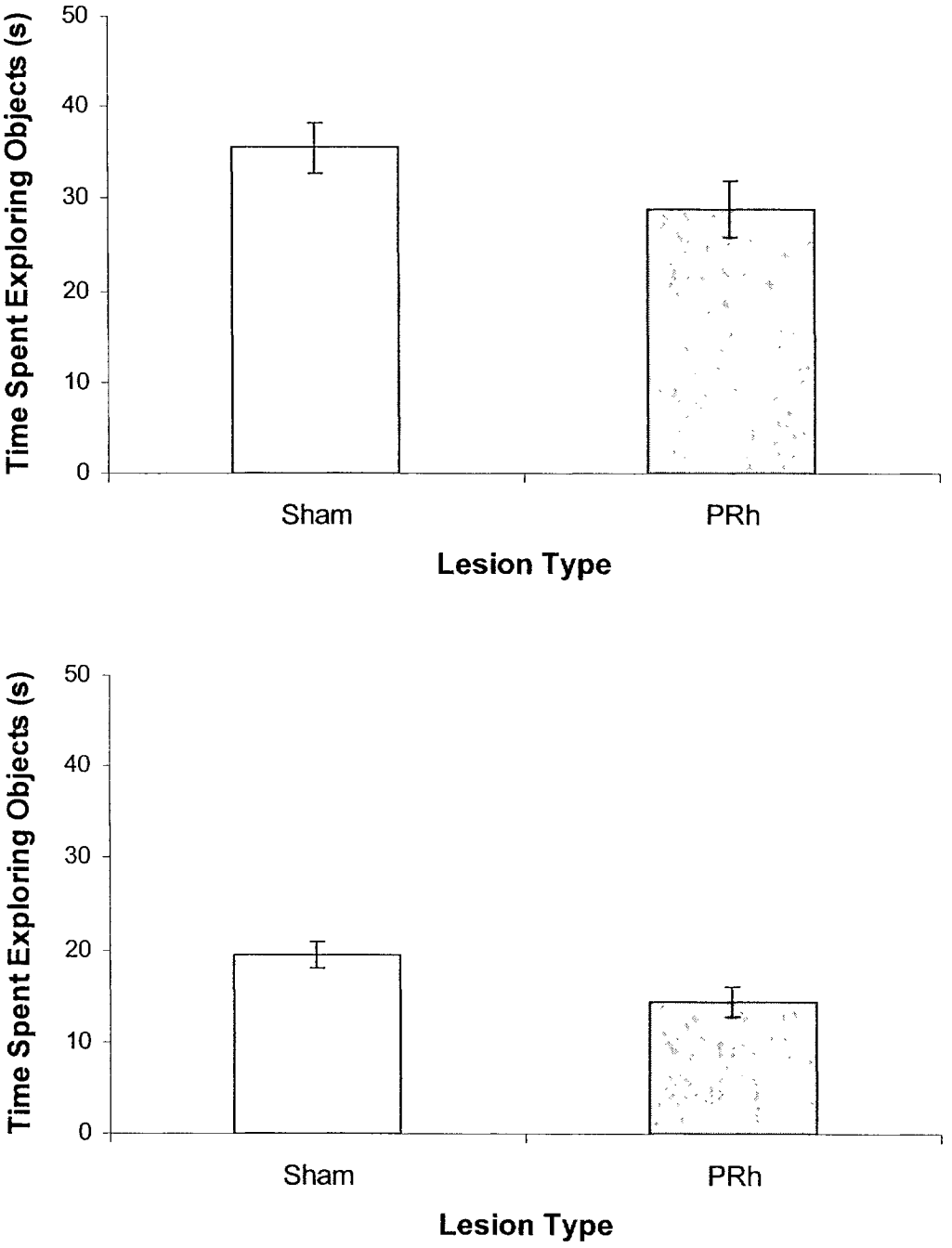

Figure C-4. The mean time Sham and PRh rats spent exploring objects during the first 2 minutes of the test session is shown in the top panel. The bottom panel shows the mean time Sham and PRh rats spent exploring objects during the first minute of the retention test. The error bars represent S.E.M.

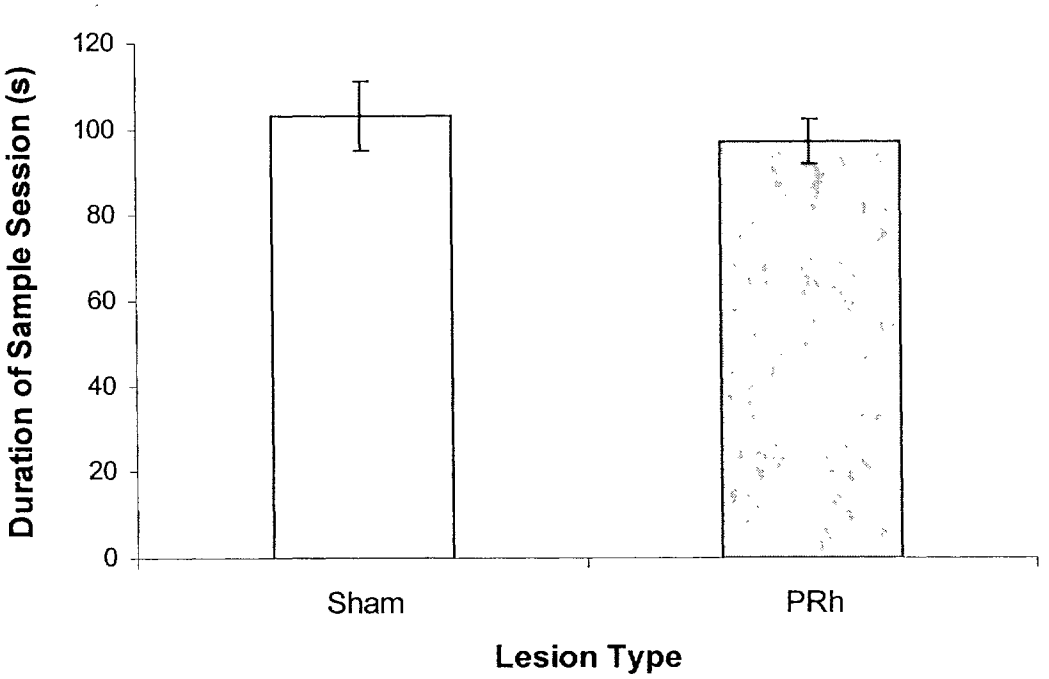

Figure C-3. The mean time required for Sham and PRh rats to accumulate a total of 20 seconds of exploration with the sample objects. The error bars represent S.E.M.

However, PRh rats spent significantly less time than Sham rats exploring objects during the first minute of the test session ($t[26] = 2.349$, $p = .027$).

C-4.2.3 Summary of object exploration in Experiment 9

In Experiment 9, Sham rats and rats with aspiration lesions of the PRh were tested on the standard and modified version of NPT. The main finding from the standard version was that both Sham and PRh rats displayed a preference for the moved object during the first 2 minutes of the test session. The present analysis revealed that both groups spent similar amounts of time exploring objects during the sample session and during the first 2 minutes of the test session.

The findings from the modified place version of NPT were not as clear. PRh rats did not show a substantial preference for the moved object during the first 2 minutes of the test session, but they did show a significant preference during the first minute. Sham rats showed a significant preference during the first minute and the first 2 minutes of the test session. The present analysis showed that Sham and PRh rats took similar amounts of time to explore the sample objects for 20 seconds. However, during the test session, PRh rats tended to spend less time exploring objects. The difference in time spent exploring objects between Sham and PRh rats was statistically significant for the first minute, but not for the first 2 minutes. Since PRh rats spent less time exploring objects than Sham rats, this could account for the tendency for this group to display a preference for the moved object during the first minute only.

C-4.3 Experiment 10: Retrograde memory for object location following aspiration lesions of the PRh

C-4.3.1 Presurgery object exploration

Figure C-5 shows the total time Sham and PRh rats spent exploring the sample objects during the 5 5-minute sample sessions conducted prior to surgery. There was a tendency for PRh rats to spend less time than Sham rats exploring objects, but the difference was not statistically significant ($t[26] = 1.674$, $p = .106$).

C-4.3.2 Postsurgery object exploration

Figure C-6 shows the total amount of time Sham and PRh rats spent exploring objects during the first 2 minutes of the retention test. In this case, PRh rats spent significantly more time exploring objects than Sham rats ($t[26] = -2.759$, $p = .010$).

C-4.3.3 Summary of object exploration in Experiment 10

In Experiment 10, all rats received 5 5-minute sample sessions during the week before surgery. Rats then received either Sham surgery or aspiration lesions of the PRh. The main finding from the postsurgery test session was that Sham, but not PRh, rats showed a significant preference for the moved object. In the present analysis, the rats that received PRh lesions tended, overall, to spend less time than Sham rats exploring the sample objects before surgery. It is possible that this difference could account for their lack of preference for the moved object. However, PRh rats still spent approximately 300 seconds exploring the sample objects. Descriptions of object exploration in Appendix B revealed that PRh rats in Experiments 7 and 8 spent approximately 250 and 350 seconds exploring objects, respectively. These rats displayed a preference for a novel object during the postsurgery test

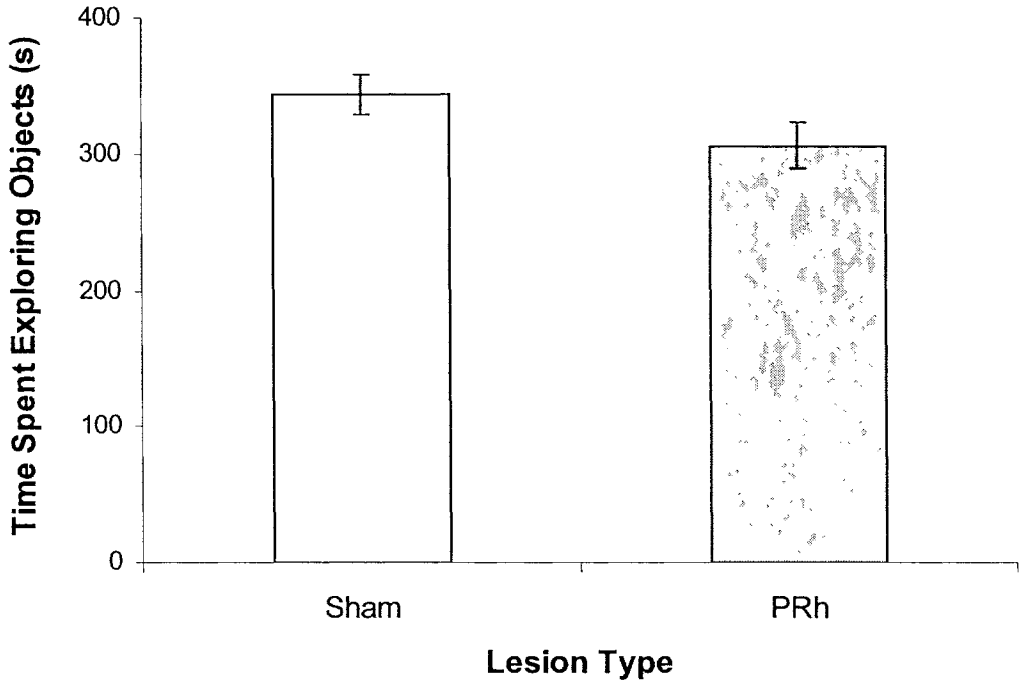

Figure C-5. The mean time Sham and PRh rats spent exploring sample objects during the presurgery sample sessions. The error bars represent S.E.M.

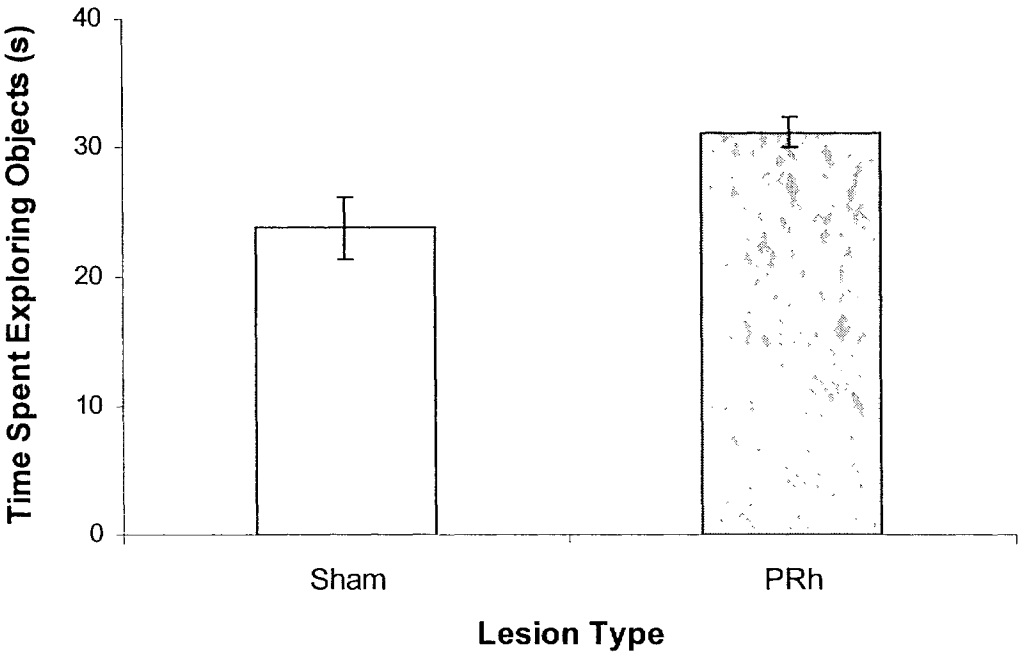

Figure C-6. The mean time Sham and PRh rats spent exploring objects during the first 2 minutes of the test session. The error bars represent S.E.M.

session, suggesting that the 300 seconds reported here is adequate exploration for subsequent discrimination.

Contrary to their presurgery object exploration, PRh rats spent more time than Sham rats exploring objects during the first 2 minute of the retention test. Given that the lack of a preference for the moved object by the PRh rats was demonstrated both by a comparison to chance performance and the Sham group, no further analyses were conducted. However, because PRh rats spend more time exploring objects it is possible that they do display a preference at an earlier time point than Sham rats. Thus, Figure C-7 shows the amount of time Sham and PRh rats spent exploring the moved and unmoved objects during the first minute of the retention test.

The same pattern of results found for the first 2 minutes of the test were observed in the first minute: Sham rats spend more time exploring the moved object than the unmoved object, whereas PRh rats spend similar amounts of time with both objects. Also, as in the first 2 minutes, it is evident from Figure C-7 that PRh rats spend more time, overall, exploring objects relative to Sham rats in the first minute of the retention test. However, PRh rats spend approximately 16 seconds exploring objects during the first minute, and analyzing an even smaller slice of their initial exploration is unlikely to yield meaningful results. Therefore, it seems evident that PRh rats are not showing a preference for the moved object.

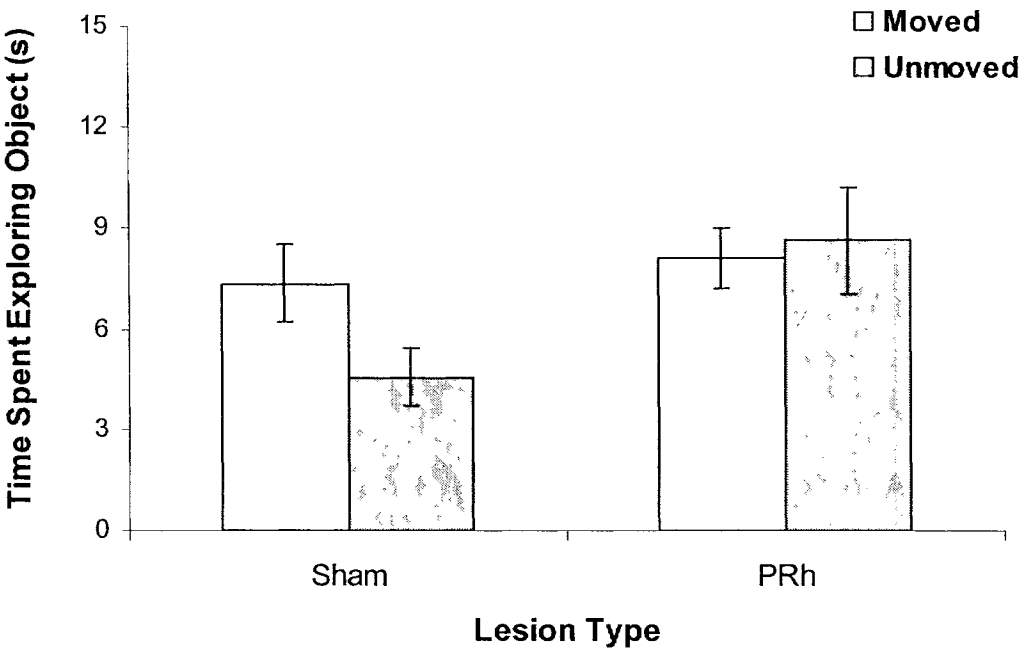

Figure C-7. The mean time Sham and PRh rats spent exploring the moved and unmoved objects during the first minute of the test session. The error bars represent S.E.M.