Contributions of the Hippocampus, Perirhinal Cortex, and Amygdala to Object-Fear Conditioning

Hugo Lehmann

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the degree of Doctor of Philosophy at
Concordia University
Montreal, Quebec, Canada

October 2004

Your file Votre référence
ISBN: 0-494-04049-1
Our file Notre référence
ISBN: 0-494-04049-1

ABSTRACT

Contributions of the Hippocampus, Perirhinal Cortex, and Amygdala to Object-Fear Conditioning

Hugo Lehmann
Concordia University, 2004

Fear conditioning refers to the ability to learn and remember that a stimulus predicts the occurrence of a fear-eliciting event. The neurobiology of fear conditioning to auditory, olfactory, and visual stimuli and to contexts has been extensively studied, but the neurobiology of fear conditioning to an object has not been thoroughly investigated. Thus, the focus of the present thesis was to determine whether object-fear conditioning depends on the hippocampus, perirhinal cortex, and amygdala. It was hypothesized that pre- and post-training lesions to either structure in rats would cause amnesia for an association between an object and a fear-eliciting event because each is involved in object recognition and/or fear conditioning. Accordingly, rats' memory was tested in the shock-probe fear-conditioning test, which required them to remember that a small plastic probe protruding from the bottom of a wall of a testing chamber was associated with a mild shock. Several behaviours, such as burying, avoidance, and changes in stress hormone were assessed during the test to comprehensively evaluate fear conditioning. It was found that hippocampal lesions caused both anterograde and retrograde amnesia. However, the anterograde amnesia resulted from a contextual fear-conditioning deficit, whereas the retrograde amnesia resulted, at least in part, from an object-fear conditioning impairment. By contrast, it was found that perirhinal cortex lesions did not cause memory deficits. Albeit, pre and post-training perirhinal cortex lesions altered avoidance and burying evoked by the object and context during the test, suggesting that the latter

structure is likely involved in appraising stimuli that guide fear responses. It was also found that amygdala lesions caused retrograde, but not anterograde, amnesia for object-fear conditioning. The findings are discussed within the context of current theory of the neural basis of cued and contextual fear conditioning and it is concluded that object-fear conditioning involves memory systems that may be distinct from those of other types of fear conditioning.

ACKNOWLEDGMENTS

Needless to say, I am grateful to my Ph. D. supervisor, Dr. Dave G. Mumby, for the mentorship and the support that he provided during the completion of this research. More importantly, I am thankful for the friendship that resulted from our collaboration. Dave, the fact that I will not be seeing you and your family on a regular basis is very saddening and I can only console myself in the thought that our careers will lead our paths to cross again, and hopefully many times.

I am also indebted to Dr. Barbara Woodside. We collaborated, shared ideas and many laughs. Barbara, you always made me think, always understood, and always had a word of encouragement. Thank you.

A special "thank you" to Melissa Glenn, Stephane Gaskin, Alfonso Abizaid, and Naomi Popeski for making my days at Concordia University more than memorable. You all left before I did and all I can say is that I missed you and I will continue to miss you.

I am also grateful for the assistance and friendship provided by many students that worked in the Mumby and Woodside labs during my stay. In particular, thank you to Guillaume Poirier, Valerie Lecluse, Carolina Cook, Stephanie Yamin, Laura Peronace, and Norman O'Brien.

I would also like to thank Dr. Dominique Walker for her valuable contribution to the experiment examining stress hormone levels in this thesis.

Finally, I am extremely thankful to my wife. Heather, you were always supportive and understanding, even at the worst of times. Thank you for your love.

"Un gros meuble à tiroirs encombré de bilans,
De vers, de billets doux, de procès, de romance,
Avec de lourd cheveux roulés dans des quittances,
Cache moins de secrets que mon triste cerveau."

- Charles Baudelaire, Spleen (II)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

A threatening, traumatic, or fear-eliciting event always occurs in a specific context and in the presence of distinct cues, such as sounds, odours, and objects. When encountered again, these cues and contexts typically evoke, in humans and other animals, a variety of behaviours from which we infer a central state of fear, thus indicating that the original fear-eliciting event was remembered. For example, if an individual victim of a car accident later returned to the scene of the incident, he or she might experience a sudden onset of symptoms, such as increased heart rate, increased respiration, jumpiness, and fidgeting, which would suggest a fearful state. Similarly, if the individual later encountered the same model car as the one involved in the accident, he or she might display symptoms that would suggest a state of fear. Thus, the site of the incident (i.e., context) and the car model (i.e., object) are now associated with a fear-eliciting event and have become stimuli that evoke fear in the individual.

The ability to learn and remember the relationships between stimuli and a fear-eliciting event is an adaptive capacity essential to the survival of all animals, because it enables them to avoid or to prepare themselves for dangerous situations. Studies of the neural mechanisms of this ability in laboratory animals utilize a paradigm termed "fear conditioning", in which the subject is required to learn and remember that a discrete stimulus (i.e., cue), or configuration of stimuli (i.e., context), predicts the occurrence of a subsequent fear-eliciting event (Maren, 2001b; Rudy & O'Reilly, 2001). Moreover, the cues or context previously associated with the fear-eliciting event now also evoke behavioural responses that suggest that the subject is a general state of fear. There is

currently much interest in determining the neural circuitry and mechanisms that mediate fear conditioning. While much is known about the brain structures involved in fear conditioning to stimuli such as auditory cues and contexts, very little is known about fear conditioning to an object. The aim of this thesis was to delineate the role of the hippocampus (HPC), perirhinal cortex (PRH), and amygdala (AMY) in object-fear conditioning.

A study conducted by Kluver and Bucy (1937) was pivotal in the emergence of research focusing on the neural correlates of fear conditioning. They found that medial-temporal-lobe (MTL) damage produced hyperorality, hypersexuality, visual agnosia, reduced neophobia, and loss of fear in monkeys. These findings suggested that structures within the MTL are involved in the mediation of fear, and considerable interest developed in understanding the contributions of specific MTL structures to the processing of a fear-eliciting event. In an attempt to further understand the role of the MTL in fear, researchers started analyzing the effects of MTL damage in several species on fear-conditioning tasks. It was found that damage to the MTL in monkeys, cats, and rodents produced severe fear-conditioning deficits (D. C. Blanchard & Blanchard, 1972; R. J. Blanchard & Fial, 1968; Brady, Schreiner, Geller, & Kling, 1954; Delacour & Borst, 1972; Grossman, Grossman, & Walsh, 1975; Horvath, 1963; Weiskrantz, 1956). Thus, it seemed that the MTL was not only involved in fear, but also in learning and remembering information about fear-eliciting events.

The MTL is composed of the AMY, HPC, entorhinal cortex (EC), PRH, and postrhinal cortex (PORH; i.e., parahippocampal cortex in primates). Numerous studies have now examined the role of these structures in contextual fear conditioning

(Antoniadis & McDonald, 2001; D. C. Blanchard & Blanchard, 1972; Bucci, Phillips, & Burwell, 2000; Maren, 1998) and cued fear conditioning (Bucci et al., 2000; Campeau & Davis, 1995a, 1995b; Cousens & Otto, 1998; Herzog & Otto, 1997; J. J. Kim & Fanselow, 1992; Sananes & Davis, 1992). The evidence from these studies suggests that MTL structures differentially contribute to fear conditioning. For example, the AMY seems to be involved in fear conditioning to all stimuli, whereas the HPC seems predominantly involved in contextual fear conditioning.

The wide majority of studies on fear conditioning to discrete cues have used stimuli specific to one sense and have used stimuli that lack physical substance such as tones or lights. Yet, most fearful situations that occur naturally involve experiencing a fear-eliciting event in the presence of a specific object. Moreover, in some situations the object may even be the fear-eliciting stimulus. Therefore, to fully understand the involvement of MTL structures in fear conditioning, it is essential to understand their contribution to object-fear conditioning.

The lack of studies focusing on the contributions of the various MTL structures to object-fear conditioning is even more surprising since the MTL has also been shown to be important for object recognition (Mahut, Moss, & Zola-Morgan, 1981; Mishkin, 1978; Squire & Zola-Morgan, 1988; Squire, Zola-Morgan, & Chen, 1988). Extensive evidence suggests that damage to the MTL can severely disrupt representations or associations pertaining to objects (Gaskin, Tremblay, & Mumby, 2003; Mishkin, 1978; Murray & Mishkin, 1983, 1984; Saunders, Murray, & Mishkin, 1984; Squire & Zola-Morgan, 1988; Squire et al., 1988). It is even suggested that the HPC and PRH are key MTL structures involved in object recognition (Murray, Bussey, Hampton, & Saksida, 2000; Squire,

Stark, & Clark, 2004; Suzuki & Eichenbaum, 2000). Lesions circumscribed to either of these structures are often sufficient to cause object recognition deficits (Alvarez, Zola-Morgan, & Squire, 1995; Gaskin et al., 2003; Glenn & Mumby, 1996; Meunier, Bachevalier, Mishkin, & Murray, 1993; Zola-Morgan, Squire, Amaral, & Suzuki, 1989). Thus, it is reasonable to hypothesize that the MTL structures involved in object recognition could also be involved in object-fear conditioning.

The aim of this thesis was to delineate the contributions of three medial temporal lobe structures (the AMY, HPC and PRH) to object-fear conditioning. The AMY, HPC, and PRH were selected because of their important role in cued fear conditioning and/or contextual fear conditioning as well as their important contribution to object recognition.

The remaining sections of this chapter provide the background for the empirical chapters that follow. Section 1.1 describes the anatomy of the MTL. Section 1.2 reviews the known contributions of the AMY, HPC, and PRH to fear conditioning. Section 1.3 describes the involvement of these latter three structures in object recognition. Section 1.4 introduces the shock-probe fear-conditioning test, which has major advantages over the commonly used cued fear-conditioning tests and is suited for assessing object-fear conditioning. Section 1.5 presents the specific questions that were addressed by the experiments in this thesis.

1.1 ANATOMY OF THE MTL

The neuroanatomical organization of the MTL has been widely described in the rat, the monkey, and the human (Amaral, 1999; Amaral & Cowan, 1980; Amaral & Price, 1984; Amaral, Veazey, & Cowan, 1982; Burwell & Amaral, 1998a; Burwell, Witter, &

Amaral, 1995; Deacon, Eichenbaum, Rosenberg, & Eckmann, 1983; Friedman, Murray, O'Neill, & Mishkin, 1986; Guldin & Markowitsch, 1983; Lavenex & Amaral, 2000; Lavenex, Suzuki, & Amaral, 2002; A. J. McDonald, 2003; Pitkanen & Amaral, 1998; Pitkanen, Kelly, & Amaral, 2002; Stefanacci, Suzuki, & Amaral, 1996; Suzuki & Amaral, 2003; Swanson, 2003; Witter & Amaral, 1991). Although there are differences between the species, the general organization of the structures remains very similar. Given these similarities and that the experiments in the current thesis were conducted on rats, the provided description of the MTL is based on the anatomy of the rat. Moreover, the description not only explains the physiological connections, but also their proposed functional importance for information and mnemonic processing. An illustration of this description is found in Figure 1.

1.1.1 The AMY

The AMY is comprised of several distinct nuclei, including, but not limited to, the lateral, basolateral, basomedial, and central nuclei. The collectivity of the lateral, basolateral, and basomedial is referred to as the basolateral complex (BLA) and is the primary sensory interface of the AMY (Paxinos, 1995). The BLA receives convergent inputs from the various unimodal and polymodal sensory areas. For instance, inputs from the thalamus are believed to relay sensory information from all sensory modalities (Herkenham, 1978; Krettek & Price, 1977b; Ottersen & Ben-Ari, 1979; Veening, Swanson, & Sawchenko, 1984). Projections from the insular cortex are thought to send gustatory and somatic information (Saper, 1982). Inputs from cortical areas, such as the EC, PRH, PORH, send visual, olfactory, somatosensory, and auditory information

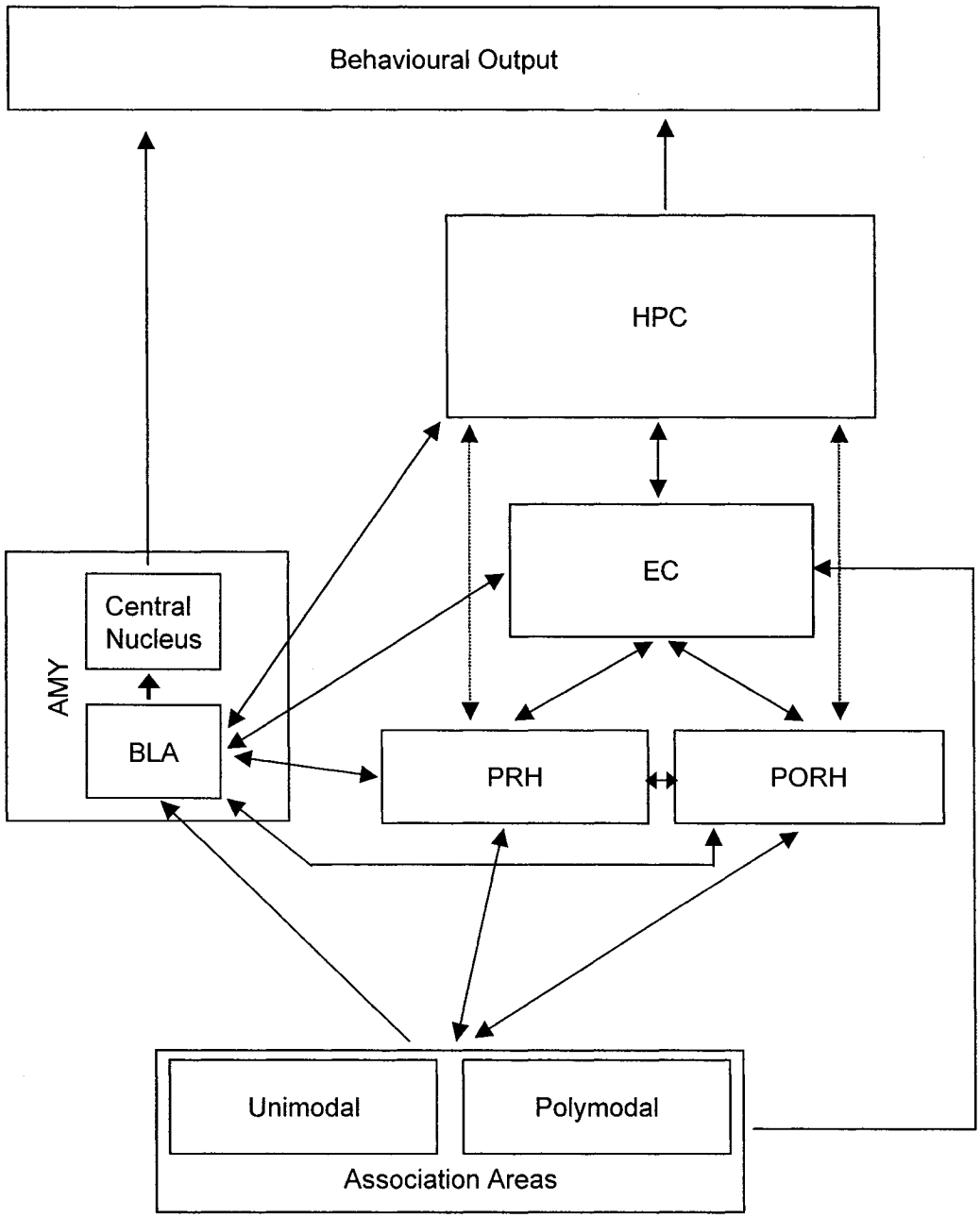

Figure 1. Major connections among the medial-temporal lobe structures that are examined in the present thesis (AMY = amygdala; BLA = basolateral complex; EC = entorhinal cortex; HPC = hippocampal formation; PORH = postrhinal cortex; PRH = perirhinal cortex).

received from sensory cortical areas (Burwell & Amaral, 1998a; Deacon et al., 1983; Ottersen, 1982). Afferents from the HPC are thought to send configural information (Maren, 2001b; White & McDonald, 2002). Projections from the substantia nigra, ventral tegmental area, nucleus accumbens, and other areas that are known to process visceral information and the rewarding or aversive properties of stimuli also reach the BLA (Beckstead, Domesick, & Nauta, 1979; Fallon & Moore, 1978a, 1978b; Ottersen, 1981; Simon, Le Moal, & Calas, 1979). Given the convergence of the inputs from sensory areas and the inputs from structures that are involved in processing the rewarding or aversive properties of stimuli, it is thought that the BLA is in an ideal position to establish associations between the various sensory properties of stimuli and affective characteristics (Maren, 2001b; White & McDonald, 2002).

The BLA has major outputs to the central nucleus of the AMY, which in turn projects to the midbrain, the hypothalamus, and bed nucleus of the stria terminalis (Berk & Finkelstein, 1981; Dong, Petrovich, & Swanson, 2001; Kelley, Domesick, & Nauta, 1982; Ottersen, 1980, 1982; Weller & Smith, 1982). Therefore, it is proposed that the BLA relays integrated sensory and affective information to the central nucleus in order to elicit various behaviours mediated by subcortical structures (Maren, 2001b). For example, projections from the central nucleus to the ventral medial hypothalamus and bed nucleus of the stria terminalis mediate increases in stress hormone release, projections to the lateral hypothalamus and dorsal motor nucleus of the vagus mediate increases in heart rate, projections to the parabrachial nucleus mediate increases in respiration, projections to the periaductal gray mediate freezing behaviour, and

projections to the nucleus reticularis points caudalis mediate potentiated startle (Maren, 2001b).

The BLA also has projections to the HPC, EC, and PRH (Krettek & Price, 1977b; Pikkarainen, Ronkko, Savander, Insausti, & Pitkanen, 1999; Pitkanen, Pikkarainen, Nurminen, & Ylinen, 2000; Shi & Davis, 2001). These projections are proposed to be important for the transmission of the rewarding or aversive properties of the stimuli that are also being processed in each of the latter structures (Campeau & Davis, 1995a; Rosen et al., 1992; White & McDonald, 2002).

1.1.2 The PRH and PORH

The PRH and PORH receive inputs from unimodal and polymodal sensory areas of the neocortex (Burwell & Amaral, 1998a; Deacon et al., 1983; Miller & Vogt, 1984; Shi & Davis, 2001). Therefore, the PRH and PORH are thought to be important for the integration of the polysensory properties of a stimulus, such as the coding of objects (Lavenex & Amaral, 2000). With regards to unimodal input, the PRH receives efferents from all sensory modalities (Burwell & Amaral, 1998a; Deacon et al., 1983; Miller & Vogt, 1984). However, olfactory inputs from the piriform cortex are most prominent (Bucci, Saddoris, & Burwell, 2002). All other unimodal cortical inputs to the PRH are roughly equally distributed across the remaining sensory modalities. The PORH mostly receives unimodal inputs from the visual and visuospatial association cortex (Burwell & Amaral, 1998a; Burwell et al., 1995). Some unimodal inputs to the PORH also originate in the auditory association areas, and from the other sensory modalities, but only weakly (Burwell & Amaral, 1998a; Burwell et al., 1995).

The PRH receives substantial polymodal inputs from the ventral temporal region and the agranular insular area (Burwell & Amaral, 1998a; Deacon et al., 1983; Guldin & Markowitsch, 1983; Ottersen, 1982; Saper, 1982). There are also weaker projections originating in the medial frontal, orbital frontal, and posterior parietal areas (Burwell & Amaral, 1998a; Deacon et al., 1983; Guldin & Markowitsch, 1983; Miller & Vogt, 1984). In contrast, polymodal inputs to the PORH come mostly from the ventro medial area (Burwell & Amaral, 1998a). To a lesser extent there are projections from the posterior parietal that terminate in PORH. There are also weak connections to the PORH that originate in the orbital frontal and agranular insular areas.

There are major outputs from the PRH and PORH to the EC and to a lesser extent the HPC (Burwell & Amaral, 1998a, 1998b; Kosel, Van Hoesen, & Rosene, 1983). It is thought that these connections relay integrated polysensory information, which the PRH and PORH received from the sensory cortical areas, for further processing and the formation of more elaborate representations (Baxter & Murray, 2001; Burwell, Saddoris, Bucci, & Wiig, 2004; Lavenex & Amaral, 2000; Murray et al., 2000). The PRH and PORH also have connections with the BLA (Burwell & Amaral, 1998a; Deacon et al., 1983; Ottersen, 1982; Saper, 1982; Shi & Davis, 2001) and these connections seem to be involved in associating sensory information of a stimulus with rewarding or aversive properties (Meunier & Bachevalier, 2002; Otto & Giardino, 2001; White & McDonald, 2002). It is also important to point out that there are strong reciprocal connections between the PRH and PORH (Burwell & Amaral, 1998b) and that both structures have projections to the unimodal and polymodal sensory areas from which they receive inputs (Burwell, 2000; Burwell et al., 1995).

1.1.3 The EC and HPC

The HPC consists of the dentate gyrus, the cell fields of the HPC proper (i.e., Ammon's horn or CA1-CA3 fields), and the subiculum and is considered to be involved in the integration of information that was already processed by the PRH, PORH, and EC (Lavenex & Amaral, 2000; Squire et al., 2004). The HPC receives direct inputs from a limited number of neocortical regions. Approximately two thirds of the HPC cortical inputs are from the EC via the perforant path (Steward, 1976; Steward & Scoville, 1976), which in turn receives major inputs from the PRH and PORH, but also from the olfactory cortex, orbital frontal cortex, insular cortex, cingulate cortex, and superior temporal gyrus (Burwell & Amaral, 1998b; Kosel, Van Hoesen, & Rosene, 1982; Kosel et al., 1983; Kosel, Van Hoesen, & West, 1981; Krettek & Price, 1977a; Steward, 1976; Steward & Scoville, 1976; Wyss, 1981). Thus, information would initially flow through the PRH and PORH, to the EC, which is thought to be a higher level of integration than the PRH and PORH, and finally to the HPC (Lavenex & Amaral, 2000; Squire & Alvarez, 1995; Squire et al., 2004; Suzuki & Eichenbaum, 2000). Moreover, it proposed that the HPC is principally involved in processing multidimensional information and/or establishing configurations between the stimuli that were processed earlier in the hierarchy (Lavenex & Amaral, 2000). The HPC also receives direct inputs from the PRH, PORH, AMY, as well as projections from subcortical structures via the fimbria-fornix (Burwell, 2000; French, Hailstone, & Totterdell, 2003; Kosel et al., 1983; Pikkarainen et al., 1999; von Bohlen und Halbach & Albrecht, 2002). The inputs from the AMY are thought to be important in providing rewarding or aversive properties to the information that is being

processed in the HPC (Anagnostaras, Gale, & Fanselow, 2001; Maren, 2001b; White & McDonald, 2002).

The HPC has major projections back to the EC, from where they are mostly distributed to the PRH and PORH (Burwell & Amaral, 1998a; Kosel et al., 1982; von Bohlen und Halbach & Albrecht, 2002). There are also projections from the HPC to the PRH and PORH (Burwell & Amaral, 1998a, 1998b). Thus, the information that was transmitted to and processed in the HPC is believed to flow back to the neocortex, such that higher order representations may interact with lower order information that is processed in the unimodal sensory cortices (Lavenex & Amaral, 2000). The HPC and EC have efferents to the AMY, which are thought to be involved in associating more highly processed information with affective properties (A. J. McDonald & Mascagni, 1997; Ottersen, 1982; Pitkanen et al., 2002; von Bohlen und Halbach & Albrecht, 2002). The HPC and EC also have projections to subcortical regions (Kosel et al., 1982). These latter connections are thought to transmit processed information from the HPC and EC to lower level regions directly involved in mediating behaviour (Squire et al., 2004; White & McDonald, 2002). In addition, the HPC has outputs to the prefrontal cortex and cingulate gyrus, which are considered involved in higher order behaviour, such as decision-making (Conde, Maire-Lepoivre, Audinat, & Crepel, 1995; Ishikawa & Nakamura, 2003; Jay, Glowinski, & Thierry, 1989).

1.2 LESION STUDIES AND FEAR CONDITIONING

The majority of our understanding about the contribution of brain structures to fear conditioning comes from studies assessing the performance of non-human animals with

localized brain lesions on fear-conditioning tests. Although lesion studies are not the only type of investigation that may elucidate the involvement of a structure in learning and memory for fear conditioning, they do offer compelling findings that are usually corroborated by evidence from other types of studies (e.g., electrophysiological and immunocytochemical studies). This section describes the findings from lesion studies examining the contributions of the AMY, HPC, and PRH to fear conditioning. The findings from other types of studies are included where appropriate.

It is important to consider that when lesion studies are conducted to determine the contribution of a brain structure to fear conditioning, retention may be assessed in subjects that received damage to the structure either before the training (i.e., pretraining lesions) or after the training (i.e., post-training lesions). When assessing fear conditioning following pretraining lesions, any deficit observed during the retention test may be attributable to anterograde amnesia, which is a deficit in remembering new information. In contrast, any deficit observed during the fear conditioning retention test following post-training lesions suggests retrograde amnesia, which is a deficit in remembering information acquired before the onset of the damage. The following section reviews the contributions of the AMY, HPC, and PRH to memory for fear conditioning based on anterograde and retrograde amnesia studies.

1.2.1 The AMY and Its Central Role In Fear Conditioning

The AMY is the brain structure that has been the most widely studied for its role in fear conditioning. Extensive evidence suggests that AMY lesions cause anterograde and retrograde amnesia for fear conditioning (Antoniadis & McDonald, 2001; Bermudez-Rattoni, Introini-Collison, Coleman-Mesches, & McGaugh, 1997; Dunn & Everitt, 1988;

M. Kim & Davis, 1993b; LaBar & LeDoux, 1996; Lee, Walker, & Davis, 1996; Liang et al., 1982; Maren, 1998, 1999a; Maren, Aharonov, & Fanselow, 1996; Muller, Corodimas, Fridel, & LeDoux, 1997; Parent, Avila, & McGaugh, 1995; Phillips & LeDoux, 1992; Poremba & Gabriel, 1999; Sananes & Davis, 1992; Wilensky, Schafe, & LeDoux, 1999). Moreover, it is thought that the AMY is involved in fear conditioning to all types of discrete stimuli (e.g., olfactory, visual, auditory) and to configural stimuli or contexts (Maren, 2001b).

Weiskrantz (1956) was the first to suggest that the AMY was involved in fear conditioning to discrete stimuli (i.e., cued fear conditioning). He reported that monkeys with MTL damage had impaired avoidance of a cue that was paired with shock. He also specified that the deficits were most pronounced when the MTL lesions included the AMY, suggesting that the AMY was the critical area involved in the conditioning. Many studies have since then investigated the effects of circumscribed AMY lesions, in a variety of species, on cued fear-conditioning tests. For instance, pre- and post-training AMY lesions typically impair avoidance of a tone or light that was associated with shock in monkeys, cats, dogs, and rats, suggesting that the AMY is involved in anterograde and retrograde memory for fear conditioning to auditory and visual stimuli (Brady et al., 1954; Fonberg, 1965; Horvath, 1963; King, 1958; Robinson, 1963; Thompson, 1981; Weiskrantz, 1956). Cued fear conditioning also seems to be impaired following AMY lesions when freezing is used as an index of fear and memory in rats. The presentation of a tone, light, or odour that was associated with shock will elicit sustained freezing in rats, if they cannot escape or avoid it. Pre- and post-training AMY lesions drastically reduce the amount of freezing evoked by these cues, suggesting that AMY damage causes both

anterograde and retrograde amnesia for an association between auditory, olfactory, or visual stimuli with a fear-eliciting event (Cousens & Otto, 1998; Gale et al., 2004; Goosens & Maren, 2001; Maren, 1998, 1999a; Muller et al., 1997; Phillips & LeDoux, 1992; Rudy, 1993). Similarly, fear-potentiated startle studies have implicated the AMY in memory for cued fear conditioning. Contrary to control rats, in the presence of a light that was previously paired with a shock, AMY-lesion rats do not startle more to a loud noise (Campeau & Davis, 1995b; M. Kim & Davis, 1993a, 1993b; Lee et al., 1996; Walker, Cassella, Lee, De Lima, & Davis, 1997). These results have been found whether the AMY was damaged before or after training, and again support the contention that AMY damage causes anterograde and retrograde amnesia for the association between a discrete visual stimulus and a fear-eliciting event. Thus, there is compelling evidence implicating the AMY in memory for fear conditioning to auditory, olfactory, and visual cues.

Extensive evidence also suggests that the AMY is involved in a memory for fear conditioning to a context (Antoniadis & McDonald, 2001; D. C. Blanchard & Blanchard, 1972; Goosens & Maren, 2001; J. J. Kim, Rison, & Fanselow, 1993; Maren, 1998, 1999a; Maren et al., 1996; Muller et al., 1997; Phillips & LeDoux, 1992). For instance, it is well established that rats will freeze when returned to a chamber in which they previously received inescapable shock (R. J. Blanchard, Dielman, & Blanchard, 1968a, 1968b; Fanselow, 1980). However, rats with either pre- or post-training AMY lesions usually freeze much less than control rats, if they freeze at all, suggesting severe anterograde and retrograde amnesia for contextual fear conditioning (Gale et al., 2004; Maren, 1998, 1999a; Maren et al., 1996; Muller et al., 1997). Similar findings have been reported when other behavioural indices have been assessed in contextual fear conditioning tests.

Specifically, AMY lesions prevent increased heart rate (Antoniadis & McDonald, 2001), increased stress hormone release (Goldstein, Rasmusson, Bunney, & Roth, 1996), and hypoalgesia (Helmstetter, 1992a; Watkins, Wiertelak, & Maier, 1993) in contexts that were associated with inescapable shock. Therefore, the AMY seems to have a crucial role in remembering that a context is associated with a fear-eliciting event.

It is important to specify that within the AMY, the BLA is thought to be the critical region involved in fear conditioning (Maren, 2001b; Maren & Fanselow, 1996). The BLA receives converging sensory, visceral, and affective (i.e., rewarding or aversive information) inputs from subcortical and cortical areas. These converging inputs are believed to make the BLA highly suitable for establishing multi-sensory associations, including associations between stimuli and fear-eliciting events (Maren, 2001b). Several lines of evidence support this contention (Clugnet & LeDoux, 1990; J. E. LeDoux, Cicchetti, Xagoraris, & Romanski, 1990; Maren, 2000; Parent & McGaugh, 1994; Quirk, Armony, & LeDoux, 1997; Quirk, Repa, & LeDoux, 1995; Rosen, Fanselow, Young, Sitcoske, & Maren, 1998). For example, neurotoxic lesions of the BLA, but not the central nucleus of the AMY, reduces freezing to a tone associated with a fear-eliciting event (Koo, Han, & Kim, 2004). Also, electrophysiological recording studies have reported increases in synaptic efficacy following cued fear conditioning in the BLA of rats (McKernan & Shinnick-Gallagher, 1997; Rogan, Staubli, & LeDoux, 1997). These changes in synaptic efficacy in the BLA are similar to those found following the induction of long-term potentiation (LTP), which is a leading mechanistic neuronal model of memory (Bliss & Collingridge, 1993). Therefore, it seems that fear conditioning is dependent on the AMY, and in particular the BLA.

Although the AMY has been widely implicated in fear conditioning, it has been proposed that the role of the AMY in memory for fear conditioning is not as critical as it seems (Cahill, Weinberger, Roozendaal, & McGaugh, 1999). This view partially stems from evidence demonstrating that AMY lesions not only disrupt conditioned fear, but also unconditioned fear responses (D. C. Blanchard & Blanchard, 1972; Burns, Annett, Kelley, Everitt, & Robbins, 1996; Dunn & Everitt, 1988; Kemble, Blanchard, & Blanchard, 1990; Kesner, Berman, & Tardif, 1992; Pesold & Treit, 1994; Vazdarjanova, Cahill, & McGaugh, 2001). For example, when presented with innately aversive stimuli, such as predators or novel situations, AMY-damaged rats avoid these stimuli less and freeze less than do control rats (D. C. Blanchard & Blanchard, 1972; Dunn & Everitt, 1988; Vazdarjanova et al., 2001). Consequently, it is difficult to determine with certainty whether deficits observed in a fear-conditioning test result from memory impairments or a general alteration in fear responses.

Evidence to support the contention that the AMY may not be critical for fear conditioning also arises from a few lesion studies that have demonstrated spared cued and contextual fear conditioning following AMY lesions (Cahill, Vazdarjanova, & Setlow, 2000; Helmstetter, 1992b; Helmstetter & Bellgowan, 1994; 2000; Lehmann, Treit, & Parent, 2003; Parent, Quirarte, Cahill, & McGaugh, 1995; Parent, Tomaz, & McGaugh, 1992; Parent, West, & McGaugh, 1994; Sutherland & McDonald, 1990; Vanderwolf, Kelly, Kraemer, & Streather, 1988). For instance, Vazdarjanova & McGaugh (1998) found that pretraining lesions of the BLA in rats did not block avoidance in a contextual fear-conditioning test. Sutherland and McDonald (1990) as well as Vanderwolf et al. (1988) have also shown that damage to the AMY does not cause anterograde amnesia for

contextual fear conditioning when the mean amount of defecation is used as an index of fear and memory.

More important to the aim of this thesis, pre- and post-training AMY damage does not impair avoidance of an object associated with a shock (Lehmann et al., 2000, 2003), suggesting that the AMY is not necessary for memory for object-fear conditioning. However, it has been argued that the mnemonic indices (i.e., latency to contact the object and number of contacts with the electrified object) used in these studies are unreliable measures of fear (Maren, 2003b). Avoidance of a cue associated with a shock does not necessarily depend on fear (Mineka, 1979; Mineka & Hendersen, 1985). One may avoid an object without experiencing emotional responses (Maren, 2003b). Thus, the assessment of other fear responses evoked by an object associated with a fear-eliciting event is needed before strong conclusions are made about the role of the AMY in object-fear conditioning.

In sum, the AMY is believed to be necessary for fear conditioning. Moreover, considerable evidence suggests that the AMY is involved in fear conditioning to stimuli, such as contexts, discrete olfactory, visual, and auditory cues. Hence, the AMY seems to have a central role in fear conditioning to all types of stimuli. However, there are cases in which AMY damage does not affect fear conditioning and, more importantly, some of these cases seem to include object-fear conditioning.

1.2.2 The HPC and Its Involvement In Contextual and Cued Fear Conditioning

The conventional view is that the HPC is necessary for memory of a context associated with a fear-eliciting event, but is not necessary for memory of a discrete stimulus associated with a fear-eliciting event (Anagnostaras et al., 2001; Fanselow,

2000; Maren, 2001a; O'Reilly & Rudy, 2001; Rudy & O'Reilly, 1999). Numerous studies have shown that pre- and post-training HPC lesions or lesions of its subregions impair contextual fear conditioning (Antoniadis & McDonald, 2001; J. J. Kim et al., 1993; Maren & Fanselow, 1997; Phillips & LeDoux, 1992, 1994; Selden, Everitt, Jarrard, & Robbins, 1991; Sutherland & McDonald, 1990). For instance, post-training lesions of the HPC in rats reduces freezing in and avoidance of a chamber that was paired with a shock (Anagnostaras, Maren, & Fanselow, 1999; Farr, Banks, La Scola, Flood, & Morley, 2000; J. J. Kim & Fanselow, 1992; Maren & Holt, 2004). In contrast, fear of a tone or light that was paired with a shock is typically spared following pre- and post-training damage to the HPC, suggesting that the HPC is not critical for learning and remembering cued fear conditioning (Anagnostaras et al., 1999; J. J. Kim & Fanselow, 1992; Phillips & LeDoux, 1992, 1994).

The theoretical explanation for the dissociation between contextual and cued fear conditioning impairments following HPC damage is that the structure is believed to be necessary for associations that involve the processing of configural representations, but not simple associations (Fanselow, 2000; Maren, 2001b; Matus-Amat, Higgins, Barrientos, & Rudy, 2004; O'Reilly & Rudy, 2001; Rudy & O'Reilly, 1999). It is thought that, during fear conditioning, the HPC processes the configuration of stimuli that are present during the fear-eliciting event, but not each stimuli independently (Fanselow, 2000; Matus-Amat et al., 2004; Rudy & O'Reilly, 1999, 2001). Moreover, it is hypothesized that the HPC transmits this configural information to the AMY through direct connections in order to establish an association with the fear-eliciting stimulus (Maren, 2001b). Thus, contextual fear conditioning would be dependent on the HPC and

the AMY because the HPC is involved in processing the configuration of the various stimuli that comprise the context during the fear-eliciting event, whereas the AMY is involved in processing the aversive properties of the event. Conversely, the HPC would not be involved in cued fear conditioning because it only involves the processing of independent stimuli.

There are studies in which HPC damage did not impair memory for contextual fear conditioning (Maren, Aharonov, & Fanselow, 1997; Richmond et al., 1999; Rudy, Barrientos, & O'Reilly, 2002). For example, Maren and colleagues (1997) showed that pretraining lesions of the HPC in rats did not reduce freezing to a context previously associated with shock, suggesting that the lesions did not induce anterograde amnesia for contextual fear conditioning. However, it has been argued that these findings were due to the rats associating the shock with a distinct feature that comprises the context, suggesting that cued fear conditioning accounted for the spared memory and not intact contextual fear conditioning (Matus-Amat et al., 2004).

One study found an absence of retrograde amnesia for contextual fear conditioning following HPC lesions in rats (McNish, Gewirtz, & Davis, 1997). Specifically, rats with post-training HPC lesions had increased startle in a context in which they previously received shock. It is unclear why HPC lesions did not impair contextual fear conditioning in this study. However, this study seems to be an exception.

Although there is a general consensus that the HPC is involved in contextual fear conditioning, the common view that the HPC is not necessary for cued fear conditioning is currently being revised. The conclusion that the HPC is not necessary for cued fear conditioning typically comes from studies in which only the dorsal HPC was damaged

(Anagnostaras et al., 1999; J. J. Kim & Fanselow, 1992; Maren et al., 1997; Phillips & LeDoux, 1992, 1994). Dorsal HPC lesions spare the ventral parts of the dentate gyrus and of the CA1-CA3 fields, and completely spare the subiculum. Recent studies that assessed the effects of lesions that encompassed the dorsal and ventral HPC or solely the ventral HPC found that the lesions not only impaired contextual fear conditioning, but also cued fear conditioning to a tone (Bast, Zhang, & Feldon, 2001b; Maren, 1999b; Maren & Holt, 2004; Richmond et al., 1999). These latter findings suggest that the HPC may have a more extensive role in fear conditioning than previously believed and that the HPC, in particular the ventral HPC, is involved in cued fear conditioning. However, impairments for cued fear conditioning following HPC lesions have only been reported for auditory stimuli. It is unclear whether damage to the HPC that includes the dorsal and ventral regions would impair cued fear conditioning to other types of discrete stimuli. Moreover, germane to this thesis, it is unknown whether complete HPC damage would impair cued fear conditioning to an object.

Interestingly, the retrograde amnesia for fear conditioning following HPC damage appears to be temporally graded (Anagnostaras et al., 1999; J. J. Kim & Fanselow, 1992; Maren et al., 1997). For example, lesions of the dorsal HPC in rats made 1-d, but not 28-d, after training in a contextual fear-conditioning test impaired freezing (J. J. Kim & Fanselow, 1992). These data were interpreted as suggesting that the HPC has a temporary role in memory for contextual fear conditioning. These findings also support the contention that the HPC is involved in consolidation of certain types of memory (Anagnostaras et al., 2001; Squire et al., 2004). However, these finding need to be interpreted with caution because, again, the lesions in these studies were limited to the

dorsal HPC. It is possible that temporally graded retrograde amnesia would not have been detected if the lesions had encompassed the entire HPC.

Overall, it is agreed that the HPC is important for contextual fear conditioning and new evidence suggests that the HPC is also important for cued fear conditioning. In addition, there is evidence that the HPC involvement in fear conditioning is temporary. However, it is unknown whether the HPC is involved in object-fear conditioning and, if it is, whether its involvement is temporary.

1.2.3 The PRH and Fear Conditioning to Discrete and Configural Stimuli

The contributions of the PRH to fear conditioning have not been as extensively studied as the contributions of the AMY and HPC. Nevertheless, the PRH seems to have an important role in fear conditioning to contexts and discrete olfactory, visual, and auditory stimuli (Burwell et al., 2004; Campeau & Davis, 1995a; Herzog & Otto, 1997, 1998; Rosen et al., 1992).

Studies have demonstrated that PRH lesions induce amnesia for cued fear conditioning to discrete olfactory stimuli (Herzog & Otto, 1997, 1998; Otto, Cousens, & Herzog, 2000; Otto & Giardino, 2001). For instance, rats with pretraining lesions of the PRH freeze significantly less than control rats when exposed to an odour that was paired with a footshock (Herzog & Otto, 1997). It is proposed that this impairment is due to the PRH lesions disrupting the processing of olfactory information. The PRH receives inputs from all sensory modalities, but approximately half of its sensory inputs are olfactory (Burwell, 2000). Moreover, it is believed that the olfactory information that is processed in the PRH is transmitted to the AMY, via direct projection, in order to establish an association between the odour and the fear-eliciting event (Otto & Giardino, 2001).

Although the anterograde amnesia for fear conditioning to an odour is believed to be a disruption in processing olfactory information, the amnesia is not likely due to an olfactory perceptual impairment. Rats with damage to the PRH are able to accurately perform an odour-guided nonmatching-to-sample task (Otto & Eichenbaum, 1992) and olfactory discriminations (Otto, Schottler, Staubli, Eichenbaum, & Lynch, 1991). These latter findings suggest that the PRH is not necessary for recognizing odours and that the deficits in fear conditioning to an odour are due to a deficit in associating the odour with the fear-eliciting event.

Unfortunately, the effects of post-training lesions of the PRH on cued fear conditioning to an odour have not been examined. Thus, it is unclear whether damage to the PRH would cause retrograde amnesia for cued fear conditioning to odour.

Evidence suggests that the PRH is also involved in cued fear conditioning to discrete visual stimuli. Post-training lesions of the PRH in rats impair fear-potentiated startle to a light that was previously paired with footshock (Campeau & Davis, 1995a; Rosen et al., 1992). However, pretraining PRH lesions do not disrupt performance on this task (Campeau & Davis, 1995a). Thus, it seems that damage to the PRH causes retrograde, but not anterograde, amnesia for fear conditioning to discrete visual cues.

This type of dissociation between retrograde and anterograde amnesia have often been reported in lesion studies and have been interpreted as suggesting that the damaged structure is normally involved in the mnemonic processing, but that the organism may meet the demands of new situations without the structure (Fanselow, 2000; Mumby & Glenn, 2000; Thornton, Rothblat, & Murray, 1997). Hence, the retrograde amnesia for fear-potentiated startle to a light would suggests that the PRH is typically involved in

processing visual information during fear conditioning. However, the absence of anterograde amnesia following fear-potentiated startle to a light would suggest that other structures are capable of supporting fear conditioning to discrete visual stimuli in the event of PRH damage.

It is proposed that the retrograde deficits for fear conditioning to visual cues following PRH lesions are due to a disruption in the processing of mnemonic visual information that is sent to the AMY (Campeau & Davis, 1995a). The PRH receives visual information from the visual cortices (Deacon et al., 1983; Miller & Vogt, 1984) and subcortical areas (Guldin & Markowitsch, 1983) and is thought to store this information (Campeau & Davis, 1995a). It is also suggested that the PRH interacts with the AMY, through direct projections (Burwell & Amaral, 1998a), to associate the visual information with the fear-eliciting event (Campeau & Davis, 1995a). Consequently, damage to the PRH after fear conditioning would disrupt the transmission of stored visual information to the AMY that was associated with the fear-eliciting event. Conversely, if the PRH is damaged before the fear conditioning, it is believed that the visual information would be stored in other areas - possibly the insular cortices or visual cortex, which also have projections to the AMY (Burwell & Amaral, 1998a; Miller & Vogt, 1984; Shi & Davis, 2001). This possibility would explain the absence of anterograde amnesia for cued fear conditioning to visual stimuli in the absence of the PRH.

Lesions of the PRH also appear to only cause retrograde amnesia for cued fear conditioning to discrete auditory stimuli. In rats, post-training lesions of the PRH impair fear-potentiated startle to a tone previously associated with footshock (Campeau & Davis, 1995a; Rosen et al., 1992). Similarly, post-training damage to the insular temporal cortex

in cats (i.e., the homologue of the perirhinal cortex) impair avoidance of auditory signal that was paired with a fear-eliciting stimulus (Guldin & Markowitsch, 1983; Neff, Diamond, & Casseday, 1975). In contrast, pretraining PRH lesions in rats do not impair fear-potentiated startle nor freezing to a tone previously paired with footshock (Campeau & Davis, 1995a; DiCara, Braun, & Pappas, 1970; Romanski & LeDoux, 1992a, 1992b).

The retrograde amnesia following PRH lesions for auditory fear conditioning is interpreted as suggesting that the PRH usually processes and stores auditory information coming from the auditory thalamus and auditory cortex and interacts with the AMY to establish an association with the fear-eliciting event (Campeau & Davis, 1995a). It is also proposed that the absence of anterograde amnesia for auditory fear conditioning following damage to the PRH may be due to other structures supporting the conditioning in the absence of the PRH. Specifically, in the event of PRH damage, it is possible that the auditory thalamus and/or auditory cortex, which have direct projections to the AMY, may sufficiently support the conditioning (Campeau & Davis, 1995a).

Several studies have implicated the PRH in contextual fear conditioning (Bucci et al., 2000; Bucci et al., 2002; Burwell et al., 2004; Corodimas & LeDoux, 1995; Sacchetti, Lorenzini, Baldi, Tassoni, & Bucherelli, 1999; Tassoni, Lorenzini, Baldi, Sacchetti, & Bucherelli, 1999). For instance, pre- and post-training lesions or inactivation of the PRH in rats reduce freezing to and avoidance of a context that was associated with a shock (Bucci et al., 2000; Bucci et al., 2002; Corodimas & LeDoux, 1995). Other studies have failed to demonstrate a role for the PRH in contextual fear conditioning (Herzog & Otto, 1997, 1998; Phillips & LeDoux, 1995). However, the lesions in these latter studies did not include damage to the entire rostral-caudal extent of the PRH and it has been

suggested that impairments would have been found if the lesions had encompassed the entire PRH (Bucci et al., 2000; Bucci et al., 2002; Burwell et al., 2004). Hence, it is believed that the PRH has an important role in contextual fear conditioning.

It was originally believed that the role of the PRH in contextual fear conditioning was possibly to serve as an interface between the sensory cortices and the HPC for the processing of configural information (Corodimas & LeDoux, 1995; Phillips & LeDoux, 1995). This belief was mostly based on anatomical data demonstrating that the PRH has strong connections with the HPC and the known role of the HPC in contextual fear conditioning. However, it has been shown that the PRH and HPC are differentially involved in the processing of configural information in spatial memory tasks, such as the water maze (Burwell et al., 2004; Glenn & Mumby, 1998; Glenn, Nesbitt, & Mumby, 2003; Mumby, Astur, Weisend, & Sutherland, 1999; Sutherland, McDonald, Hill, & Rudy, 1989). Moreover, configural information may still reach the HPC following PRH damage through parallel and redundant pathways. Thus, it is assumed that the PRH has an important role in contextual fear conditioning, which is distinct from that of the HPC (Burwell et al., 2004).

In summary, the PRH is important for fear conditioning to discrete cues, whether olfactory, visual, or auditory, and to context. However, lesions of the PRH more reliably impair fear conditioning when induced after training then before training. Despite the evidence implicating the PRH in fear conditioning, its role in object-fear conditioning has never been investigated.

1.3 OBJECT RECOGNITION

The above section described the known contributions of the AMY, HPC, and PRH to fear conditioning. Compelling evidence suggests that each of these structures are involved in fear conditioning. However, studies have seldom addressed the issue of whether these structures are involved in fear conditioning to an object. This oversight is surprising, since the AMY, HPC, and PRH have all been implicated, to a certain extent, in object recognition (Aggleton, Blindt, & Rawlins, 1989; Bachevalier, Parkinson, & Mishkin, 1985; Clark, West, Zola, & Squire, 2001; Clark, Zola, & Squire, 2000; Gaffan, Parker, & Easton, 2001; Gaskin et al., 2003; Mahut, Zola-Morgan, & Moss, 1982; Murray et al., 2000; Zola et al., 2000; Zola-Morgan, Squire, Amaral et al., 1989) and that the association between an object and fear-eliciting event possibly involves object-recognition processes.

Object recognition refers to the ability to discriminate between objects that have previously been encountered and objects that have never been encountered (Mumby, 2001). It is well established that combined damage to the AMY, HPC, and PRH causes object-recognition impairments in humans, monkeys, and rats (see Mumby, 2001; Squire et al., 2004). Moreover, damage limited to either the PRH or HPC is sufficient to disrupt object recognition (Buffalo et al., 1999; Bussey, Saksida, & Murray, 2003; Clark et al., 2001; Clark et al., 2000; Glenn & Mumby, 1996; Mumby & Pinel, 1994; Zola et al., 2000; Zola-Morgan, Squire, Amaral et al., 1989).

It was initially proposed that the HPC was the central structure for object recognition (Gaffan, 1974; Mishkin, 1978). Evidence suggested that lesions of the HPC, in monkeys, produced impairments on an object delayed nonmatching-to-sample task, in

which monkeys, after a delay, were required to select a novel object over a previously encountered object in order to receive a food reward (Bachevalier & Mishkin, 1989; Gaffan, 1974; Mishkin, 1978; Zola-Morgan & Squire, 1985). It was also shown that HPC lesions impaired object delayed matching-to-sample performance, in which monkeys had to select a previously encountered object over a novel object in order to obtain a food reward (Gaffan, 1974). Thus, it seemed that the HPC was essential for remembering information about objects.

However, the role of the HPC in object recognition was overemphasized. The brain lesions in the early studies implicating the HPC in object recognition not only included damage to the HPC, but also damage to the surrounding structures, such as the AMY, PRH, PORH, and EC (Bachevalier & Mishkin, 1989; Gaffan, 1974; Mahut et al., 1982; Mishkin, 1978; Zola-Morgan & Squire, 1985; Zola-Morgan, Squire, & Amaral, 1989b; Zola-Morgan, Squire, & Mishkin, 1982). Therefore, it was unclear whether the object-recognition deficits observed in the early studies resulted specifically from damage to the HPC, damage to the other structures, or combined damage to the HPC and neighboring structures.

In the past few years, it has been shown that damage limited to the HPC, in monkeys, do not cause object-recognition deficits that are as severe as lesions limited to neighboring structures, such as the PRH (Baxter & Murray, 2001). In addition, lesions restricted to the HPC in rats typically cause little, if any, deficits in the delayed non-matching-to-sample test, suggesting that object-recognition is intact following HPC damage in rats (Mumby, 2001; c.f., Squire, Clark, & Knowlton, 2001). Combined, these

findings suggest that the HPC is not as critical as originally believed for object recognition.

Yet, the evidence demonstrating that the HPC is not essential for object recognition stems from anterograde amnesia tests. For instance, the commonly used delayed nonmatching -to-sample task is designed to assess memory for information acquired after the onset of HPC damage and not information acquired before the onset of the damage. Therefore, the HPC may play a role in object recognition for information acquired before the damage. In fact, damage to the HPC can cause retrograde amnesia for object-recognition appears to be dependent on the HPC. A recent study assessed the effects of pre- and post-training HPC lesions on object-recognition test and found retrograde, but not anterograde, amnesia in rats (Gaskin et al., 2003). Specifically, Gaskin and colleagues found that rats with post-training, but not pretraining, HPC damage did not show the normal preference for a novel object over a previously encountered object. These latter findings suggest that the HPC has a role in object recognition, but predominantly for objects that were encountered before the onset of the damage.

In the early object-recognition studies, it was also noticed that damage to the HPC caused greater deficits when it included extensive damage to the AMY (Aggleton et al., 1989; Bachevalier et al., 1985; Murray & Mishkin, 1984; Saunders et al., 1984). The synergistic effect of damage to the HPC and AMY was interpreted as suggesting that the AMY and HPC were critically related for the processing of object-recognition information (Saunders et al., 1984). Thus, it seemed that the AMY was an important structure for object recognition. However, again in these studies, the excision of the AMY and the HPC also included damage to the overlying cortex and efferent fibers from

the PRH that course the AMY. Therefore, it was unclear whether the AMY indeed contributed to object recognition.

It is now clear that the AMY is not significantly involved in object recognition (Mumby, Pinel, Kornecook, Shen, & Redila, 1995; Murray & Mishkin, 1998; Zola-Morgan, Squire, & Amaral, 1989a). When the AMY is damaged through techniques that spare the surrounding fibers and cortical structures (cytotoxic lesions) object recognition performance is intact (Murray & Mishkin, 1998). It has also been demonstrated that cytotoxic lesions of the AMY do not enhance the object-recognition deficits that follow HPC lesions (Murray & Mishkin, 1998).

The PRH is currently believed to be critical for object recognition (Murray et al., 2000). The PRH is adjacent to the HPC and was typically damaged in the initial studies implicating the HPC in object recognition. Research thus focused on the effects of lesions restricted to the PRH on object recognition tests and extensive evidence suggests that PRH lesions severely disrupt object recognition (Buffalo et al., 1999; Bussey et al., 2003; Glenn & Mumby, 1996; Thornton et al., 1997; Zola-Morgan, Squire, Amaral et al., 1989). For instance, lesions of the PRH in monkeys and rats impair delayed non-matching-to-sample performance (Buffalo et al., 1999; Gaffan & Murray, 1992; Glenn & Mumby, 1996; Zola-Morgan, Squire, Amaral et al., 1989). Moreover, pre- and post-training lesions of the PRH impair novel object preference (Bussey, Duck, Muir, & Aggleton, 2000; Bussey, Muir, & Aggleton, 1999; Ennaceur, Neave, & Aggleton, 1997; Mumby, Glenn, Nesbitt, & Kyriazis, 2002). Therefore, it seems that the PRH is necessary for object-recognition.

In addition to the evidence from lesion studies implicating the PRH in object recognition, evidence from electrophysiological and immuno-histochemical studies suggests that the PRH is important in determining the familiarity of an object (Brown & Xiang, 1998; Fahy, Riches, & Brown, 1993; Wan, Aggleton, & Brown, 1999). Generally, neurons in the PRH of monkeys respond with their highest firing rate when exposed to a novel visual stimulus, such as an object, and this response decreases as the stimulus becomes more familiar over repeated exposure (Brown & Xiang, 1998; Fahy et al., 1993). Also, there is greater expression of the immediate early gene c-fos in the PRH of rats exposed to a novel object than a familiar object (Wan et al., 1999). Therefore, the findings from several types of studies suggest that the PRH is involved in object recognition.

In summary, the PRH and HPC seem to be important for object-recognition. In contrast, object recognition appears to be independent of the AMY. The contribution of the PRH and HPC to object recognition has important implications for object-fear conditioning. Given that object-fear conditioning may involve object-recognition processes, damage to neural structures, such as the PRH and HPC, that are implicated in object recognition has the potential to disrupt object-fear conditioning.

1.4 THE SHOCK-PROBE FEAR-CONDITIONING TEST

An ideal test to assess learning and memory of a fear-eliciting event associated with an object is the shock-probe fear-conditioning test. The shock-probe test has been widely used to study defensive responses. It involves placing a rat into a chamber with bedding material or another loose substrate (e.g., sand) covering the floor (see Figure 2). A small-

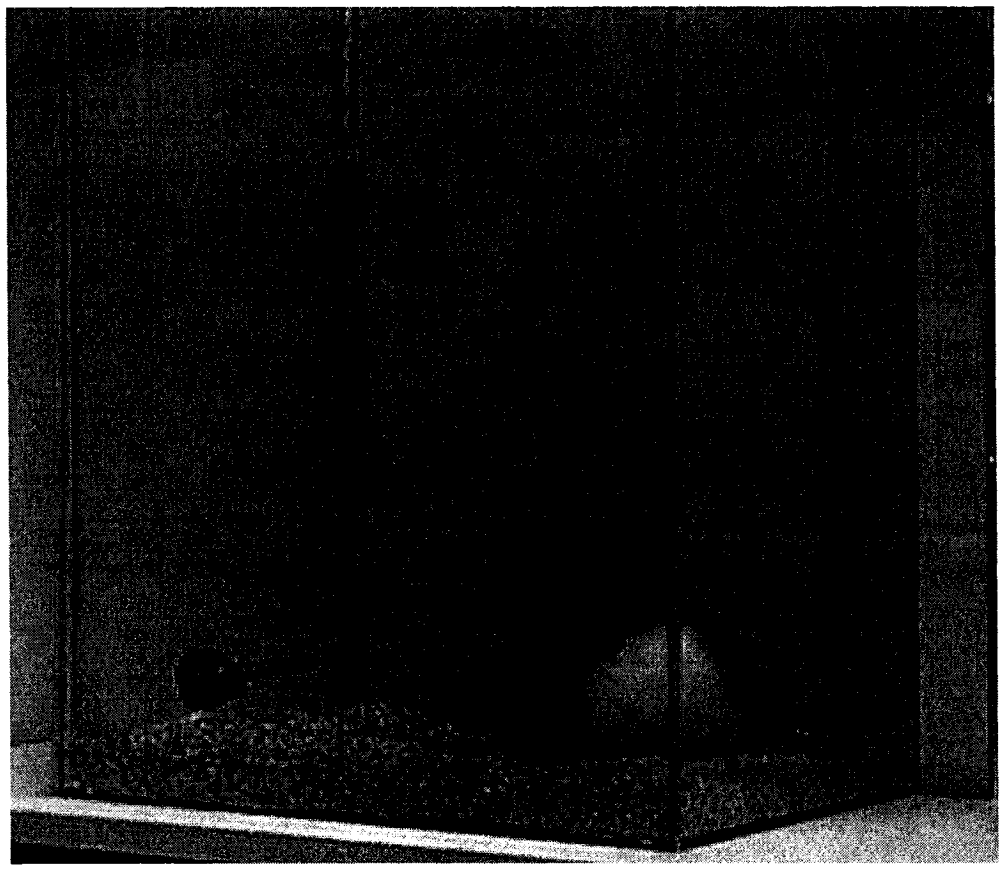

Figure 2. Illustration of the shock-probe fear-conditioning apparatus

electrified probe (i.e., object) protrudes from one wall slightly above the floor of the chamber, such that if the rat contacts the probe with its paw or snout it receives a mild shock. Typically, after being shocked, rats will bury the probe with the bedding material and avoid contacting it again (Kopchia, Altman, & Commissaris, 1992; Pinel & Treit, 1978; Terlecki, Pinel, & Treit, 1979; Treit, Pesold, & Rotzinger, 1993; Treit, Pinel, & Fibiger, 1981).

Burying behaviour in rats is typically considered to be an adaptive response to cope with dangers or threats (Moser & Tait, 1983; Pinel & Treit, 1978; Terlecki et al., 1979). For instance, in the wild, rats will bury the entrance of their underground nests in the presence of a territorial threat (Moser & Tait, 1983). Others believe that the response is indicative of nesting behaviour (Fanselow, Sigmundi, & Williams, 1987). However, situations that typically elicit sustained burying are situations that involve aversive stimuli, suggesting that it is largely a fear or defensive response. Burying is also considered an innate response in rats because it can be elicited in rats that were reared in environmental conditions in which they were deprived of any substrate with which to bury (Pinel, Symons, Christensen, & Tees, 1989).

Compelling evidence suggests that the burying and avoidance responses elicited in the shock-probe test are behaviours from which we can reliably infer a general state of fear in rats. Anxiolytic drugs, such as diazepam, midazolam, and pentobarbital significantly reduce burying and avoidance of the electrified probe (Menard & Treit, 1999; Treit, 1985, 1990; Treit & Fundytus, 1988; Treit et al., 1981; Tsuda, Ida, & Tanaka, 1988). Conversely, drugs that increase anxiety, such as yohimbine and pentylenetretrazol typically increase burying and avoidance in this test (Treit, 1990;

Tsuda et al., 1988). Similar findings have been found with compounds that directly affect stress hormone release (Korte, Korte-Bouws, Bohus, & Koob, 1994).

The shock-probe test can be used to assess fear conditioning. Rats will bury the probe and avoid contacting it when re-exposed to it at a later time (Lehmann et al., 2000, 2003; Pinel & Treit, 1978; Roozendaal, Oldenburger, Strubbe, Koolhaas, & Bohus, 1990; Terlecki et al., 1979). These latter findings suggest that the association between the probe and the shock is remembered and that the mere re-exposure to the probe without being shocked elicits fear. In addition, it has been established that, in the shock-probe test, rats do not incidentally bury the probe because it is the most salient feature in the chamber. When tested in a chamber with two probes and where only one was associated with a shock, rats selectively bury the probe that was the source of the aversive stimulus (Pinel & Treit, 1978). Moreover, the amount of time spent burying is significantly reduced if the appearance of the probe is changed once the rats have been shocked from it, suggesting the visual properties of the probe are associated with the shock (Pinel, Treit, & Wilkie, 1980).

The shock-probe fear-conditioning test has many advantages. It does not involve extensive training, several overt behaviours may be assessed non-obtrusively (burying, the latency to contact the object, and even where the rats spend their time in the chamber) as indices of memory and fear, and it involves the assessment of behaviours that rats naturally engage in when encountering fearful situations in the wild. The shock-probe conditioning test also has advantages over more commonly used cued fear-conditioning tests. Generally cued fear-conditioning tests, such as auditory fear conditioning or fear-potentiated startle involve the use of intangible stimuli that are never the source of the

aversive stimulation (e.g., a tone associated with a shock that is delivered through the floor). Thus, in these tests, the cue and aversive stimulation are not spatially contiguous. In contrast, the delivery of the shock in the shock-probe fear-conditioning test is contiguous with the probe, since the object is the actual source of the aversive stimulus.

In sum, the shock-probe fear-conditioning test involves rat's remembering that an object is associated with a fear-eliciting event. It is also a test that enables the assessment of several overt behavioural responses, so that fear conditioning to an object can be comprehensively evaluated.

1.5 SUMMARY AND MAIN QUESTIONS

Fear conditioning refers to the ability to learn and remember that a stimulus predicts the occurrence of a fear-eliciting event. The neurobiology of fear conditioning to auditory, olfactory, and visual stimuli and to contexts has been extensively studied, but the neurobiology of fear conditioning to an object has not been thoroughly investigated. It is unclear which brain structures contribute to the learning and memory of an association between an object and a fear-eliciting event.

It is reasonable to hypothesize that the neural structures that are critical for cued fear conditioning and even contextual fear conditioning may also be involved in fear conditioning to an object. Moreover, the neural structures that play a role in object-recognition possibly also contribute to object-fear conditioning. Thus, the focus of this thesis was to determine whether memory for an association between a fear-eliciting event and an object depends on the AMY, HPC, or PRH – three structures that have been shown to be important in object recognition and/or fear conditioning.

The following three chapters describe and discuss the results of a series of experiments that were designed to assess object-fear conditioning following, HPC, PRH, and AMY lesions. Overall, it was hypothesized that pre- and post-training lesions of each structure would impair memory for an association between an object and a fear-eliciting event in the shock-probe fear-conditioning test.

Specifically, the main goal of the experiments described in Chapter 2 was to determine whether lesions of the HPC induce anterograde and retrograde amnesia for object-fear conditioning. It has been clearly established that the HPC is necessary for contextual fear conditioning and recent findings have also implicated the HPC in cued fear conditioning. Further, the evidence from object-recognition studies suggests that the HPC has an important role in object-recognition. Combined, this evidence raised the possibility that the HPC is central to object-fear conditioning and that pre- and post-training HPC lesions would impair memory in the shock-probe fear-conditioning test.

The experiments described in Chapter 3 were aimed at finding whether the PRH damage would cause anterograde and retrograde amnesia for object-fear conditioning. It is well established that the PRH is necessary for object-recognition and the findings from several studies suggest that the PRH is also essential for fear conditioning to different types of stimuli. Therefore, it seemed likely that the PRH would be involved in object-fear conditioning and it was hypothesized that pre- and post-training lesions of the PRH would cause memory impairments in the shock-probe fear-conditioning test.

The experiments described in Chapter 4 focused on establishing whether the AMY is necessary for object-fear conditioning. The AMY has been shown to be crucial for fear conditioning to many types of stimuli, raising the possibility that it would also be

involved in object-fear conditioning. However, the findings of two recent studies suggest that object-fear conditioning is not dependent on the AMY. Perhaps the conclusions of the latter two studies are premature, since only avoidance of the object was assessed and it is argued that avoidance does not necessarily involve the expression of fear. Consequently, it remained possible that the AMY would be involved, and the goal of the experiments in Chapter 4 was to determine the effects of pre- and post-training AMY lesions on object-fear conditioning.

CHAPTER 2

THE HPC AND OBJECT-FEAR CONDITIONING

The goal of the experiments in this chapter was to evaluate the contribution of the HPC to object-fear conditioning. Evidence from many fear-conditioning studies suggests that the HPC is not necessary for memory of an association between a discrete cue and a fear-eliciting event (Anagnostaras et al., 1999; J. J. Kim & Fanselow, 1992; Phillips & LeDoux, 1992, 1994). However, this evidence stems from lesion studies in which the damage was limited to the dorsal HPC. Recent studies have demonstrated that damage to the ventral HPC or the entire HPC produce memory deficits for an auditory cue associated with a fear-eliciting event (Bast, Zhang, & Feldon, 2001a; Maren, 1999b; Maren & Holt, 2004; Richmond et al., 1999). But, whether the HPC is involved in fear conditioning to a non-auditory cues is unknown.

It has also been shown that the HPC is involved in object recognition (Clark et al., 2001; Gaskin et al., 2003). This latter evidence combined with the recent evidence implicating the HPC in cued fear conditioning raise the possibility that the HPC may play an important role in object-fear conditioning.

The experiments included in this chapter were aimed at extending the findings implicating the HPC in cued fear conditioning by assessing the effects of HPC lesions (encompassing both the dorsal and ventral HPC) on memory for object-fear conditioning. The main purpose of Experiment 1 was to determine whether HPC lesions caused anterograde amnesia for an association between an object (i.e., a probe) and a shock in the shock-probe fear-conditioning test. Another aim of Experiment 1 was to evaluate anterograde amnesia for contextual fear conditioning in the shock-probe fear-

conditioning test following HPC damage. It was found that HPC lesions did not cause anterograde amnesia for object-fear conditioning, but did impair contextual fear conditioning in the shock-probe test.

Experiment 2 was designed to determine whether HPC lesions caused retrograde amnesia for object-fear conditioning in the shock-probe fear-conditioning test and whether this amnesia, if any, would be temporally graded. It was found that HPC damage caused severe memory deficits for cued fear conditioning and that these deficits were long lasting.

2.1 EXPERIMENT 1: The Effects of Pretraining Lesions of the HPC In the Shock-Probe Fear-Conditioning Test

Recent studies that assessed the effects of lesions of the ventral HPC or the entire HPC found that the lesions not only impaired contextual fear conditioning, but also cued fear conditioning (Bast et al., 2001a; Maren, 1999b; Maren & Holt, 2004; Richmond et al., 1999). These latter findings suggest that the HPC may have a more extensive role in fear conditioning than previously believed. Consequently, shock-probe fear conditioning was used to investigate whether the lesions of the HPC caused anterograde amnesia for object-fear conditioning.

To test the role of the HPC in learning and remembering object-fear conditioning, rats were given two sessions in the shock-probe test. The first session served as the conditioning session, whereas the second session assessed memory for the association between the probe and the shock. Rats were either tested for retention in the same context they were trained in or in a different context. This procedure enabled the assessment of the fear conditioning to the discrete cue and enabled the evaluation of contextual fear conditioning.

2.1.1 METHODS

2.1.1.1 Subjects

Male Long-Evans rats (Charles River, Quebec; 300-350 g) were housed individually in standard laboratory cages, kept on a 12:12 light-dark cycle (lights on at 08:00), provided with food and water *ad libitum*. Rats were acclimated to vivarium

conditions for at least one week prior to the surgery and all testing was conducted during the light phase.

2.1.1.2 Surgery

Rats were anesthetized with sodium pentobarbitol (Somnotol, 65 mg/kg, i.p.; Bimeda-MTC, Cambridge, Ontario) or isoflurane (Janssen, Toronto, Ontario) in 0.8 L/min oxygen at 14.7 PSIA at 21°C (Benson Medical Industries, Markham, Ontario). They were then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA) and a midline scalp incision was made to expose the top of the skull. The HPC lesions were made by intrahippocampal infusions of *N*-methyl-D-aspartic acid (NMDA; 7.5 μg/μl; Sigma Chemical Co., St. Louis, MO) at 10 sites bilaterally (see Table 1 for coordinates) The infusions were done sequentially through an injection needle attached to a 10 μl Hamilton syringe via polyethylene tubing (PE-50). At each site, a total volume of .4 μl was infused at a flow rate of .15 μl per minute. The injection needle was left in place for an additional 2.5 min following the injection to facilitate diffusion. Following the lesions, the scalp incision was closed using wound clips and an antibiotic powder (Cicatrin, GlaxoWellcome) was applied to the wound. As the rats began awakening, they were given a dose of diazepam (.2cc; 10mg/ml, i.p.; Hoffman-La Roche, Mississauga, Ontario) as a prophylaxis against seizures. The same surgical procedures were used for the SHAM rats, except that no damage was done to the skull or brain of these rats. All the rats were allowed to recover for a minimum of two weeks before beginning behavioural testing.

2.1.1.3 Behavioural Procedure

Prior to testing, the rats were habituated to one of two shock-probe chambers. Each chamber was made of Plexiglas and measured 40 cm long X 30 cm wide X 40 cm

TABLE 1. Injection coordinates relative to Bregma (in mm) for neurotoxic (NMDA) lesions of the HPC

Anteroposterior (AP)	**Mediolateral (ML)**	**Dorsoventral (DV)**
-3.1	±1.0	-3.6
-3.1	±2.0	-3.6
-4.1	±2.0	-4.0
-4.1	±3.5	-4.0
-5.0	±3.0	-4.1
-5.0	±5.2	-5.0
-5.0	±5.2	-7.3
-5.8	±4.4	-4.4
-5.8	±5.1	-6.2
-5.8	±5.1	-7.5

high with 2 cm of bedding material covering the floor. To make the two chambers different (i.e., different contexts), three of the walls of one chamber were opaque, whereas all the walls of the other chamber were transparent and they were located in different testing rooms. Each rat was given four consecutive daily 15-min habituation sessions during the week that preceded the acquisition session. The probe was not present during the habituation sessions and the rats randomly assigned to a specific chamber for habituation.

No more than 2-d and no less than 1-d after the completion of habituation, rats were given an acquisition session. For this session, each rat spent 15-min in the shock-probe chamber with which they had been familiarized. From the start of the session a wire-wrapped Plexiglas probe (6.0 × 0.5 × 0.5 cm) was protruding from the center of one of the walls, 3 cm above the floor bedding. For some of the rats the probe was constantly electrified (3 mA) and for the others it was not (NAÏVE rats). The total number of contact-induced shocks and the amount of time spent burying was measured in each rat exposed to the electrified probe. In addition, the rats' behavioural reaction to each shock was scored according to a four-point scale that ranges from a score of 1 for a flinch involving head or forepaw to a score of 4 for a whole body flinch and jump (all four paws in the air) followed by running to the opposite end of the chamber (Treit, Pesold et al., 1993).

Twenty-four hours later, retention performance was assessed in a second 15-min shock-probe session. The procedure was the same as for the acquisition session with the exception that the probe was not electrified for any of the rats and the rats were either tested in the same chamber they received their acquisition session in (CONGRUENT) or

the other chamber (INCONGRUENT). The behaviour of each rat was videotaped for both the acquisition and retention sessions. Indices of fear and measures of retention included the latency to initially contact the probe, the amount of time spent with all four paws in the half area of the chamber where the probe was located, and the amount of time spent burying. The amount of time the rats spent immobile (no skeletal movement except for breathing) was also measured manually using a stopwatch and used as an inverse measure of activity. The probe and the walls of the chamber were washed with acetone, and urine or feces was removed from the bedding after each test.

2.1.1.4 Histology

Upon completion of behavioural testing, all rats received an overdose of sodium pentobarbital (1 cc; 65 mg/ml, i.p.), and were perfused intracardially with 0.9% saline followed by 10% formalin. Their brains were excised and stored in a 10% formalin-30% sucrose solution for at least 48 hr and then sectioned (40 μm), mounted on gelatin-coated slides, and stained with cresyl violet. The stained sections were examined through a light microscope (Leica, Germany) to examine the extent of the lesions. The data from rats that did not have damage to both the ventral and dorsal HPC bilaterally were excluded from the statistical analyses.

2.1.1.5 Statistical Analyses

Planned-comparisons were conducted with the nonparametric one-tailed Mann-Whitney U-tests because homogeneity of variance and normality of the distribution were occasionally violated for the acquisition session and the retention test data. Although some behavioural measures did not violate any of the assumptions required for parametric analyses, the pattern of results did not change using either parametric or

nonparametric tests. Hence, for ease of communication, nonparametric analyses were used in all instances. Also, the immobility data can be found in Appendix A and was analyzed with the non-parametric Kruskal-Wallis test because this behaviour was not used as an index of fear or memory, but rather as a measure of activity, and did not involve specific predictions.

2.1.2 RESULTS

2.1.2.1 Histology

Figure 3 illustrates the extent of the HPC lesions for the rats that were exposed to the electrified probe during acquisition and tested in the CONGRUENT or INCONGRUENT context. The NMDA injections produced extensive cell loss in all principle subfields of the HPC, dentate gyrus, and subiculum for each lesion rat. The damage to the dorsal HPC was pronounced in all rats, but in 6 rats there was some minor sparing of dentate granule cells and CA1 pyramidal neurons in the most temporal portions of the HPC. Cells in the dorsal lateral CA2 and CA3 fields were also partially spared unilaterally in 7 rats. The extent of damage to the ventral HPC was also extensive, where there was bilateral loss of cells in this area in each rat. However, in 9 rats there was some minor sparing of the CA fields unilaterally and sparing of cells in the most posterior part of the subiculum in most rats. In all HPC rats there was also some damage to the posterior parietal cortex where the injection cannulae were inserted. Some rats also sustained minor damage to fimbria/fornix. No damage was found in the thalamus, AMY, or rhinal cortex. Four rats were excluded from the study because there was too little

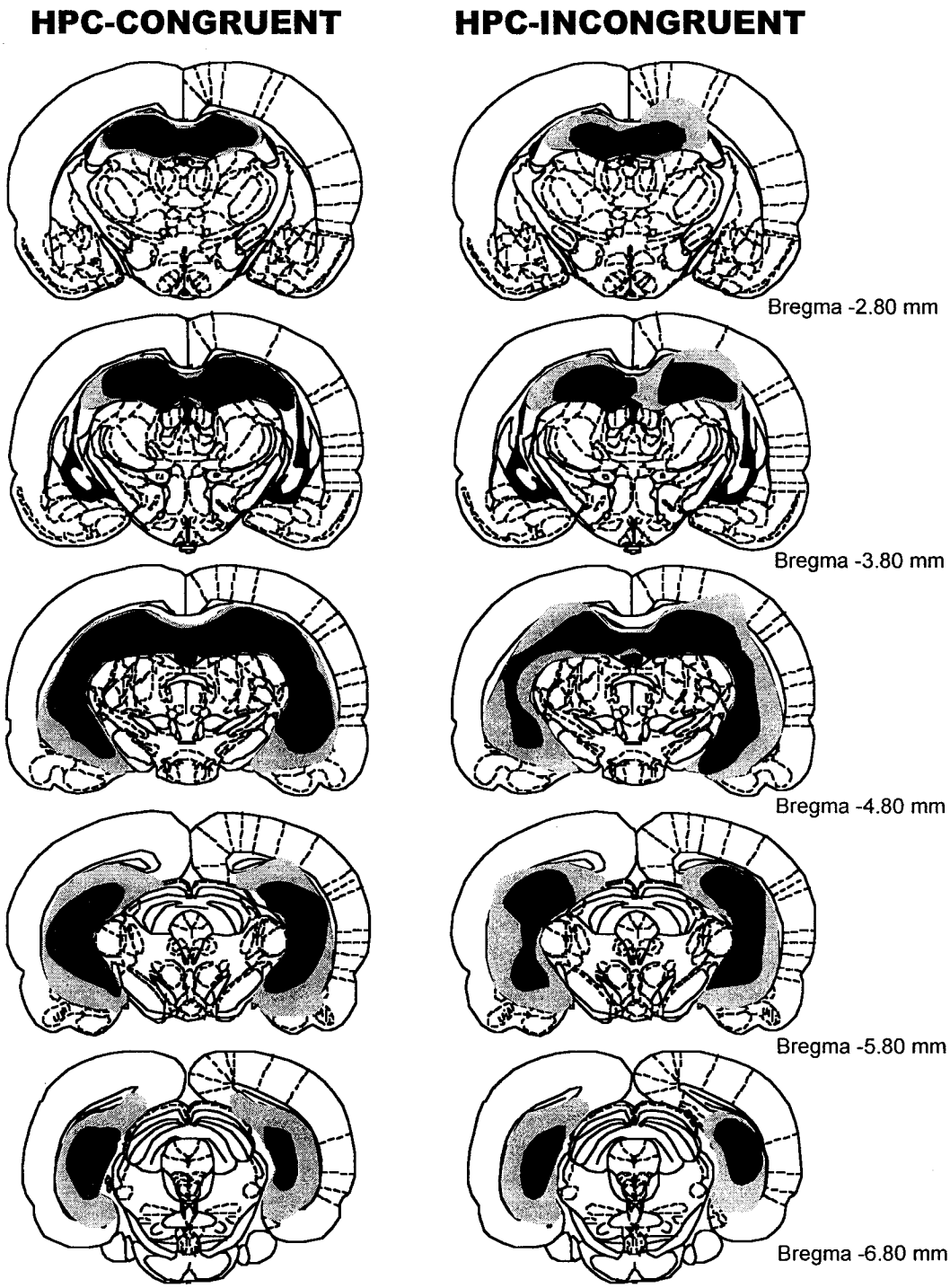

Figure 3. Illustrations of the smallest (dark grey) and largest (light gray) lesion observed bilaterally through the rostral and caudal extent of the HPC for HPC-CONGRUENT and HPC-INCONGRUENT rats. Atlas plates are from Paxinos and Watson (1997).

damage to either the dorsal or ventral HPC in one hemisphere. Another three rats were excluded from the study because there was extensive cortical damage.

2.1.2.2 Behavioural Results

The data from one rat in the HPC-CONGRUENT group were excluded from the experiment because its burying score on the retention test was more than four standard deviations above the group mean.

The data from the four NAÏVE control groups (SHAM or HPC and CONGRUENT or INCONGRUENT) were pooled because their scores on the acquisition session and retention test were not statistically different (see appendix B). Consequently, the analytical comparisons of the data involved five groups. The NAÏVE group (n= 36), which included all the rats exposed to the non-electrified probe during acquisition, and four groups of rats exposed to the electrified probe during acquisition: SHAM-CONGRUENT (n = 29), SHAM-INCONGRUENT (n = 23), HPC-CONGRUENT (n= 20), and HPC-INCONGRUENT (n = 16).

2.1.2.2.1 ***Acquisition.*** Figure 4 shows the number of contact-induced shocks the rats received during the acquisition session. There was a tendency for an increased number of shocks for HPC-CONGRUENT rats when compared to SHAM-CONGRUENT rats, $U = 225.0, p = .083$. However, this tendency was not evident when HPC-INCONGRUENT rats were compared to SHAM-INCONGRUENT rats, $U = 182.5, p = .483$.

Figure 5 shows the amount of time spent burying during the acquisition session. The rats that were exposed to the electrified probe buried more than rats that were exposed to the non-electrified probe (SHAM-CONGRUENT vs. NAÏVE, $U = 116.5, p < .001$; SHAM-INCONGRUENT vs. NAÏVE, $U = 85.0, p < .001$; HPC-CONGRUENT vs..

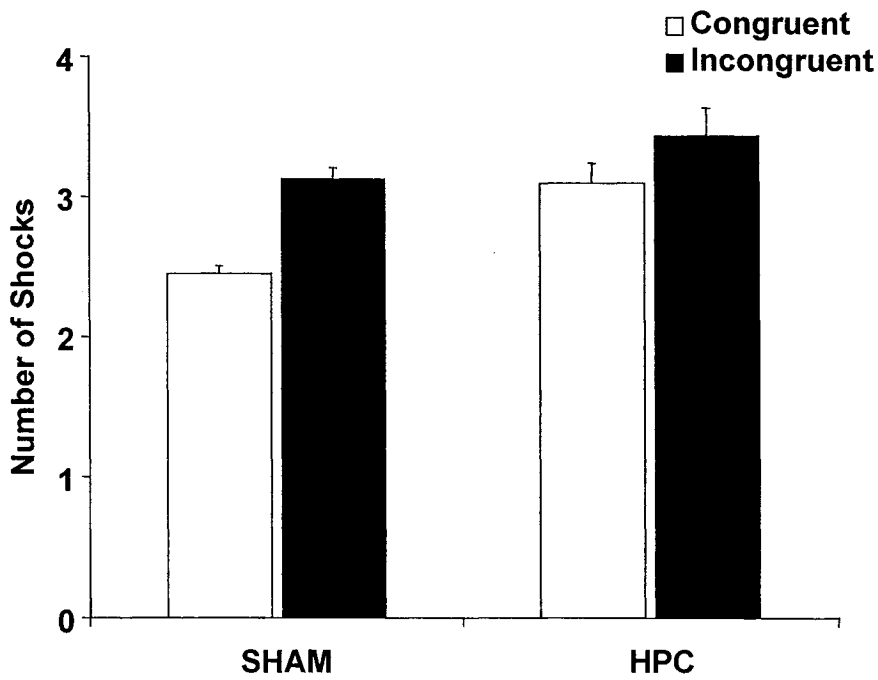

Figure 4. Mean (± *SEM*) number of contact-induced shocks received during the acquisition session by SHAM and HPC rats that were exposed to the electrified probe.

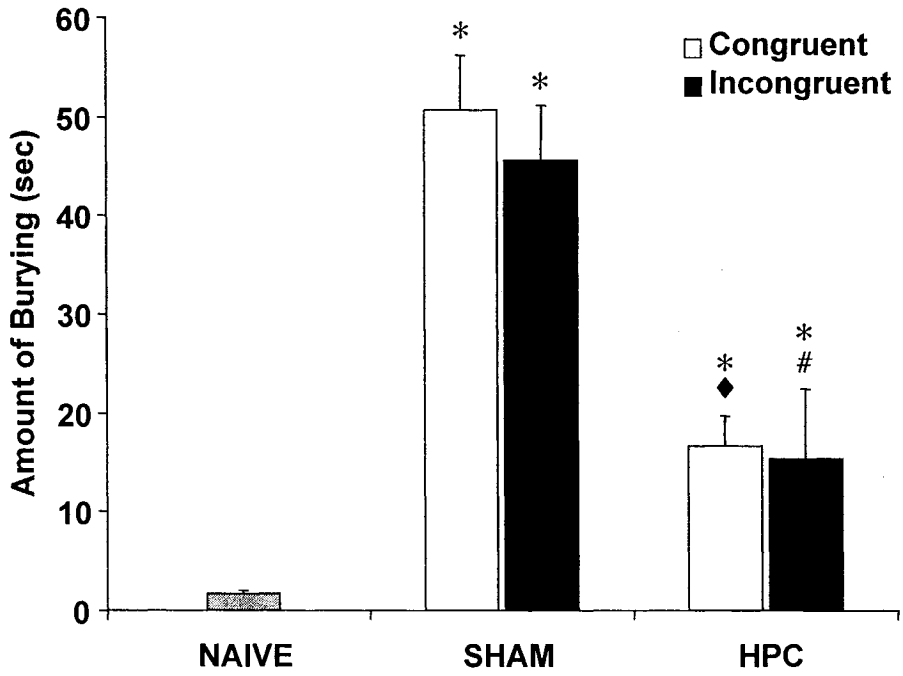

Figure 5. Mean (± *SEM*) amount of time spent burying during the acquisition session by NAÏVE, SHAM, and HPC rats (*$p < .05$ versus NAIVE; ♦$p < .05$ versus SHAM-CONGRUENT; #$p < .05$ versus SHAM-INCONGRUENT).

NAIVE, $U = 289.5, p < .05$; HPC-INCONGRUENT vs. NAIVE, $U = 224.0, p < .05$). However, the HPC rats did not bury as much as the SHAM rats (HPC-CONGRUENT vs. SHAM-CONGRUENT, $U = 143.0, p < .01$; HPC-INCONGRUENT vs. SHAM-INCONGRUENT, $U = 62.5, p < .001$). There was no significant difference in shock reactivity between SHAM-CONGRUENT rats ($M = 2.21$, $SEM = 0.04$) and HPC-CONGRUENT rats ($M = 2.31$, $SEM = 0.04$), $U = 287.0, p = .475$, nor between SHAM-INCONGRUENT ($M = 2.21$, $SEM = 0.05$) and HPC-INCONGRUENT rats ($M = 2.46$, $SEM = 0.06$), $U = 144.0, p = .132$.

2.1.2.2.2 *Retention.* Figure 6 shows the amount of time spent burying during the retention test. Regardless of the context they were tested in, SHAM rats buried significantly more than NAÏVE rats (SHAM-CONGRUENT vs. NAIVE, $U = 296.0, p < .001$; SHAM-INCONGRUENT vs. NAÏVE, $U = 223.5, p < .001$). In contrast, the HPC rats did not bury more than NAÏVE rats (HPC-CONGRUENT vs. NAIVE, $U = 315.5, p <= .175$; HPC-INCONGRUENT vs. NAIVE, $U = 245.5, p = .127$). Moreover, HPC rats buried significantly less than their respective SHAM comparison group (HPC-CONGRUENT vs. SHAM-CONGRUENT, $U = 191.0, p < .05$; HPC-INCONGRUENT vs. SHAM-INCONGRUENT, $U = 85.0, p < .001$). The change of context on the retention test did not affect burying for either SHAM or HPC rats. No significant differences in time spent burying were found between the SHAM-CONGRUENT and -INCONGRUENT rats, $U = 331.0, p = .481$, nor between HPC-CONGRUENT and -INCONGRUENT rats, $U = 124.0, p = .066$.

Figure 7A illustrates the latency to initially contact the probe on the retention test. Regardless of the context they were tested in, SHAM and HPC rats had significantly

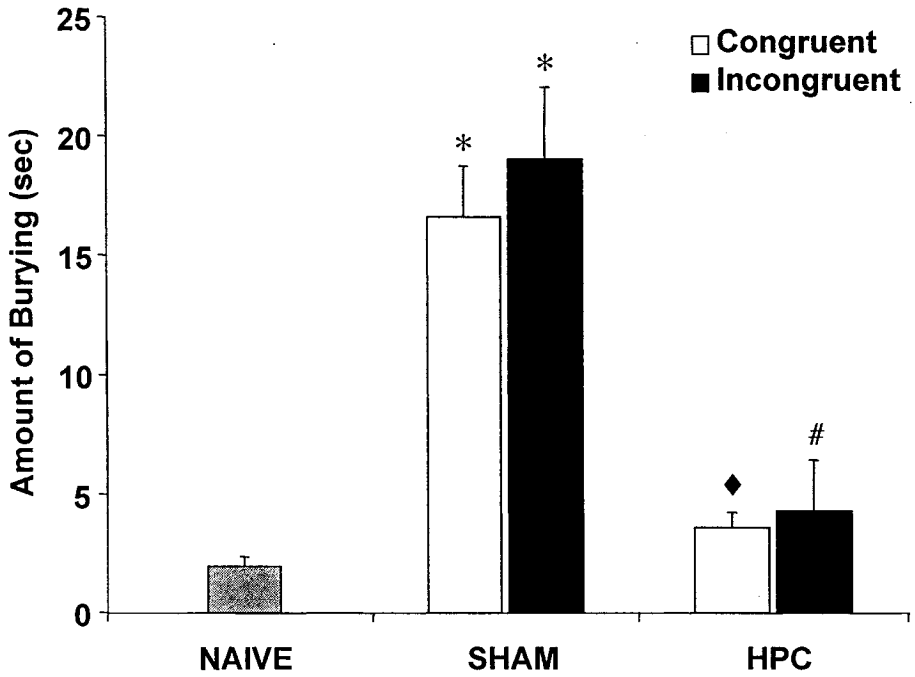

Figure 6. Mean (± *SEM*) amount of time spent burying during the retention test by NAÏVE, SHAM, and HPC rats tested in the CONGRUENT and INCONGRUENT contexts (*$p < .05$ versus NAIVE; ♦$p < .05$ versus SHAM-CONGRUENT; #$p < .05$ versus SHAM-INCONGRUENT).

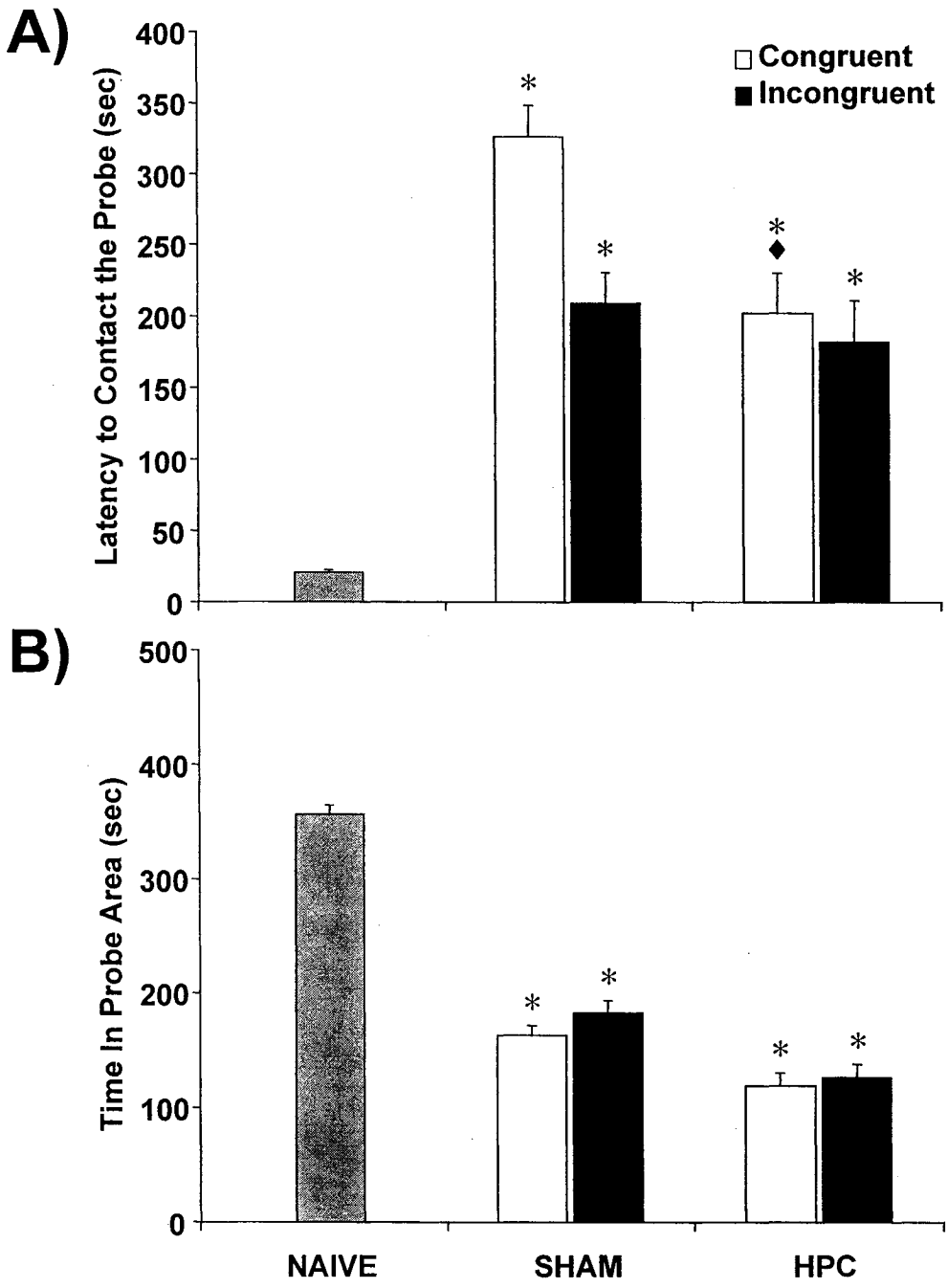

Figure 7. Mean (± *SEM*) **(A)** latency to contact the probe and **(B)** amount of time spent in the probe area of the chamber during the retention test for NAÏVE, SHAM, and HPC rats tested in the CONGRUENT and INCONGRUENT contexts (*$p < .05$ versus NAIVE; ♦$p < .05$ versus SHAM-CONGRUENT).

longer latencies than NAÏVE rats (SHAM-CONGRUENT vs. NAÏVE, $U = 54.0, p < .001$; SHAM-INCONGRUENT vs. NAÏVE, $U = 122.0, p < .001$; HPC-CONGRUENT vs. NAÏVE, $U = 73.5, p < .001$; HPC-INCONGRUENT vs. NAÏVE, $U = 142.0, p < .01$). Interestingly, the latencies to contact the probe differed between SHAM and HPC rats, but this effect was context dependent. Specifically, HPC-CONGRUENT rats contacted the probe more quickly than the SHAM-CONGRUENT rats, $U = 200.0, p < .05$, whereas no difference was found between the latencies of HPC-INCONGRUENT and SHAM-INCONGRUENT rats, $U = 157.0, p = .221$. The change of context on the retention test did not significantly reduce the latencies to contact the probe for SHAM rats (SHAM-INCONGRUENT vs. SHAM-CONGRUENT, $U = 255.5, p = .076$), nor HPC rats (HPC-INCONGRUENT vs. HPC-CONGRUENT, $U = 138.0, p = .242$).

Figure 7B illustrates the amount of time spent in the half of the chamber where the probe was located. Regardless of the testing context, SHAM and HPC rats spent significantly less time in the probe area than NAÏVE rats (SHAM-CONGRUENT vs. NAÏVE, $U = 169.0, p < .001$; SHAM-INCONGRUENT vs. NAÏVE, $U = 147.5, p < .001$; HPC-CONGRUENT vs. NAIVE, $U = 66.0, p < .001$; HPC-INCONGRUENT vs. NAÏVE, $U = 56.0, p < .001$). In addition, the amount of time spent in the probe area did not differ between HPC and SHAM rats (HPC-CONGRUENT vs. SHAM-CONGRUENT rats, $U = 206.0, p = .097$; HPC-INCONGRUENT vs. SHAM-INCONGRUENT, $U = 140.0, p = .109$). The change of context on the retention test did not affect the amount of time spent in the probe area for the SHAM nor the HPC rats (SHAM-CONGRUENT vs. SHAM-INCONGRUENT, $U = 229.5, p = .335$; HPC-CONGRUENT vs. HPC-INCONGRUENT, $U = 138.0, p < .322$).

2.1.3 DISCUSSION

The findings of the present experiment suggest that lesions of the HPC cause anterograde amnesia in the shock-probe fear-conditioning test. Rats with HPC lesions tested in the same context in which they had been trained (i.e., congruent context) did not bury more than rats that had never been shocked and they contacted the probe more quickly than SHAM rats. However, the amnesia seemed mild because even though the HPC rats did not perform as well as SHAM rats, they did not contact the probe as quickly as the NAÏVE rats and they spent very little time in the area of the chamber where the probe was located suggesting that they retained some information. The amnesia also appeared to be related to a contextual fear conditioning impairment and not an object-fear conditioning impairment. The probe avoidance for HPC and SHAM rats that were tested solely for fear conditioning to the probe in the incongruent context did not differ. Thus, the removal of contextual information associated with shock eliminated the impairment that was apparent in the congruent context.

The contextual fear conditioning impairment adds to the extensive evidence implicating the HPC in contextual learning (Antoniadis & McDonald, 2001; J. J. Kim et al., 1993; Maren & Fanselow, 1997; Phillips & LeDoux, 1992, 1994; Selden et al., 1991; Sutherland & McDonald, 1990). In contrast, the finding that HPC rats were able to successfully avoid the probe in the incongruent context does not support the recent findings from several studies suggesting that the HPC and especially the ventral HPC is necessary for cued fear conditioning (Bast et al., 2001b; Maren, 1999b; Maren & Holt, 2004; Richmond et al., 1999). However, all these studies used an auditory cue, whereas an object was used in the current experiment. In shock-probe fear-conditioning, different

features of the probe (e.g., visual, olfactory) may account for the learning and memory of the association between the probe and the shock. Although it is unclear what features are learned and remembered, the findings demonstrate that the HPC is not necessary for avoidance of an object that was associated with shock.

Unfortunately, the burying response could not be used as a reliable index of fear conditioning because the HPC lesions impaired the response during both the acquisition and retention sessions. Even though HPC rats buried during the acquisition session, they buried much less than SHAM rats. Consequently, the minimal amounts of burying by HPC rats during the retention test are probably due to a floor effect and not a fear conditioning impairment. It is important to note that the ventral HPC seems to be the region of the HPC involved in the burying response in the shock-probe test. Lesion studies have shown that damage to the dorsal HPC does not impair burying in the shock-probe test (Degroot & Treit, 2004; Treit & Menard, 1997), but that lesions of the ventral HPC significantly impair the behaviour (Degroot & Treit, 2004). Combined, these findings suggest that the burying deficits in the current experiment are likely the result of ventral HPC damage.

It is interesting that the HPC lesions only impaired one of the two avoidance measures that were assessed. The lesions reduced the latencies to contact the probe in the congruent context, but had no effect on the amount of time the rats spent in the half area of the chamber where the probe was located. The innate motivation of rats to explore their environment and the objects in it (Renner, Bennett, & White, 1992; Renner & Seltzer, 1991) is most likely accountable for this dissociation. Avoidance of the probe area does not require the rats to inhibit this exploration. The rats may explore this area

and yet overall spend very little time in it. However, avoiding contacting the probe requires greater inhibition of this innate motivation because they cannot fully investigate the object. When avoiding contact with the probe, the rats cannot touch or chew it like they typically do when exploring an object. Consequently, it may require more fear and a stronger memory to avoid contacting the probe than it does for avoiding the area in which it is located.

The absence of anterograde amnesia for the association between the probe and the shock is not likely due to incomplete lesions of the HPC. In all the lesion rats there was extensive damage to both the dorsal and ventral regions of the HPC. There was occasionally a little sparing of the ventral HPC, which is believed to be the area of the HPC essential for cued fear conditioning (Bast et al., 2001a). However, the damage to the ventral HPC was comparable to that of other studies that found impairments in auditory fear conditioning (Maren, 1999b; Richmond et al., 1999). In addition, the multiple 0.4μl injections of NMDA throughout the extent of the ventral HPC probably damaged more cells than the number of cells inactivated by a single 0.5μl infusion of the sodium channel blocker tetrodotoxin that has been shown to be effective in impairing cued fear conditioning (Bast et al., 2001b).

It is difficult to imagine that the increased activity levels that are often observed in HPC rats (D. C. Blanchard, Blanchard, Lee, & Fukunaga, 1977; Douglas & Isaacson, 1964; Maren, 1999b; Maren et al., 1997; Maren & Fanselow, 1997; Richmond et al., 1999) could account for the contextual fear conditioning impairment in the shock-probe fear-conditioning test. In fact, the HPC rats tended to spend more time immobile during the retention test (see Appendix A) than SHAM rats, suggesting that the HPC rats were

not hyperactive. In addition, the HPC rats were able to avoid the probe as well as SHAM rats when they were tested in the incongruent context. Even in the congruent context, the HPC rats spent very little time in the area of the chamber where the probe was located. This latter finding suggests that HPC rats were not more likely to contact the probe because of their location in the chamber than SHAM rats or HPC rats tested in the incongruent context. This finding also suggests that the HPC rats did not randomly navigate through the chamber and contact the probe because of an ambulation impairment. Therefore, the avoidance impairment of HPC rats tested in the congruent context seems to be caused by an inability to learn and remember that the chamber is associated with shock.

In sum, the present findings demonstrate that pretraining HPC lesions do not impair avoidance of an object associated with a shock. However, the findings suggest that pretraining HPC lesions impair the ability of contextual fear conditioning to modulate avoidance of an object associated with a fear-eliciting event. Therefore, damage to the HPC does not cause anterograde amnesia for object-fear conditioning, but does induce anterograde amnesia for contextual fear conditioning.

2.2 EXPERIMENT 2: The Effects of Post-Training Lesions of the HPC In the Shock-Probe Fear-Conditioning Test

According to the findings of the previous experiment, the HPC is not necessary for object-fear conditioning. However, There are many cases of dissociations between retrograde and anterograde amnesia in the literature (Campeau & Davis, 1995a; Fanselow, 2000; Mumby & Glenn, 2000; Thornton et al., 1997). Thus, the absence of anterograde amnesia does not imply that HPC lesions will not cause retrograde amnesia.

There is evidence suggesting that post-training HPC damage does cause memory deficits for an association between a discrete cue and a fear-eliciting event (Anagnostaras et al., 1999; J. J. Kim & Fanselow, 1992; Maren et al., 1997). This evidence is based on lesion studies that limited the damage to the dorsal HPC and several studies have demonstrated that lesions that encompass the entire HPC formation are more likely to impair memory than partial HPC lesions (Richmond et al., 1999). Moreover, a very recent study demonstrated that damage to the ventral HPC resulted in retrograde amnesia for an association between an auditory stimulus and a fear-eliciting event (Maren & Holt, 2004). Hence, the possibility remains that post-training damage that encompassed the entire HPC formation would cause retrograde amnesia for object-fear conditioning.

In support of the possibility that HPC lesions will cause retrograde amnesia for object-fear conditioning is evidence from a retrograde amnesia study implicating the HPC in object-recognition (Gaskin et al., 2003). In addition, damage to the HPC occasionally causes temporally graded retrograde amnesia, in which memory for information acquired soon before the damage is forgotten and information acquired long before the damage is spared (see Squire & Alvarez, 1995). More importantly, temporally

graded retrograde amnesia has been reported in contextual fear conditioning tests following HPC damage (Anagnostaras et al., 1999; J. J. Kim & Fanselow, 1992; Maren et al., 1997). Hence, it seems that the HPC has a temporary role in memory for stimuli associated with a fear-eliciting event. These latter findings raise the possibility that HPC damage induced only soon after, but not long after, learning object-fear conditioning will impair memory.

Consequently, the goal of Experiment 2 was to investigate whether lesions of the HPC would cause retrograde amnesia for object-fear conditioning and whether such amnesia would be temporally graded. Specifically, memory was assessed following damage of the entire HPC induced either 1-d or 2-wk after training in the shock-probe fear-conditioning test. These two delays were selected because contextual fear conditioning findings suggest that these intervals are sufficiently long to detect evidence of a temporal gradient for the retrograde amnesia following HPC lesions (J. J. Kim & Fanselow, 1992).

2.2.1 METHODS

2.2.1.1 Subjects

Male Long-Evans rats (Charles River, Quebec; 300-350 g) served as subjects. Their housing conditions were the same as in Experiment 1 with the exception that they were provided with approximately 30 g of food daily.

2.2.1.2 Behavioural Procedure

2.2.1.2.1 ***Habituation and Acquisition.*** The procedures for habituation and acquisition were identical to those of Experiment 1 with the exception that only the transparent shock-probe chamber was used.

2.2.1.2.2 ***Surgery.*** Following acquisition, rats were assigned to either the RECENT or REMOTE condition and then assigned to either receive SHAM or HPC lesions. Rats in the REMOTE condition received surgery 14 days following the acquisition session, whereas rats assigned to the recent condition received surgery 1 day following the acquisition session. The rats that were exposed to the electrified probe during the acquisition session were roughly matched according to the number of shocks they received, their average shock reactivity, and the amount of time they spent burying prior to being assigned to either condition or surgical group.

The same surgical procedures as in Experiment 1 were used with the exception that the rats were limited to a 13-14 days recovery period and food was available *ad libitum* only during the first 7 days of this period.

2.2.1.2.1 ***Retention***. After recovery from surgery, each rat's retention performance was assessed in a second 15 min shock-probe session. The rats were all tested in the same chamber (i.e., transparent chamber) in which they received conditioning (i.e., acquisition session) and the probe was not electrified for any of the rats. Measures of fear and indices of retention were the same as those in Experiment 1.

The behaviour of each rat was videotaped for both the acquisition and retention sessions. The probe and the walls of the chamber were washed with acetone, and urine or feces was removed from the bedding after each test.

2.2.1.4 Histology

The same procedures as in Experiment 1 were used.

2.2.1.5 Statistical Analyses

The same procedures as in Experiment 1 were used.

2.2.2 RESULTS

2.2.2.1 Histology

Figure 8 illustrates the extent of the HPC lesions for the rats that were exposed to the electrified probe during the acquisition session in the REMOTE and RECENT conditions. The NMDA injections produced extensive cell loss in all principle subfields of the HPC, as well as in the dentate gyrus and subiculum for each lesion rat. The damage to the dorsal HPC was pronounced in all rats, but in 2 rats there was some unilateral sparing of dentate granule cells and CA1-CA3 pyramidal neurons in the most temporal portions of the HPC. The extent of damage to the ventral HPC was also extensive, where there was bilateral loss of cells in this area in each rat. However, there was some minor sparing of the most ventral part of the HPC in 4 rats unilaterally and in 7 rats bilaterally. There was also some sparing of cells in the most posterior part of the subiculum in 4 rats. In all HPC rats there was some damage to the posterior parietal cortex where the injection cannulae were inserted. Some rats also sustained minor damage to fimbria/fornix . No damage was found in the thalamus, AMY, or rhinal cortex. Three rats were excluded from the study because there was too little damage to either the dorsal or ventral HPC in one or both hemispheres.

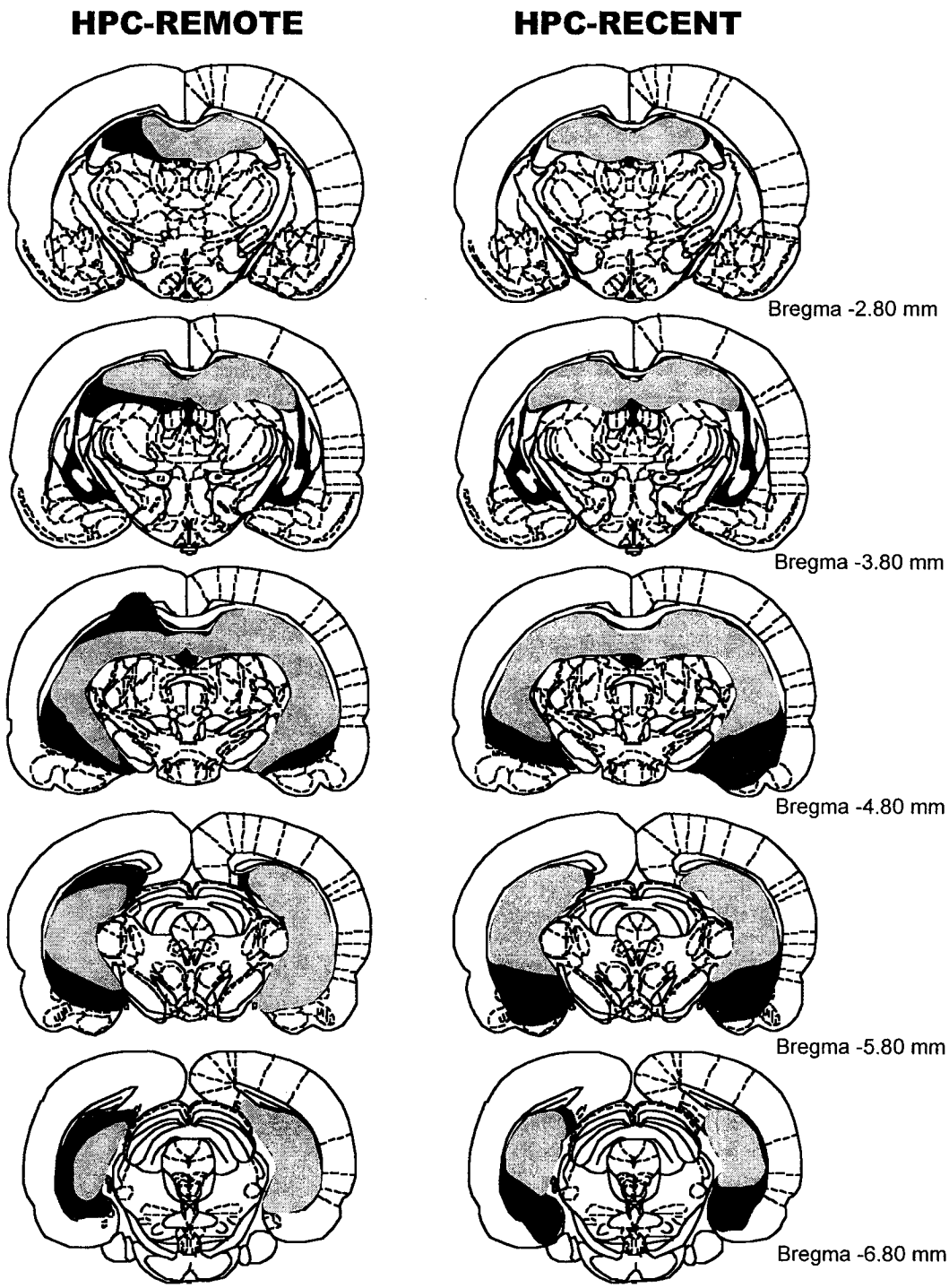

Figure 8. Illustrations of the smallest (dark grey) and largest (light gray) lesion observed bilaterally through the rostral and caudal extent of the HPC for HPC-REMOTE and HPC-RECENT rats. Atlas plates are from Paxinos and Watson (1997).

2.2.2.2 Behavioural Results

The data from one rat in the HPC-REMOTE group were excluded from the study because the rat's score was 900 sec, whereas the group mean is less than 18 sec when this rat is excluded.

The data from the four NAÏVE control groups (SHAM or HPC and REMOTE or RECENT conditions) were pooled because their scores on the acquisition session and retention test were not statistically different (see appendix B). Consequently, the analytical comparisons of the data involved five groups. The NAÏVE group (n= 12), which included all the rats exposed to the non-electrified probe during acquisition, and four groups of rats exposed to the electrified probe during acquisition: SHAM-REMOTE (n = 8), SHAM-RECENT (n = 9), HPC-REMOTE (n= 7), and HPC-RECENT (n = 8).

2.2.2.2.1 ***Acquisition.*** Figure 9 shows the number of contact-induced shocks the rats received during the acquisition session. The HPC rats received a similar number of contact-induced shocks as SHAM rats (HPC-REMOTE vs. SHAM-REMOTE, $U = 26.5$, $p = .428$; HPC-RECENT vs. SHAM-RECENT, $U = 29.5$, $p = .255$).

Figure 10 shows the amount of time spent burying during the acquisition session. The rats that were exposed to the electrified probe buried more than rats that were exposed to the non-electrified probe (SHAM-REMOTE vs. NAÏVE, $U = 25.5$, $p < .05$; SHAM-RECENT vs. NAÏVE, $U = 18.0$, $p < .01$; HPC-REMOTE vs. NAIVE, $U = 19.0$, $p < .05$; HPC-RECENT vs. NAIVE, $U = 16.5$, $p < .01$). No differences were found between the HPC rats the SHAM rats (HPC-REMOTE vs. SHAM-REMOTE, $U = 27.5$, $p = .477$; HPC-RECENT vs. SHAM-RECENT, $U = 34.5$, $p = .443$).

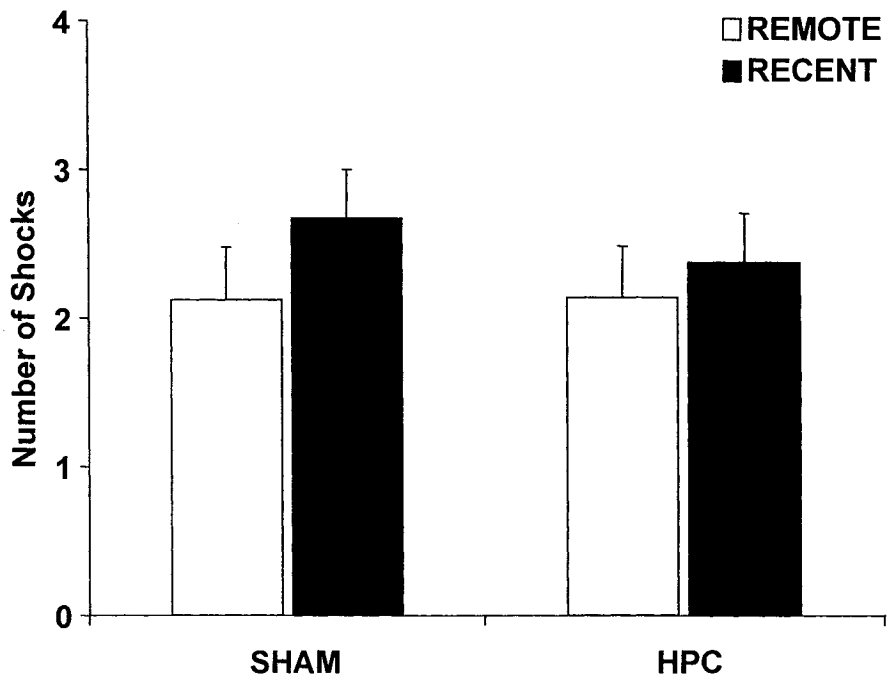

Figure 9. Mean (± *SEM*) number of contact-induced shocks received during the acquisition session by SHAM and HPC rats that were exposed to the electrified probe.

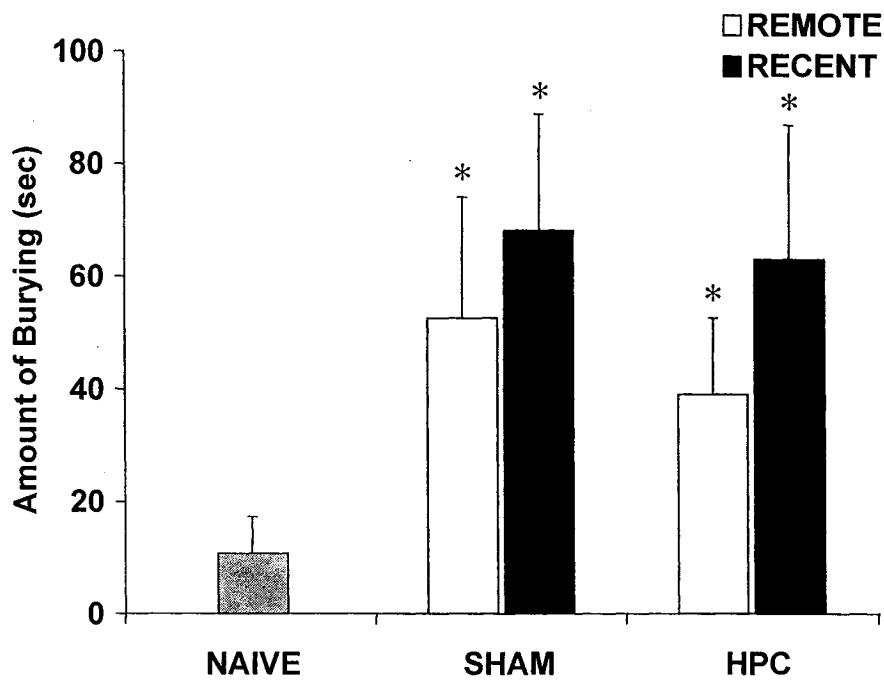

Figure 10. Mean (± *SEM*) amount of time spent burying during the acquisition session by NAÏVE, SHAM, and HPC rats (*$p < .05$ versus NAÏVE).

There was no significant difference in shock reactivity between SHAM-REMOTE rats (M= 1.87, SEM= 0.17) and HPC-REMOTE rats (M= 2.07, SEM= 0.21), U = 19.0, p = .136, nor between SHAM-RECENT (M= 2.19, SEM= 0.11) and HPC-RECENT rats (M= 1.96, SEM= 0.13), U = 23.5, p = .106.

2.2.2.2.2 *Retention.* Figure 11 shows the amount of time spent burying during the retention test. The SHAM rats buried significantly more than NAÏVE rats (SHAM-REMOTE vs. NAÏVE, U = 13.0, p < .01; SHAM-RECENT vs. NAÏVE, U = 14.0, p < .01). In contrast, the HPC rats did not show significant amounts of burying (HPC-REMOTE vs. NAIVE, U = 39.0, p = .419; HPC-RECENT vs. NAIVE, U = 32.5, p = .119). Also, HPC-RECENT rats buried significantly less than their respective SHAM-RECENT rats, U = 18.0, p < .05, but no significant difference was found between HPC-REMOTE rats and SHAM-REMOTE rats, U = 14.0, p = .06. Moreover, the interval between conditioning and surgery did not influence the amount of burying on the retention test since no significant differences were found between SHAM-REMOTE and -RECENT, U = 27.0, p = .212, nor between HPC-REMOTE and -RECENT, U = 23.0, p = .307.

Figure 12A illustrates the latency to initially contact the probe on the retention test. The SHAM rats had significantly longer latencies than NAÏVE rats (SHAM-REMOTE vs. NAÏVE, U = 3.0, p < .001; SHAM-RECENT vs. NAÏVE, U = 0.0, p < .001). The HPC-REMOTE rats also had significantly longer latencies than NAÏVE rats, U = 19.5, p < .05, but not the HPC-RECENT rats, U = 38.5, p = .23. The HPC rats were significantly faster in contacting the probe than SHAM rats (HPC-REMOTE vs. SHAM-REMOTE, U = 3.0, p < .01; HPC-RECENT vs. SHAM-RECENT, U = 0.0, p < .001). In addition, the

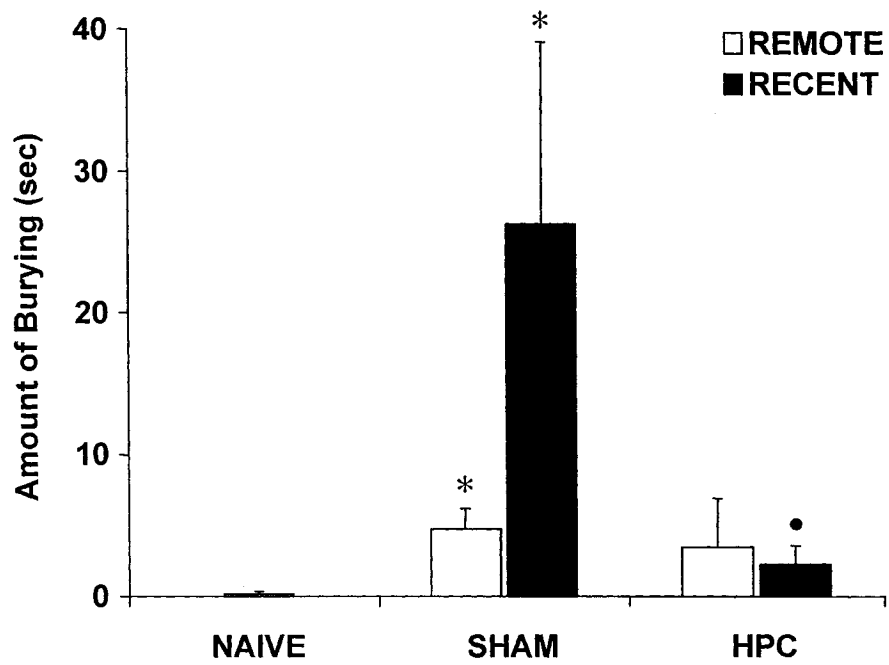

Figure 11. Mean (± *SEM*) amount of time spent burying during the retention test by NAÏVE, SHAM, and HPC rats in the REMOTE and RECENT conditions (*$p < .05$ versus NAIVE; • $p < .05$ versus SHAM-RECENT).

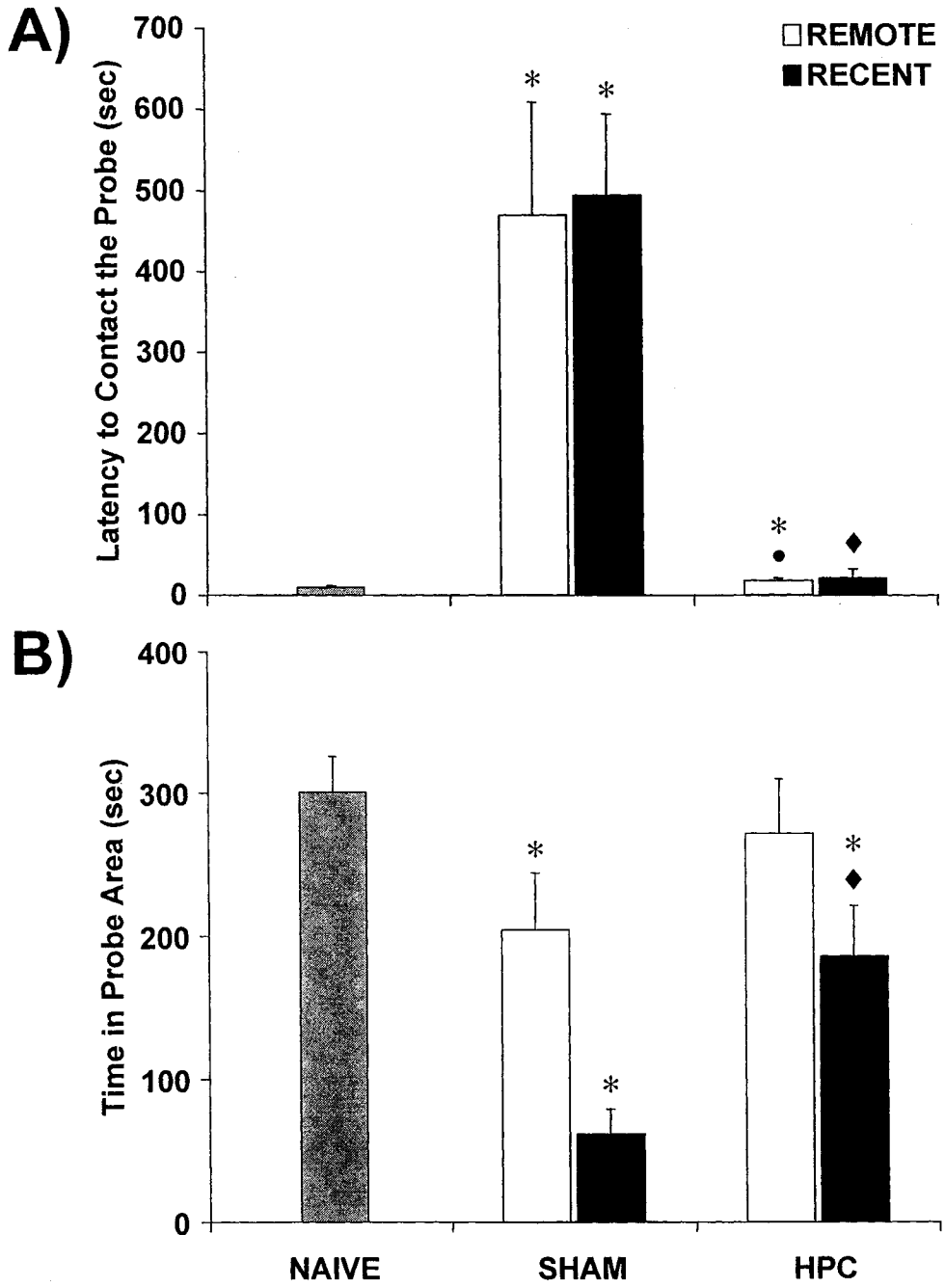

Figure 12. Mean (± *SEM*) **(A)** latency to contact the probe and **(B)** amount of time spent in the probe area of the chamber during the retention test for NAÏVE, SHAM, and HPC rats in the REMOTE and RECENT conditions (*$p < .05$ versus NAIVE; •$p < .05$ versus SHAM-REMOTE; ♦$p < .05$ versus SHAM-RECENT).

interval between conditioning and surgery did not have a significant effect on the latencies to contact the probe. Specifically, the SHAM-REMOTE and -RECENT rats latencies did not significantly differ, $U = 32.0, p = .349$), nor did the HPC-REMOTE and -RECENT rats latencies, $U = 20.5, p = .192$).

Figure 12B illustrates the amount of time spent in the half of the chamber where the probe was located. Both groups of SHAM rats spent significantly less time in the probe area than the NAÏVE rats (SHAM-REMOTE vs. NAÏVE, $U = 25.5, p < .05$; SHAM-RECENT vs. NAÏVE, $U = 0.0, p < .001$). In contrast, the HPC-REMOTE rats did not spend less time in the probe area than the NAÏVE rats, $U = 31.0, p = .176$, but the HPC-RECENT rats did, $U = 20.0, p < .05$. However, the HPC-RECENT avoided the probe area significantly less than the SHAM-RECENT rats, $U = 7.0, p < .01$. The amount of time spent in the probe area did not differ between HPC and SHAM rats from the REMOTE condition, $U = 18.0, p = .124$, but this comparison is not very meaningful since the HPC-REMOTE rats did not spend less time in this area than NAÏVE rats. Interestingly, the interval between conditioning and surgery affected the amount of time spent in the probe area. The SHAM-REMOTE rats did not avoid the probe area as much as SHAM-RECENT rats, $U = 8.0, p < .01$, but this difference was not significant for the HPC rats, $U = 16.0, p = .083$).

2.2.3 DISCUSSION

The findings of the present experiment suggest that lesions of the HPC cause severe and lasting retrograde amnesia in the shock-probe fear-conditioning test. Rats with HPC lesions contacted the probe more quickly and spent more time in the area of the chamber

than SHAM rats suggesting that the HPC lesions caused amnesia. Moreover, the HPC-RECENT rats did not take more time to contact the probe than the NAÏVE rats, whereas the HPC-REMOTE rats took only slightly more time than the NAÏVE rats. These latter results suggest that the HPC rats remembered little, if any, information about the association between the probe and the shock. There was also no evidence of a temporal gradient. The avoidance deficits were similar both between the HPC-REMOTE- and -RECENT rats when each was compared to their respective SHAM group.

HPC lesions also impaired the burying response during the retention test. The HPC rats buried significantly less than the SHAM rats and no more than the NAÏVE rats. However, the burying impairment on the retention test cannot be interpreted as strong evidence for retrograde amnesia. Findings from Experiment 1 clearly showed that HPC lesions impair the expression of burying and that this behavioural response cannot be reliably used for inferences about learning and memory in HPC-damaged rats.

The present findings contrast with the results that have been reported about the effects of post-training HPC lesions on cued fear conditioning. Typically, HPC lesions do not cause retrograde amnesia for cued fear conditioning (Anagnostaras et al., 1999; J. J. Kim & Fanselow, 1992; Maren et al., 1997), whereas the current experiment found significant memory impairments. The divergent findings may be due to differences in the type of discrete stimulus for the fear conditioning (i.e., object vs. tone) or methodology (i.e., footshock vs. receiving shock from the object) that lead to different types of learning. However, the divergence in findings is most likely due to the extent of the HPC lesions. All the studies that have found an absence of retrograde amnesia for cued fear conditioning limited the lesions to the dorsal HPC (Anagnostaras et al., 1999; J. J. Kim &

Fanselow, 1992; Maren et al., 1997), whereas the lesions in this experiment included both the dorsal and ventral HPC. Indeed, a recent study found that post-training ventral HPC lesions caused retrograde amnesia for auditory fear conditioning (Maren & Holt, 2004). Combined these findings suggest that the HPC is involved in cued fear conditioning and that it is dependent on the ventral and not the dorsal region of the HPC.

According to some studies, the HPC has a temporary role in memory for fear conditioning (Anagnostaras et al., 1999; J. J. Kim & Fanselow, 1992; Maren et al., 1997). For instance, lesions of the HPC made 24 hr, but not 28 days, after contextual fear conditioning impaired memory, suggesting that HPC damage causes temporally-graded retrograde amnesia (J. J. Kim & Fanselow, 1992). However we did not find any evidence of a temporal gradient to the retrograde amnesia for object-fear conditioning. Lesions of the HPC, induced up to 2-wk after shock-probe fear conditioning, impaired memory as much as lesions made 1-d after conditioning. The amount of time required for memories to be consolidated may vary according to the type of information to be remembered (see Nadel & Moscovitch, 1997), and it is not known how long it takes for object-fear conditioning to become consolidated in long-term memory. Consequently, the 2-wk interval between conditioning and surgery in the shock-probe fear-conditioning test might have been too short for the memory to have undergone significant consolidation before the HPC lesions were made. Thus, it is possible that temporally graded retrograde amnesia would have been found in the shock-probe fear-conditioning test if the lesions had been made more than 2-wk after the conditioning. However, lesions made 7-d following contextual fear conditioning already showed evidence of a temporal gradient to the retrograde amnesia (J. J. Kim & Fanselow, 1992). This latter finding suggests that the

interval between conditioning and surgery in the current experiment was likely sufficient to detect evidence of a temporal gradient to the retrograde amnesia for object-fear conditioning, if there were any.

The absence of a temporal gradient to the retrograde amnesia for fear conditioning in the present experiment, in contrast to other studies, is likely due to the extent of the HPC damage or to the type of stimulus involved in the conditioning. Given that temporally-graded retrograde amnesia has been found only in contextual fear conditioning tests and no other type of fear conditioning test (Anagnostaras et al., 1999; J. J. Kim & Fanselow, 1992; Maren et al., 1997), then it is possible that retrograde amnesia for fear conditioning is only temporally graded when it involves an association between contextual information and a fear-eliciting event. However, it is more likely that temporally graded retrograde amnesia for fear conditioning is the result of partial HPC damage. If the HPC lesions in the contextual fear conditioning studies had encompassed the entire HPC, then there might have been a flat gradient to the retrograde amnesia similar to the one found in this experiment.

It could be argued that the retrograde amnesia in shock-probe fear conditioning following HPC lesions is the result of a memory impairment of a configural representation. Extensive evidence suggests that the HPC is necessary for configural representations (Fanselow, 2000; R. J. McDonald & White, 1993; O'Reilly & Rudy, 2001; Rudy & O'Reilly, 1999, 2001; Sutherland et al., 1989; White & McDonald, 2002). However, for this possibility to be true, the rats, during conditioning, would have needed to encode the probe as part of a configural representation in which the conditioning occurred and not as a distinct cue. Only in this case would the memory deficits following

post-training HPC lesions in the shock-probe fear-conditioning test not be attributable to a cued fear conditioning impairment, but to a contextual fear conditioning impairment. This is most unlikely since the procedure used to condition the rats minimized this possibility. The rats were habituated to the shock-probe chamber prior to the presentation of the probe on the conditioning day. Consequently, the probe was probably perceived as a novel and distinct feature during the acquisition session. Evidence supports this hypothesis: The SHAM rats, in Experiment 1, tested in a different context (i.e., incongruent) than the one in which they received conditioning still avoided the probe suggesting that the rats encoded the probe as a distinct feature associated with a shock. Thus, it most likely that the retrograde amnesia in the shock-probe fear-conditioning test is due, in part, to an inability of HPC-damaged rats to remember that a distinct cue is associated with a fear-eliciting event.

There is a possibility that the post-training HPC lesions not only impaired memory for object-fear conditioning, but also contextual fear conditioning in the shock-probe test. Again, findings from Experiment 1 showed that shock-probe fear conditioning involves cued and contextual fear conditioning. Given that the HPC rats did not perform better than the NAÏVE rats in the present experiment it is likely that the retrograde amnesia reflects a memory deficit for both cued and contextual fear conditioning. Despite the impossibility of dissociating the impairment for the two types of fear conditioning in the current experiment, the findings still suggest that the HPC is involved in memory for cued fear conditioning.

In summary, lesions of the HPC made up to 2-wk after conditioning impaired avoidance of an object associated with a fear-eliciting event in the shock-probe fear-

conditioning test. Thus, the HPC has a lasting role in memory for object-fear conditioning.

2.3 SUMMARY AND IMPLICATIONS

The conventional view about HPC function in fear conditioning is that the HPC is necessary for learning and remembering that configural representations, but not independent cues, are associated with a fear-eliciting event (Anagnostaras et al., 2001; Fanselow, 2000; Maren, 2001b; Rudy & O'Reilly, 1999, 2001). The absence of anterograde amnesia for object-fear conditioning, but impaired contextual fear conditioning following HPC lesions in the shock-probe fear-conditioning test reported here is consistent with this view. In Experiment 1, fear conditioning impairments were apparent when HPC rats were tested in the congruent context, which involved the configuration of several features. However, the HPC rats successfully avoided the probe in the incongruent context, which only involved the assessment of fear conditioning to a distinct cue or feature that was part of the original conditioning context. Although several studies have found similar findings (Frankland, Cestari, Filipkowski, McDonald, & Silva, 1998; Maren et al., 1997; Phillips & LeDoux, 1994; Rudy et al., 2002), this is the first, to my knowledge, that shows this dissociation in rats with complete HPC lesions.

The retrograde amnesia for cued fear conditioning following HPC lesions challenges the conventional view however. Lesions of the HPC, in Experiment 2, severe retrograde amnesia for object-fear conditioning suggesting that the HPC is implicated in cued fear conditioning. Therefore, the HPC is involved in learning and remembering an association between a distinct cue and a fear-eliciting event and the contention that the HPC is not involved in cued fear conditioning appears to have been reached prematurely.

Likewise, Bast et al. (2001a) recently proposed that cued fear conditioning is dependent on the ventral HPC. This idea stems from anterograde amnesia findings

implicating the ventral HPC in auditory fear conditioning (Bast et al., 2001b; Maren, 1999b; Richmond et al., 1999). The experiments in this chapter do not directly assess whether the ventral HPC is the region involved in object-fear conditioning because the lesions in the Experiment 1 and 2 included both the dorsal and ventral HPC. Nonetheless, the findings in this chapter extend and support the new view that the HPC, and in particular the ventral region, is involved in cued fear conditioning.

The dissociation between anterograde and retrograde amnesia for object-fear conditioning following HPC lesions is similar to that found for object recognition memory. Lesions of the HPC cause retrograde amnesia for object recognition, but not anterograde amnesia (Gaskin et al., 2003). Given that the pattern of memory deficits in object-fear conditioning parallels the pattern of deficits in object recognition following HPC damage, it is possible that the memory impairment in object-fear conditioning is due to an object recognition deficit and not an impairment in remembering the association between the cue and the fear-eliciting event. Specifically, object-fear conditioning involves an association between an object and a fear-eliciting event and any memory impairment for this conditioning may be the result of a deficit in recognizing the object, remembering the fear-eliciting event, or remembering the association between the two stimuli.

It is impossible to directly assess whether the retrograde amnesia for object-fear conditioning is specifically due to a deficit in remembering the association between the object and the fear-eliciting event, the fear-eliciting event itself, or in recognizing the object. However, if the deficits were the result of an object-recognition deficit, then one would expect that damage to another structure involved in object recognition would also

cause object-fear conditioning impairments. Since damage to the PRH has been shown to induce anterograde and retrograde amnesia for object recognition, it would be expected that PRH damage would also cause anterograde and retrograde amnesia for object-fear conditioning. Therefore, the next chapter aims at determining the effects of PRH damage in object-fear conditioning in the shock-probe fear-conditioning test.

CHAPTER 3

THE PRH AND OBJECT-FEAR CONDITIONING

The findings from the previous chapter suggest that the HPC is involved in memory for object-fear conditioning. However, it is unclear whether this involvement is specific to the memory of the association between the object and the fear-eliciting event, to the memory of the fear-eliciting event, or to recognition of the object. It is reasonable to assume that if the involvement of the HPC in memory for object-fear conditioning is due to object recognition functions, then damage to another neural structure involved in object recognition would cause similar object-fear conditioning deficits as HPC lesions. Given this assumption, and the extensive evidence implicating the PRH in object recognition (Buffalo et al., 1999; Bussey et al., 1999; Bussey, Saksida, & Murray, 2002; Ennaceur & Aggleton, 1997; Gaffan & Murray, 1992; Glenn & Mumby, 1996; Meunier et al., 1993; Mumby et al., 2002; Mumby & Pinel, 1994; Murray & Bussey, 1999; Thornton et al., 1997; Zola-Morgan, Squire, Amaral et al., 1989), the goal of the experiments in this chapter was to assess whether PRH lesions would cause anterograde and retrograde amnesia for object-fear conditioning.

The primary purpose of Experiment 3 was to determine whether aspiration lesions of the PRH would cause anterograde amnesia for the association between an object and a fear-eliciting event in the shock-probe fear-conditioning test. A second purpose of Experiment 3 was to assess the effects of pretraining PORH lesions on memory for object-fear conditioning in the shock-probe fear-conditioning test because this latter structure is believed to have similar mnemonic functions as the PRH (Bucci et al., 2000; Bucci et al., 2002; Burwell et al., 2004; Murray et al., 2000). The results indicated that

the PRH lesions reduced burying, but enhanced avoidance of the probe, whereas PORH lesions did not significantly affect either response. These findings suggest that neither lesions to the PRH nor the PORH induce anterograde amnesia in the shock-probe fear-conditioning test. However, the findings suggest that the PRH may be involved in mediating avoidance and burying evoked by an object associated with a fear-eliciting event.

The aim of Experiment 4 was to assess whether the burying impairment observed in PRH-damaged rats in Experiment 3 was the result of a performance deficit, such as an inability to perform the motor response. Specifically, rats with PRH lesions were trained and tested in a modified shock-probe chamber (i.e., smaller chamber) that elicited substantially more burying than the typical shock-probe testing apparatus. In this context, the PRH lesions did not impair burying during the retention test, suggesting that rats with PRH aspiration lesions are capable of burying.

Experiment 5 was designed to evaluate whether neurotoxic lesions of the PRH would cause retrograde amnesia in the shock-probe test. Since the lesion method used to damage and evaluate the role of the PRH in memory in Experiment 5 was different than the method used in Experiment 3 and 4, it also seemed important to assess the effects of pretraining neurotoxic lesions of the PRH in the shock-probe fear-conditioning test. The value of this latter assessment is further supported by evidence suggesting that the method used to damage the PRH may have differential effects on memory and behaviour in other tests (Campeau & Davis, 1995a; Mumby & Glenn, 2000). It was found that rats with post-training neurotoxic lesions of the PRH buried during the retention test, suggesting that the PRH is not necessary for memory in the shock-probe fear-

conditioning test. It was also found that pretraining neurotoxic lesions of the PRH did not impair burying, but like aspiration lesions, enhanced avoidance.

3.1 EXPERIMENT 3: The Effects of Pretraining Lesions of the PRH and PORH In the Shock-Probe Fear-Conditioning Test

Extensive evidence suggests that the PRH is involved in object-recognition. For instance, pretraining damage to the PRH impairs object-recognition performance on the object delayed non-matching to sample task (Gaffan & Murray, 1992; Glenn & Mumby, 1996; Meunier et al., 1993; Mumby & Pinel, 1994; Nemanic, Alvarado, & Bachevalier, 2004; Zola-Morgan, Squire, Amaral et al., 1989) and the novel-object-preference test (Aggleton, Keen, Warburton, & Bussey, 1997; Bussey et al., 2000; Bussey et al., 1999; Ennaceur & Aggleton, 1997). Moreover, electrophysiological studies in monkeys and immuno-histochemical studies in rats have demonstrated strong neuronal responses to novel objects in the PRH (Brown & Xiang, 1998; Fahy et al., 1993; Wan et al., 1999). Thus, the PRH seems to be important for processing information pertaining to objects and raises the possibility that it may be essential for memory for object-fear conditioning.

Lesions of the PRH have also been shown to cause anterograde amnesia for associations that involve a fear-eliciting event (Burwell et al., 2004; Otto et al., 2000; Otto & Giardino, 2001). For example, pretraining lesions of the PRH can impair contextual fear conditioning (Bucci et al., 2000; Bucci et al., 2002) and cued fear conditioning (Campeau & Davis, 1995a; Herzog & Otto, 1997, 1998). This latter evidence further supports the hypothesis that the PRH may be involved in memory for object-fear-conditioning.

The PORH, which is adjacent to the PRH, is thought to play a similar mnemonic role as the PRH (Bucci et al., 2000; Bucci et al., 2002; Burwell et al., 2004; Murray et al., 2000), even though the cytoarchitectural and connectional differences between the two

structures suggest that functional differences have simply not yet been found (Burwell & Amaral, 1998a; Burwell et al., 1995). Specifically, like PRH damage, PORH damage impairs object recognition in several paradigms (Murray et al., 2000) and also causes anterograde amnesia for contextual fear conditioning (Bucci et al., 2000; Bucci et al., 2002). The similarities between the effects of PRH and PORH lesions on mnemonic functioning suggests that pretraining damage to the PORH may parallel the effects of the PRH lesions on object-fear conditioning. Thus, it also seemed pertinent to determine the effects of pretraining PORH lesions in the shock-probe fear-conditioning test.

In sum, the main purpose of this experiment was to determine the effects of pretraining PRH and PORH lesions on object-fear conditioning in the shock-probe fear-conditioning test. The rats were also tested on an object-investigation test and in the elevated plus-maze. The object-investigation test enabled an examination of the effects of the lesions on investigation of objects that are not explicitly paired with a fear-eliciting event, whereas the elevated plus-maze test allowed an examination of the effects of the lesions on unconditioned avoidance. Rats naturally avoid open spaces, such as the arms of the elevated-plus maze, and it is believed that this behaviour is related to fear (File, Zangrossi, Viana, & Graeff, 1993; Pellow, Chopin, File, & Briley, 1985; Pellow & File, 1986; Treit, Menard, & Royan, 1993). Indeed, brain lesions or drugs that affect avoidance in elevated-plus maze typically have parallel effects in other anxiety tests that involve avoidance (Degroot & Treit, 2002; Shah & Treit, 2004; Treit & Menard, 1997).

3.1.1 METHODS

3.1.1.1 Subjects

Male Long-Evans rats (Charles River, Quebec; 300-350 g) served as subjects. Their housing conditions were the same as in Experiment 1.

3.1.1.2 Surgery

Rats were anesthetized with isoflurane (Janssen, Toronto, Ontario) in 0.8 L/min oxygen at 14.7 PSIA at 21°C (Benson Medical Industries, Markham, Ontario). For PRH and PORH rats, a scalp incision was made and the muscle overlying the temporal skull was displaced. A portion of skull overlying the target region 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. PRH lesions were restricted to tissue within 2,5 and 8 mm posterior to Bregma. PORH lesions were restricted to tissue 8 mm posterior to Bregma. 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 a minimum of two weeks before behavioural training commenced.

3.1.1.3 Behavioural Procedures

3.1.1.3.1 ***Shock-Probe Test.*** The procedures for habituation, acquisition, and retention were the same as those in Experiment 1 with the exception that only the transparent shock-probe chamber was used.

3.1.1.3.2 ***Object-Investigation Test.*** The object-investigation test was conducted in an open-field arena (70 X 70 X 60 cm) constructed of grey PVC plastic. A stainless steel tray served as the floor of the arena and was covered with bedding material. The rats

were habituated to the open-field arena prior to the object investigation test by being placed in the apparatus for 15 min on four different days.

For the test, each rat was individually placed in the arena and allowed to freely explore and investigate for 5 min. However, two identical objects were now fixed to the floor of the arena 27 cm from two opposing corners. The objects were made of glass or porcelain that varied in height between approximately 5 cm and 15 cm and in width between approximately 4 cm and 10 cm. The objects were counterbalanced between the groups. The behaviour of each rat was recorded on videotape, which was then scored for the amount of time the rat spent investigating the objects. A rat was considered to be investigating an object if its head was located within a 3 cm distance and a 45° angle of the object. Chewing the object and climbing over it were not considered investigation. Following each test, the bedding covering the floor was changed and the floor and walls of the arena and the objects were washed with water.

3.1.1.3.3 ***Elevated Plus-Maze.*** The plus-maze was made of wood and consisted of two opposing open arms (50 X 10 X 1 cm) and two opposing closed arms (50 X 10X 39 cm), elevated to a height of 52 cm. For the test each rat was placed individually in the center of the maze and allowed 5 min of free exploration. The behaviour of the rats was recorded by a video camera placed above the maze. Following each test, the maze was cleaned of feces and urine with water. The maze exploration of the rats was analyzed by calculating the number of entries with all four paws into each type of arm (i.e., closed and open) as well as the total time the rats spent with all four paws in each type of arm. If a rat fell from the maze, then it was excluded from the test.

3.1.1.4 Histology

The same procedures as in Experiment 1 were used.

3.1.1.5 Statistical Analyses

The same procedures as in Experiment 1 were used.

3.1.2 RESULTS

3.1.2.1 Histology

The location and extent of the smallest and largest PRH and PORH lesions are shown in Figure 13. One PRH and 2 PORH rats were excluded from the experiment because they had extensive damage outside the target area. Rats in the PRH group sustained substantial and nearly complete, bilateral damage to the PRH. There was unilateral sparing of the rostral portions of the PRH in 3 rats, and bilateral sparing in 2 rats. In terms of the caudal extent of the lesion, there was unilateral sparing of PRH tissue in only 1 rat. Rats in the PORH group sustained extensive bilateral damage centered on the PORH; some bilateral sparing of the PORH in the rostral and caudal portions was evident in all rats in this group.

There was bilateral damage to the lateral EC in all PRH rats; 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. Damage to the anterior portion of the PORH occurred in only 2 PRH rats, and damage was minimal in both cases. Damage was found unilaterally in the temporal association cortex of 3 rats and minimal damage was found bilaterally in 1 rat. The lateral amygdala was damaged unilaterally in 3 rats and bilaterally in 1 rat. One rat had significant unilateral damage to the piriform cortex. Minor

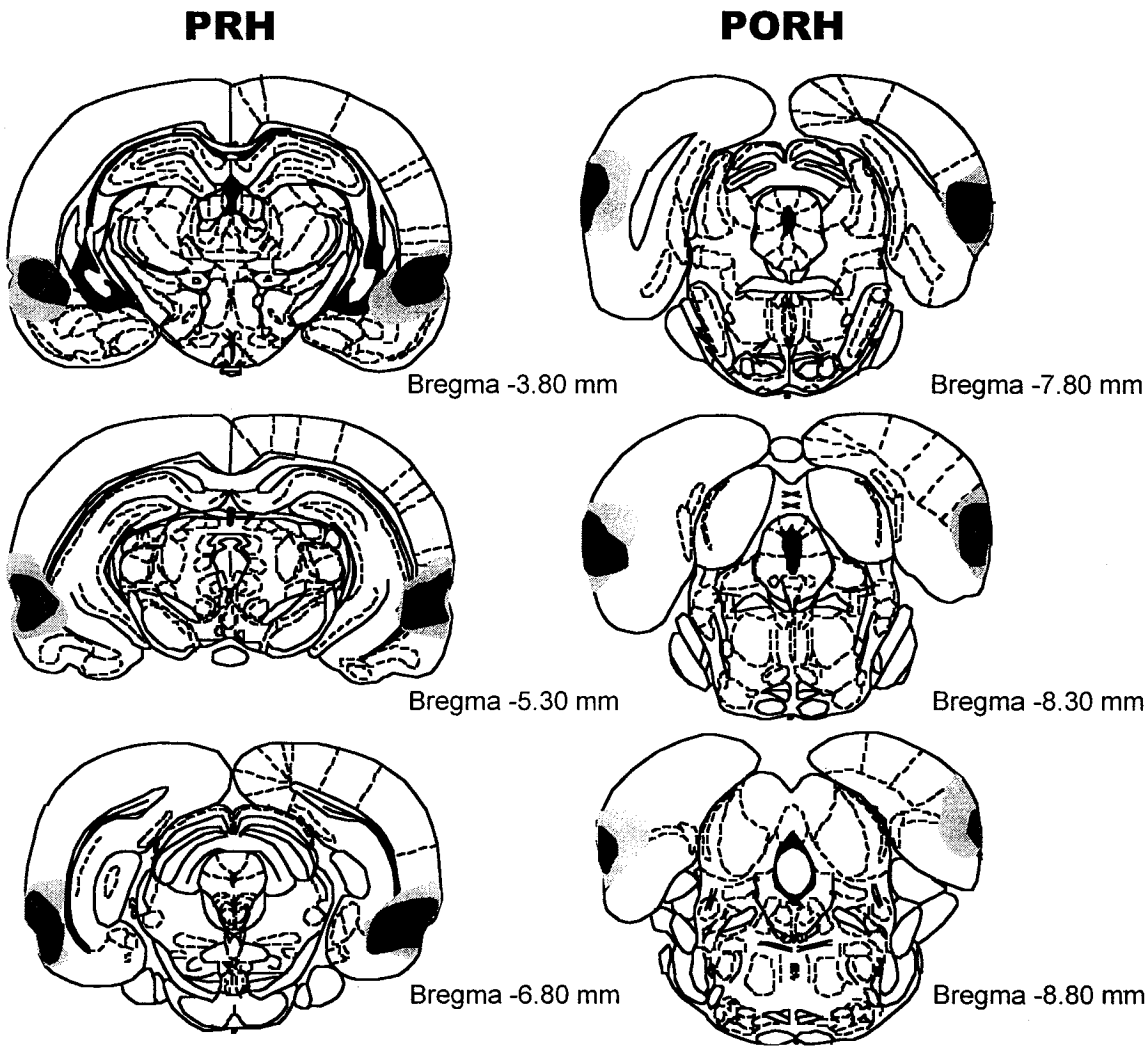

Figure 13. Illustrations of the smallest (dark grey) and largest (light gray) lesion observed bilaterally through the rostral and caudal extent of the PRH and PORH rats. Atlas plates are from Paxinos and Watson (1997).

damage of the CA1 subfield of the HPC was seen unilaterally in 4 rats and bilaterally in 2 rats. The ventral portion of the subiculum was slightly damaged unilaterally in 6 rats, and bilaterally in 1 rat.

The PORH lesions also included some damage to the adjacent EC and PRH. Bilateral encroachment on the medial EC was evident in 8 of the 11 PORH rats and unilateral encroachment was evident in the remaining 3 rats. This latter damage was minimal in most rats and more substantial in 2 rats. Four PORH lesions included bilateral damage and 3 PORH lesions included unilateral damage to the lateral EC and PRH. The remaining 4 lesions did not extend to these areas. Nine of the 11 PORH lesions included some damage to the adjacent temporal association cortex, though this damage was minor in all cases and bilateral in only 4 of the 9 rats.

3.1.2.2 Behavioural Results

3.1.2.2.1 Shock-Probe Test

The data from one rat in the PRH group were excluded from the study because its burying score on the retention test was more than three standard deviations away from the group mean.

The data from the three NAÏVE control groups (SHAM, PRH, and PORH) were pooled because their behavioural results on the acquisition session and retention test were not statistically different (see Appendix B). Consequently, the analytical comparisons of the data involved four groups. The NAÏVE group (n= 11), which included all the rats exposed to the non-electrified probe, and the three groups of rats exposed to the electrified probe: SHAM (n = 13), PRH (n = 11), and PORH (n = 10).

3.1.2.2.1.1 ***Acquisition.*** Figure 14 shows the number of contact-induced shocks the rats received during the acquisition session. There was no significant difference in the number of contact induced-shocks between PORH and SHAM rats, $U = 53.0, p = .445$, nor between PRH and SHAM rats, $U = 48.5, p = .153$. There was no significant difference in shock reactivity between SHAM ($M = 2.16$, $SEM = 0.17$) and PORH rats ($M = 2.23 \pm SEM = 0.16$), $U = 55.0, p = .522$, nor between SHAM and PRH rats ($M = 2.29 \pm 0.13$), $U = 52.0, p = .240$.

Figure 15 shows the amount of time spent burying during the acquisition session. With the exception of the PRH rats, the rats exposed to the electrified probe buried more than the ones that were exposed to the non-electrified probe (SHAM vs. NAÏVE, $U = 9.0$, $p < .001$; PORH vs. NAÏVE, $U = 15.0, p < .01$; PRH vs. NAIVE, $U = 43.0, p = .118$), The PRH rats buried significantly less than SHAM rats, $U = 22.5, p < .01$, whereas no significant difference was found between PORH and SHAM rats, $U = 47.5, p < .277$.

3.1.2.2.1.2 ***Retention.*** Figure 16 shows the amount of time spent burying on the retention test. The SHAM rats buried significantly more than the NAÏVE rats, $U = 28.0, p < .01$) and the PORH also rats tended to bury more than the NAÏVE rats, $U = 32.5, p = .069$. In contrast, the PRH rats did not bury more than NAÏVE rats, $U = 57.0, p = .754$. Moreover, the PRH rats buried significantly less than the SHAM rats, $U = 30.0, p < .01$, whereas no significant difference was found between the PORH and SHAM rats, $U = 44.0, p = .183$.

Figure 17A illustrates the latency to initially contact the probe on the retention test. All the groups that were exposed to the electrified probe during the acquisition session had significantly longer latencies than NAÏVE rats (SHAM vs. NAÏVE, $U = 4.0, p <$

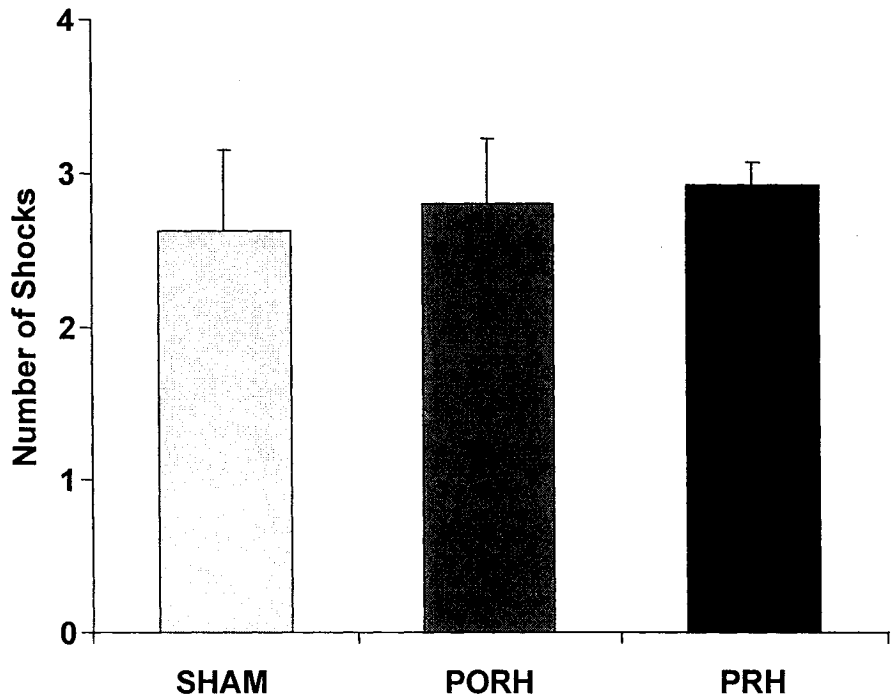

Figure 14. Mean (± *SEM*) number of contact-induced shocks received during the acquisition session by SHAM, PORH, and PRH rats that were exposed to the electrified probe.

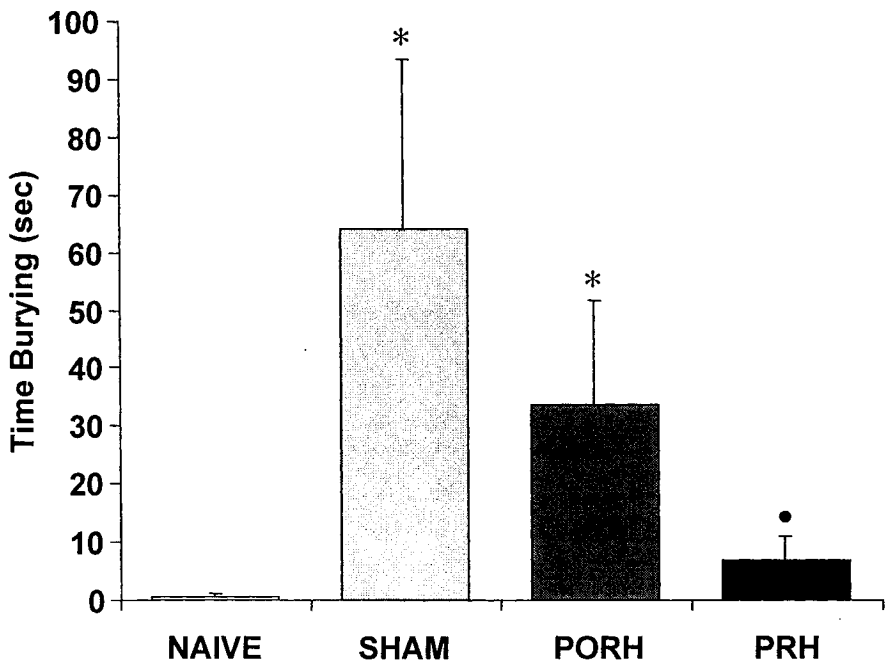

Figure 15. Mean (± *SEM*) amount of time spent burying during the acquisition session by NAÏVE, SHAM, PORH, and PRH rats (*$p < .05$ versus NAIVE; • $p < .05$ versus SHAM).

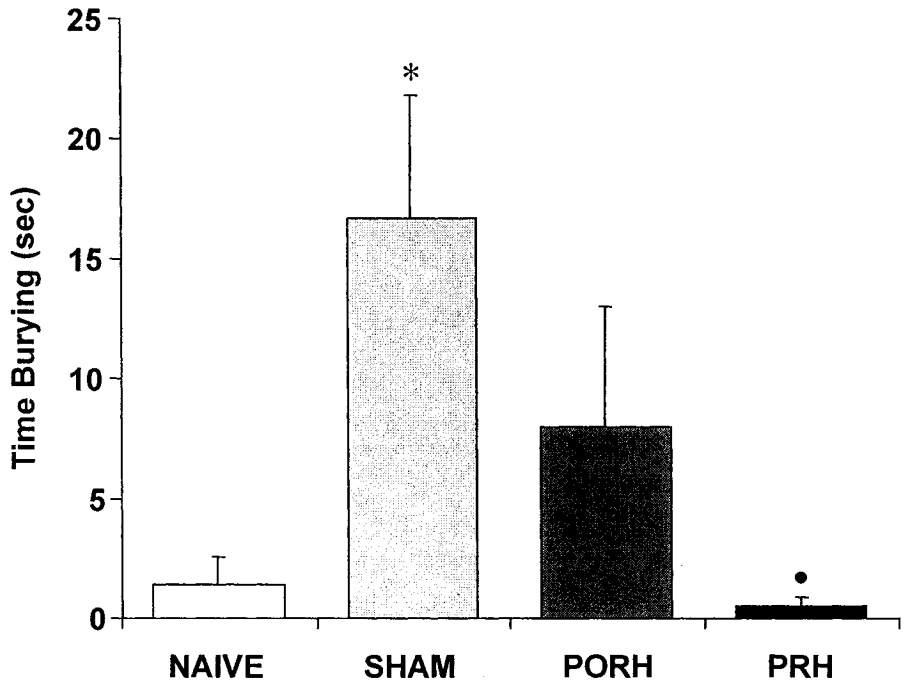

Figure 16. Mean (± *SEM*) amount of time spent burying during the retention test by NAÏVE, SHAM, PORH, and PRH rats (*$p < .05$ versus NAIVE; • $p < .05$ versus SHAM).

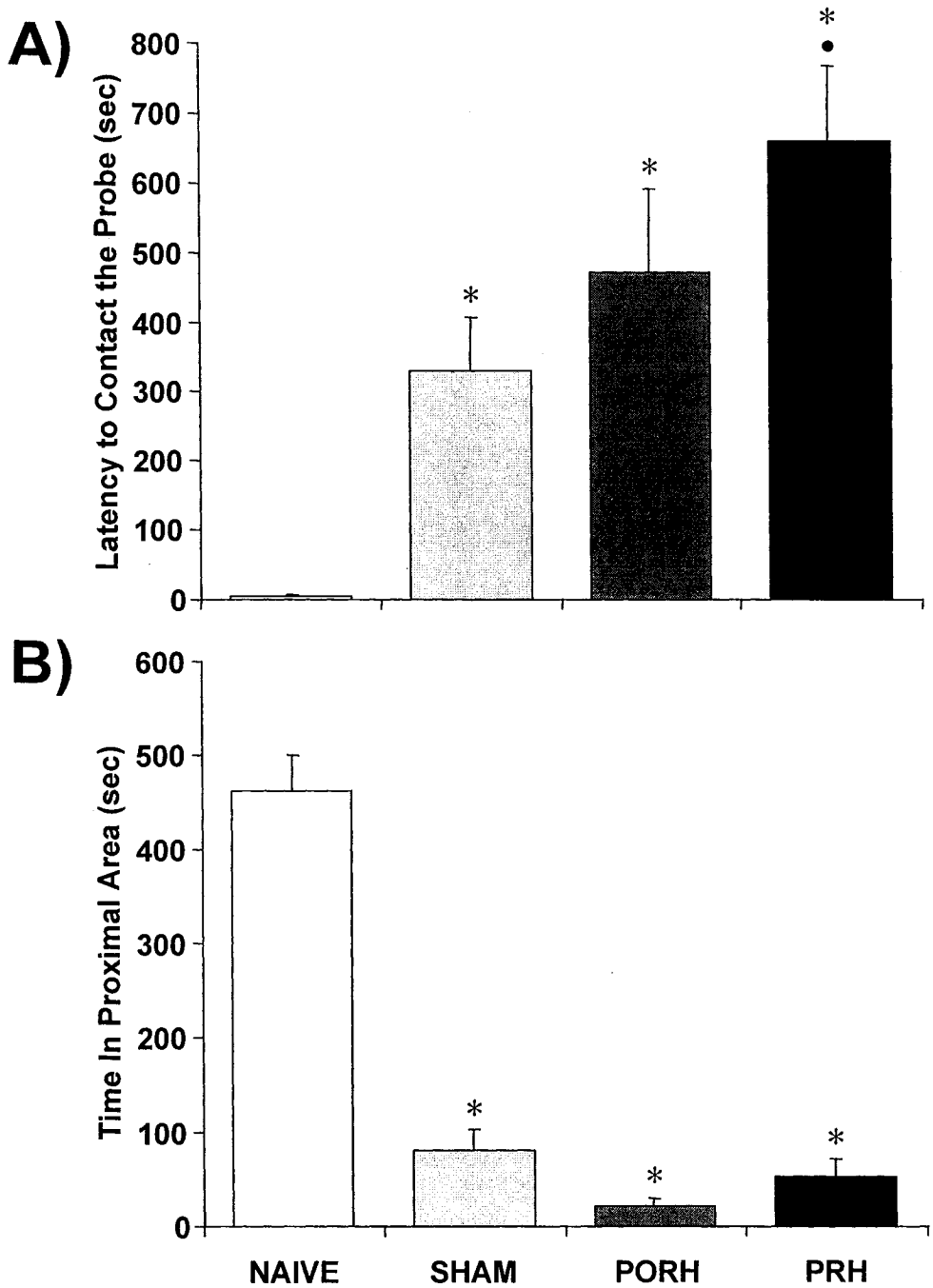

Figure 17. Mean (± *SEM*) **(A)** latency to contact the probe and **(B)** amount of time spent in the probe area of the chamber during the retention test for NAÏVE, SHAM, PORH, and PRH rats (*$p < .05$ versus NAIVE; • $p < .05$ versus SHAM).

.001; PORH vs. NAÏVE, $U = 0.0, p < .001$; PRH vs. NAÏVE, $U = 0.0, p < .001$). Interestingly, PRH had significantly longer latencies than SHAM rats, $U = 34.0, p < .05$. No differences were found between PORH and SHAM rats, $U = 48.5, p = .305$.

Figure 17B illustrates the amount of time spent in the half area of the chamber where the probe was located. The SHAM, PORH, and PRH rats spent significantly less time in the probe area than NAÏVE rats (SHAM vs. NAÏVE, $U = 1.0, p < .001$; PORH vs. NAÏVE, $U = 0.0, p < .001$; PRH vs. NAÏVE, $U = 0.0, p < .001$). In addition, the amount of time spent in the probe area tended to differ between PORH and SHAM rats, $U = 32.5$, $p = .068$, but not between PRH and SHAM rats, $U = 53.0, p = .423$.

3.1.2.2.2 Object Investigation Test

The PRH rats investigated the objects significantly more than the SHAM rats, $U = 46.0, p < .05$. No differences were found between PORH and SHAM rats, $U = 28.0, p = .166$. Specifically, the SHAM rats investigated the objects for $M = 66.14 \pm 5.46$ sec, the PRH rats for $M = 94.77 \pm 7.81$ sec, and PORH rats for $M = 78.64 \pm 7.25$ sec.

3.1.2.2.3 Elevated Plus-Maze

The open-arm activity in the elevated plus-maze of PORH and PRH rats was similar to that of SHAM rats. Neither PRH nor PORH lesions affected the proportion of time the rats spent in the open-arms (PORH vs. SHAM, $U = 57.0, p = .520$; PRH vs. SHAM, $U = 68.0, p = .801$). Specifically, the SHAM rats spent $M = 2.3\% \pm 1.2$, the PORH $M = 1.5\% \pm 1.3$, and the PRH $M = 5.9\% \pm 4.8$ of their time in the open arms. The lesions also did not have an effect on the proportion of entries into the open-arms (PORH vs. SHAM, $U = 57.0, p = .521$; PRH vs. SHAM, $U = 70.0, p = .914$). The SHAM rats entered the open-arms $M = 7.7\% \pm 3.6$ times, the PORH $M = 4.0 \pm 2.9$ times, and the PRH $M = 6.3\% \pm 3.8$

times. However, the PRH lesions significantly increased the overall entries into the open- and closed-arms, $U = 36.5, p < .05$, whereas the PORH lesions did not, $U = 43.5, p = .179$. Specifically, the SHAM rats entered the open- and closed-arms $M = 3.9 \pm 0.8$ times, the PORH $M = 5.4 \pm 0.9$ times, and the PRH $M = 6.5 \pm 0.9$ times.

3.1.3 DISCUSSION

Damage to the PRH and PORH did not cause anterograde amnesia in the shock-probe fear-conditioning test. During the retention test, the PRH rats took significantly longer to contact the probe than SHAM rats and spent as little time in the probe area. The PORH rats did not differ from the SHAM rats either in latency to contact the probe or burying.

The current finding of spared memory for object-fear conditioning following pretraining PRH lesions is inconsistent with the studies that have implicated the PRH in cued fear conditioning (Herzog & Otto, 1997, 1998). However, visual and olfactory features of the probe likely guide performance during the shock-probe fear-conditioning test and it has been demonstrated that pretraining PRH lesions do not disrupt fear conditioning to a visual cue (Campeau & Davis, 1995a). Therefore, the absence of anterograde amnesia following PRH damage in the present experiment may be attributable, at least in part, to intact fear conditioning to the visual features of the probe.

Only one previous study has assessed the role of the PORH in cued fear conditioning and found that pretraining lesions of the PORH did not impair memory for an association between a tone and a shock (Bucci et al., 2000). The intact object-fear conditioning following pretraining PORH lesions, in the current experiment, extends the

latter findings and suggests that memory for cued fear conditioning is independent of the PORH. However, inputs to the PORH are almost exclusively visual (Burwell & Amaral, 1998a) and there are no studies that have investigated the role of the PORH in fear conditioning to a discrete visual cue. The shock-probe test is thought to be partially dependent on visual information, but other features of the probe may also have supported the fear conditioning. More studies, especially ones solely involving visual cues, are needed before strong conclusions can be made about the role of the PORH in cued fear conditioning.

It is important to note that the absence of anterograde amnesia for object-fear conditioning following PRH or PORH damage in this experiment is unlikely due to incomplete lesions. The rats in both groups had lesions that were as extensive as those in previously published studies reporting impaired fear conditioning or object recognition (Bucci et al., 2000; Bucci et al., 2002; Herzog & Otto, 1997, 1998; Mumby et al., 2002).

Even though the pretraining PRH lesions did not impair object-fear conditioning, the lesions severely disrupted defensive responses. The PRH lesions enhanced avoidance of the probe, yet they impaired burying during the retention test, and almost completely abolished this response during the acquisition session. These results suggest that the lack of burying during the retention test reflected a change in the coping repertoire rather than a memory deficit.

Although the decreased burying seen in the PRH rats may be attributable to a general performance deficit, the increase in avoidance behaviour cannot be explained by a general behavioural change. The PRH rats did not receive fewer contact-induced shocks than the SHAM rats during the acquisition session, suggesting that PRH lesions do not

affect unconditioned avoidance. Nor is the increased avoidance of the probe likely to be the consequence of a reduction in object investigation because the PRH lesions increased rats' normal interaction with objects in the object-investigation test. In addition, the increased avoidance could not be the result of a decrease in activity levels because the PRH rats did not spend more time immobile (see Appendix A) or spend less time in the probe area of the chamber than the SHAM rats during the shock-probe fear-conditioning retention test. Actually, the PRH lesions might have increased activity levels because the PRH rats shuttled between the closed-arms of the elevated-plus maze significantly more than the SHAM rats.

Overall, the present findings suggest that pretraining PRH and PORH lesions do not impair memory for object-fear conditioning. The findings also suggest that the PRH is involved in mediating avoidance and burying evoked by an object associated with a fear-eliciting event.

3.2 EXPERIMENT 4: The PRH and Burying In the Shock-Probe Fear-Conditioning Test

In Experiment 3, during the acquisition session and retention test, rats with PRH damage did not bury more than NAÏVE rats. Hence, it is possible that damage to the PRH eliminated this response from their coping repertoire. Considering this possibility, the aim of this experiment was to determine whether rats with PRH lesions are capable of burying in the shock-probe test.

It has been shown that rats bury more in a small apparatus than in a bigger one (Pinel, Treit, Ladak, & MacLennan, 1980). Thus, it was rationalized that rats with PRH damage would be more likely to bury, if indeed they can bury, in a shock-probe chamber that was significantly smaller than the one used in Experiment 3.

3.2.1 METHODS

The procedures in Experiment 4 were the same as those in Experiment 3 with a few exceptions. First, rats were only tested in the shock-probe test, and only SHAM ($n = 7$) and PRH ($n = 6$) rats were tested. Second, the shock-probe apparatus was smaller (40 X 20 X 20.5 cm) and had a lid to prevent the rats from escaping. This procedure was followed to maximize the likelihood of eliciting burying behaviour (Pinel, Treit, Ladak et al., 1980). Third, the rats were not habituated to the apparatus to reduce familiarity of the context, which could potentially increase fear and burying. Finally, we did not include a NAÏVE group - all the rats were exposed to the electrified probe during the acquisition session.

3.2.2 RESULTS

3.2.2.1 Histology

The location and extent of the largest and smallest PRH lesions are shown in Figure 18. There was substantial and nearly complete, bilateral damage to the PRH in each lesion rat and were similar to those in Experiment 3. All lesions included bilateral damage to the lateral entorhinal cortex; this damage was minimal in 3 of the 6 rats, and moderate in the remaining rats. Overall, there was very little damage to the PORH; only 1 rat showed some bilateral damage, and the remaining rats showed minor unilateral damage.

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 2 rats sustained unilateral damage. There was bilateral damage to the ventral portions of the CA1 cell field and subiculum in 2 of the 6 rats, and an additional 3 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 rats it was unilateral.

3.2.2.2 Behavioural Results

3.2.2.2.1 ***Acquisition.*** There was no significant difference in the number of contact-induced shocks between PRH rats ($M = 3.17 \pm 0.60$) and SHAM rats ($M = 3.57 \pm 0.61$), $U = 18.5, p = .715$. There was also no significant difference in shock reactivity between PRH rats ($M = 2.01 \pm 0.12$) and SHAM rats ($M = 1.85 \pm 0.20$), $U = 18.5, p = .716$. Figure 19 shows the amount of time spent burying during the acquisition session. Although there

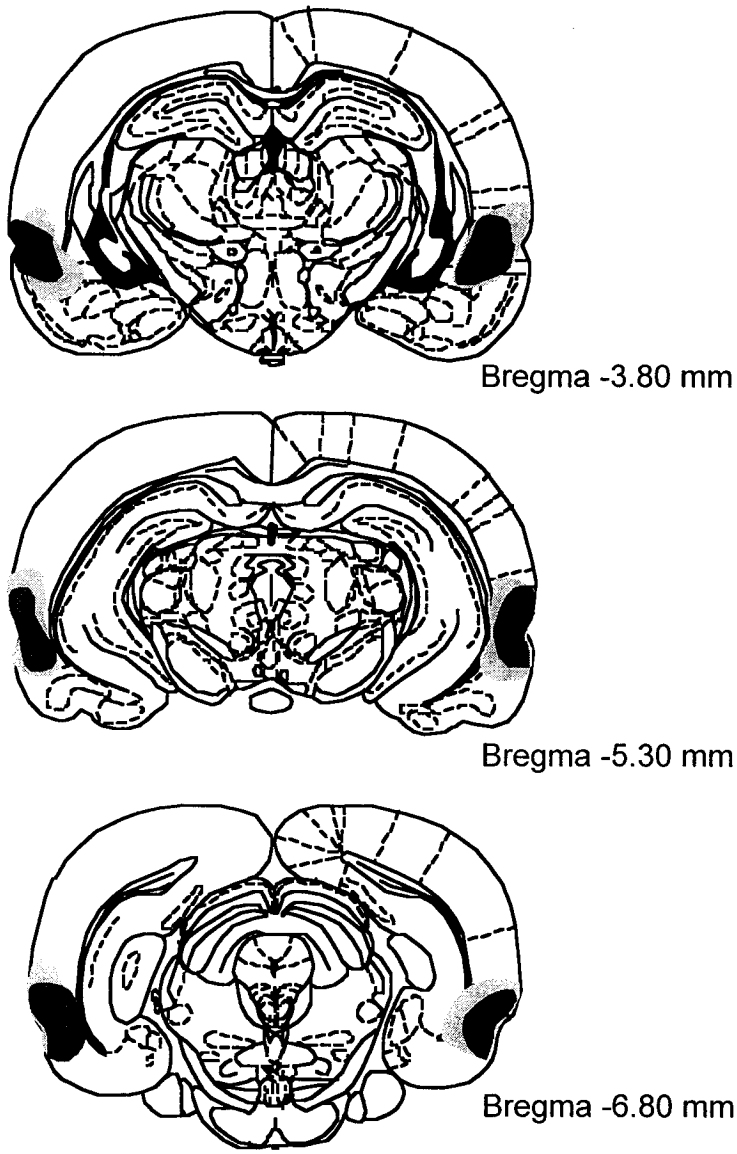

Figure 18. Illustrations of the smallest (dark gray) and largest (light gray) lesion observed bilaterally through the rostral and caudal extent of the PRH rats. Atlas plates are from Paxinos and Watson (1997).

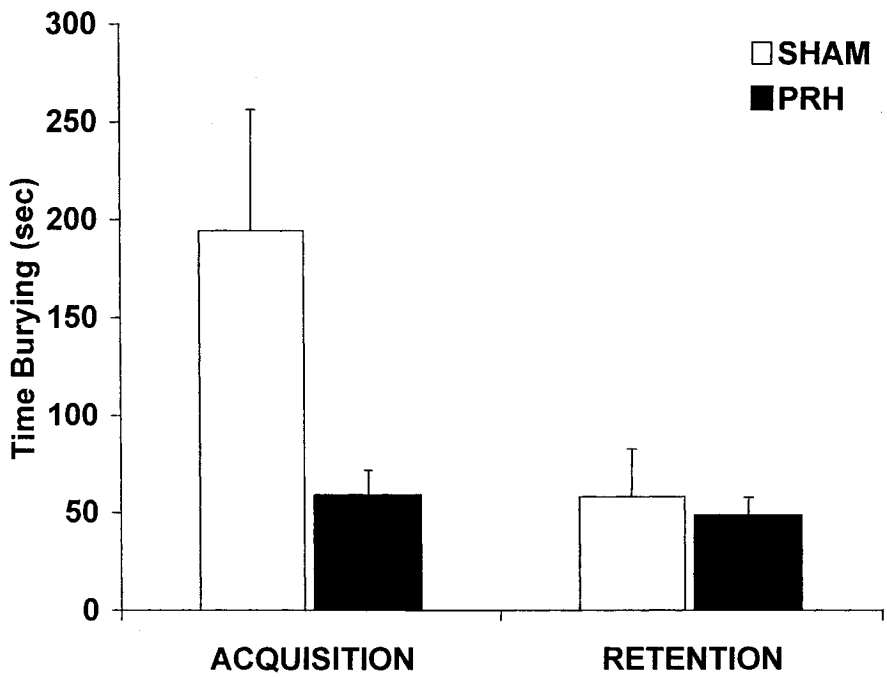

Figure 19. Mean (± *SEM*) amount of time spent burying during the acquisition session and retention test by SHAM and PRH rats.

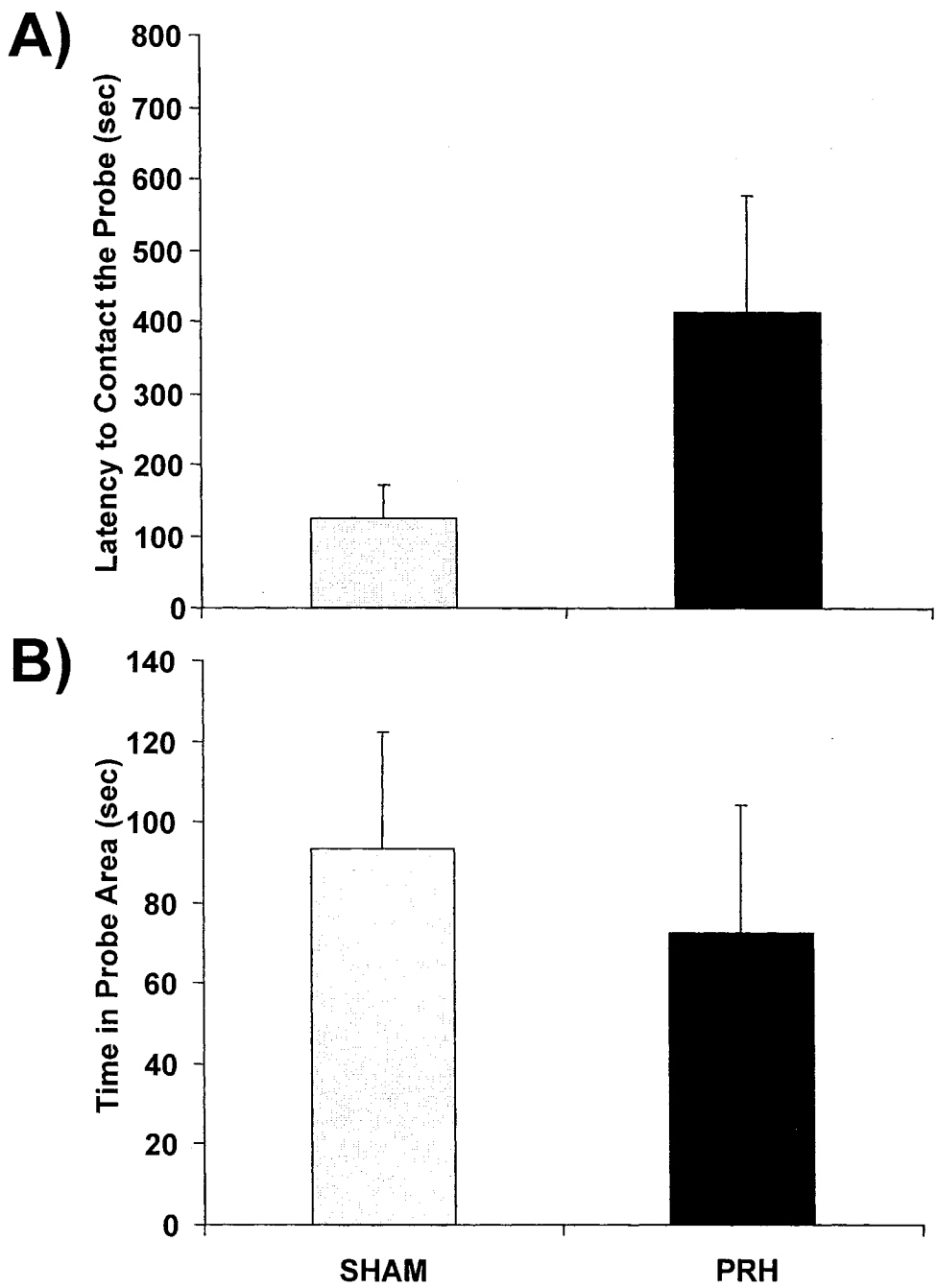

Figure 20. Mean (± *SEM*) **(A)** latency to contact the probe and **(B)** amount of time spent in the probe area of the chamber during the retention test for SHAM and PRH rats.

was a tendency for the PRH rats to bury less than SHAM rats, a one-tailed test failed to find a significant difference between the two groups, $U = 13.0, p = .127$.

3.2.2.2.2 *Retention.* Figure 19 shows the amount of time spent burying on the retention test and the PRH rats did not bury less than SHAM rats, $U = 20.0, p = .443$. Figure 20 illustrates the latency to initially contact the probe and the amount of time spent in the probe area of the chamber on the retention test. The latencies to contact the probe did not significantly differ between the PRH and SHAM rats, $U = 14.0, p = .158$, nor did the amount of time spent in proximity of the probe, $U = 16.0, p = .238$.

3.2.3 DISCUSSION

The present findings suggest that the PRH is not a critical structure for the expression of burying evoked by an object associated with a fear-eliciting event. The PRH rats buried for approximately 60-s during both the acquisition session and retention test and, more importantly, they did not bury significantly less than the SHAM rats during the retention test. In addition, the findings of the current experiment combined with those of Experiment 3 suggest that the proposed role of the PRH in mediating avoidance and burying is dependent on the environmental conditions in which the rats encounter the object associated with the fear-eliciting event. In the present experiment, unlike in Experiment 3, the PRH lesions did not significantly reduce the amount of burying and did not significantly enhance the two avoidance responses during the retention test. However, the testing chamber was much smaller than the one used in the previous experiment as well as less familiar because the rats were not habituated to it prior to the acquisition session. Thus, the PRH might be involved in appraising the

environmental conditions that influence the expression of avoidance and burying rather than directly involved in mediating these responses.

Furthermore, the findings in this experiment support the conclusion, from Experiment 3, that the pretraining PRH lesions do not cause anterograde amnesia for object-fear conditioning, because the PRH rats successfully buried and avoided the probe during the retention test.

3.3 EXPERIMENT 5: The Effects of Pre- and Post-Training Neurotoxic Lesions of the PRH In the Shock-Probe Fear-Conditioning Test

Although the findings of Experiment 3 and 4 suggest that PRH lesions do not cause anterograde amnesia for object-fear conditioning, it does not necessarily imply that the PRH is not involved in memory of an association between an object and a fear-eliciting event. In the previous chapter, lesions of the HPC had dissociable effects on anterograde and retrograde amnesia for object-fear conditioning. Moreover, there is evidence that PRH lesions may cause retrograde amnesia for object recognition (Mumby & Glenn, 2000). Thus, PRH lesions may cause retrograde amnesia for object-fear conditioning and the principal goal of this experiment was to investigate whether post-training lesions of the PRH would cause memory deficits in the shock-probe fear-conditioning test.

It was also rationalized that neurotoxic lesions of the PRH would be more appropriate to investigate the effects of post-training PRH damage on object-fear conditioning. Neurotoxic lesions typically spare fibers of passage, whereas aspiration lesions do not. Studies have occasionally shown that lesion that include damage to fibers of passage are more likely to cause mnemonic deficits that are not attributable to the actual functions of the target structure (Campeau & Davis, 1995a; Maren et al., 1997; Meunier, Bachevalier, Murray, Malkova, & Mishkin, 1999). For instance, electrolytic lesions of the PRH, which damage fibers of passage, disrupted fear-potentiated startle to discrete auditory and visual stimuli, but not when the lesions were made neurotoxically (Campeau & Davis, 1995a; Rosen et al., 1992). The lesions in both cases were equally extensive and the difference in results was attributed to damage of fibers of passage within or surrounding the PRH, such as external capsule fibers or fibers that course the

PRH and target the AMY (Campeau & Davis, 1995a). It is also possible that pretraining neurotoxic lesions of the PRH may have different effects on avoidance and burying in the shock-probe fear-conditioning test than the aspiration lesions in Experiment 3. Accordingly, neurotoxic lesions were used to assess the effects of pre- and post-training PRH lesions on object-fear conditioning in the current experiment.

This experiment was also designed to investigate whether a longer retention interval (i.e., interval between acquisition and retention) than the one used in Experiment 3 and 4 would be more likely to reveal anterograde amnesia for object-fear conditioning following PRH lesions. There is evidence suggesting that lesions may cause anterograde amnesia by accelerating the decay of memories, such that memory seems intact when the memory tests involve a short retention delay, but deficits become apparent after longer retention delays (Clark et al., 2001; Clark et al., 2000; Mumby et al., 1995; Vnek & Rothblat, 1996). Although the retention intervals in Experiment 3 and 4 was limited to 1-d and did not reveal any deficits, it is possible that a substantially longer retention interval would reveal deficits. Thus, the current experiment was also designed to assess the effects of pretraining PRH lesions on memory in the shock-probe fear-conditioning test with a retention interval that lasted 14-d.

In sum, the purpose of this experiment was to assess whether neurotoxic lesions of the PRH induced 1-d after shock-probe fear conditioning would induce retrograde amnesia for object-fear conditioning. In addition, this experiment aimed at determining whether neurotoxic lesions of the PRH would cause anterograde amnesia for object-fear conditioning when the retention interval lasted 14-d. It is important to note this 14-d retention interval was selected arbitrarily as a long interval. However, this retention

interval is similar to the interval between acquisition and the retention test in the retrograde test condition. Consequently, with these retention intervals direct comparisons could be made between the performance of rats with PRH-damage in the retrograde and anterograde test conditions.

3.3.1 METHODS

3.3.1.1 Subjects

Male Long-Evans rats (Charles River, Quebec; 300-350 g) served as subjects. Their housing conditions were the same as in Experiment 1 with the exception that they were provided with approximately 30 g of food daily.

3.3.1.2 Surgery

The surgical procedures were similar to those in Experiment 1 with a few exceptions. First, all the rats were anesthetized with isoflurane. Second, the PRH lesions were made by 5 bilateral NMDA infusions (see Table 2 for coordinates) with the cannulae angled 10° laterally. Third, at each infusion site, a total volume of .2 µl was infused at a flow rate of .2 µl/min and the injection needle was left in place for an additional 4 min following the injection to facilitate diffusion. Finally, food was made available *ad libitum* only during the first 7 days of the recovery period and rats in the retrograde condition were limited to a 14-day recovery period.

3.3.1.3 Behavioural Procedures

3.3.1.3.1 ***Retrograde Shock-Probe Test (RETROGRADE condition).*** The procedures for habituation, acquisition, and retention were the same as those in

TABLE 2. Injection coordinates relative to Bregma (in mm) for neurotoxic (NMDA) lesions of the PRH

AP	ML (10°)	DV
-3.0	±5.5	-9.5
-4.0	±5.5	-9.5
-5.0	±5.5	-9.5
-6.0	±5.5	-9.5
-7.0	±5.5	-8.5

Experiment 2 with the exception that all the rats received surgery 1 day following acquisition.

3.3.1.3.2 ***Anterograde Shock-Probe Test (ANTEROGRADE condition).*** The procedures for habituation, acquisition, and retention were the same as those in Experiment 3 with the exception that the interval between acquisition and retention was 14 days.

3.3.1.4 Histology

The same procedures as in Experiment 1 were used.

3.3.1.5 Statistical Analyses

The same procedures as in Experiment 1 were used.

3.3.2 RESULTS

3.3.2.1 Histology

The location and extent of the largest and smallest neurotoxic PRH lesions in the RETROGRADE and ANTEROGRADE condition are shown in Figure 21. Three rats were excluded from the study because of minimal PRH damage. The remaining lesion rats had extensive bilateral damage through the rostral-caudal extent of the PRH. However, there was some minor sparing of the most anterior part of the PRH in most lesion rats. The majority of the lesions included minor damage to the area of the EC immediately adjacent to the PRH. The majority of the lesions also included damage to portions of the ventral temporal association cortex. This damage was minimal in 6 rats and moderate in 10 rats. Three rats sustained bilateral and 3 others unilateral damage to the PORH. There was minor unilateral damage to the ventral portions of the CA1 cell

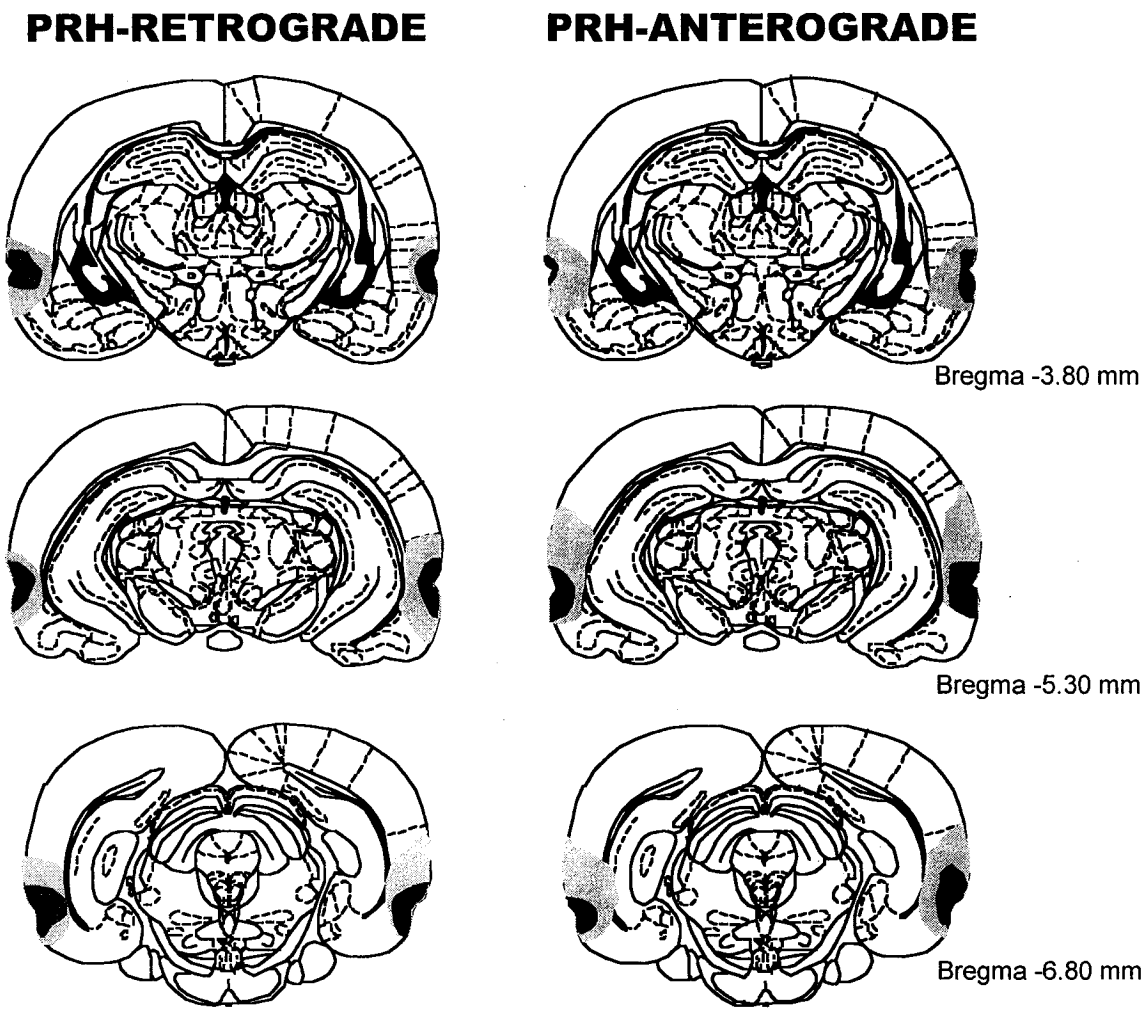

Figure 21. Illustrations of the smallest (dark grey) and largest (light gray) neurotoxic lesion observed bilaterally through the rostral and caudal extent of the PRH rats in the RETROGRADE and ANTEROGRADE condition. Atlas plates are from Paxinos and Watson (1997).

field in 2 rats. Damage was found in the posterior region of the subiculum unilaterally in 5 rats and bilaterally in 2 rats. The dorsal area of the piriform cortex was damaged bilaterally in 7 rats and unilaterally in 3 rats. In all PRH rats there was also some damage to the posterior parietal cortex where the injection needles were inserted. No damage was found in the AMY.

3.3.2.2 Behavioural Results

The data from the four NAÏVE control groups (SHAM or PRH and RETROGRADE or ANTEROGRADE) were pooled because their behavioural results on the acquisition session and retention test were not statistically different (see Appendix B). Consequently, the analytical comparisons of the data involved five groups. The NAÏVE group (n= 13), which included all the rats exposed to the non-electrified probe, and the four groups of rats exposed to the electrified probe: SHAM-RETROGRADE (n = 9), SHAM-ANTEROGRADE (n = 8), PRH-RETROGRADE (n = 8), and PRH-ANTEROGRADE (n = 6).

3.3.2.2.1 ***Acquisition.*** Figure 22 shows the number of contact-induced shocks the rats received during the acquisition session. There was no significant difference in the number of contact induced-shocks between PRH and SHAM rats (PRH-RETROGRADE vs. SHAM-RETROGRADE, $U = 32.5, p = .363$; PRH-ANTEROGRADE vs. SHAM-ANTEROGRADE, $U = 18.0, p = .215$).

There was also no significant difference in shock reactivity between SHAM-RETROGRADE ($M = 2.09$, $SEM = 0.12$) and PRH-RETROGRADE rats ($M = 2.10 \pm SEM = 0.07$), $U = 26.5, p = .153$, nor between SHAM-ANTEROGRADE ($M = 2.14 \pm 0.16$) and PRH-ANTEROGRADE rats ($M = 2.38 \pm 0.16$), $U = 15.0, p = .119$.

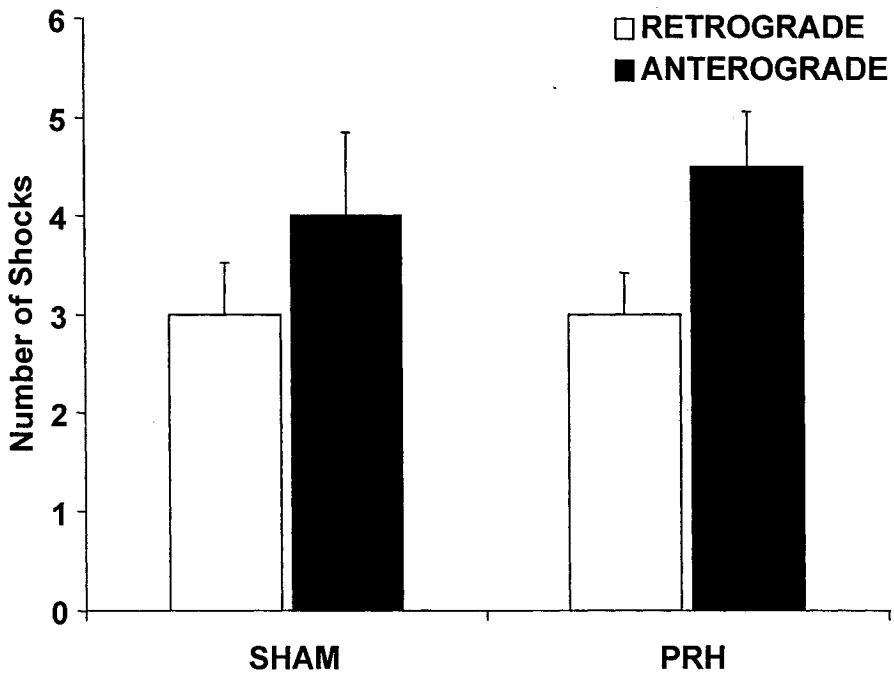

Figure 22. Mean (± *SEM*) number of contact-induced shocks received during the acquisition session by SHAM and PRH rats in the RETROGRADE and ANTEROGRADE conditions.

Figure 23 shows the amount of time spent burying during the acquisition session. The rats exposed to the electrified probe buried more than the ones that were exposed to the non-electrified probe (SHAM-RETROGRADE vs. NAÏVE, $U = 4.0, p < .001$; SHAM-ANTEROGRADE vs. NAÏVE, $U = 11.0, p < .001$; PRH-RETROGRADE vs. NAÏVE, $U = 13.0, p < .01$; PRH-ANTEROGRADE vs. NAIVE, $U = 2.0, p < .001$). No significant differences in the amount of time spent burying were found between PRH and SHAM rats (PRH-RETROGRADE vs. SHAM-RETROGRADE, $U = 28.5, p = .235$; PRH-ANTEROGRADE vs. SHAM-ANTEROGRADE, $U = 22.0, p = .398$).

3.3.2.2.2 *Retention.* Figure 24 shows the amount of time spent burying on the retention test. The SHAM and PRH rats in the RETROGRADE condition buried significantly more than the NAÏVE rats (SHAM-RETROGRADE vs. NAÏVE, $U = 24.0$, $p < .01$; PRH-RETROGRADE vs. NAÏVE, $U = 24.5, p < .05$). In the ANTEROGRADE memory condition, the SHAM-ANTEROGRADE rats did not bury significantly more than the NAÏVE rats, $U = 33.5, p = .069$, but the PRH-ANTEROGRADE rats did, $U = 14.5, p < .01$. No significant differences where found between the PRH rats and the SHAM rats in either memory test condition (PRH-RETROGRADE vs. SHAM-RETROGRADE, $U = 36.0, p = .50$; PRH-ANTEROGRADE vs. SHAM-ANTEROGRADE, $U = 18.5, p = .236$). In addition, no differences were found between the PRH rats from either condition (PRH-ANTEROGRADE vs. PRH-RETROGRADE, $U = 23.0, p = .897$)

Figure 25A illustrates the latency to initially contact the probe on the retention test. The SHAM rats and the rats in the PRH-ANTEROGRADE had significantly longer latencies than NAÏVE rats, whereas the PRH-RETROGRADE did not (SHAM-

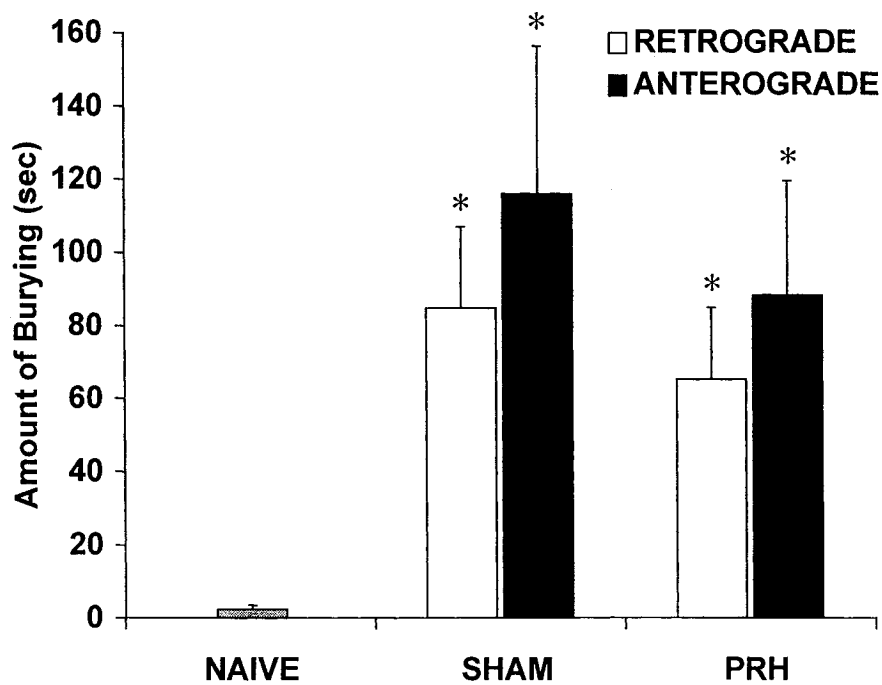

Figure 23. Mean (± *SEM*) amount of time spent burying during the acquisition session by NAÏVE, SHAM, and PRH rats in the RETROGRADE and ANTEROGRADE memory test conditions (*$p < .05$ versus NAIVE).

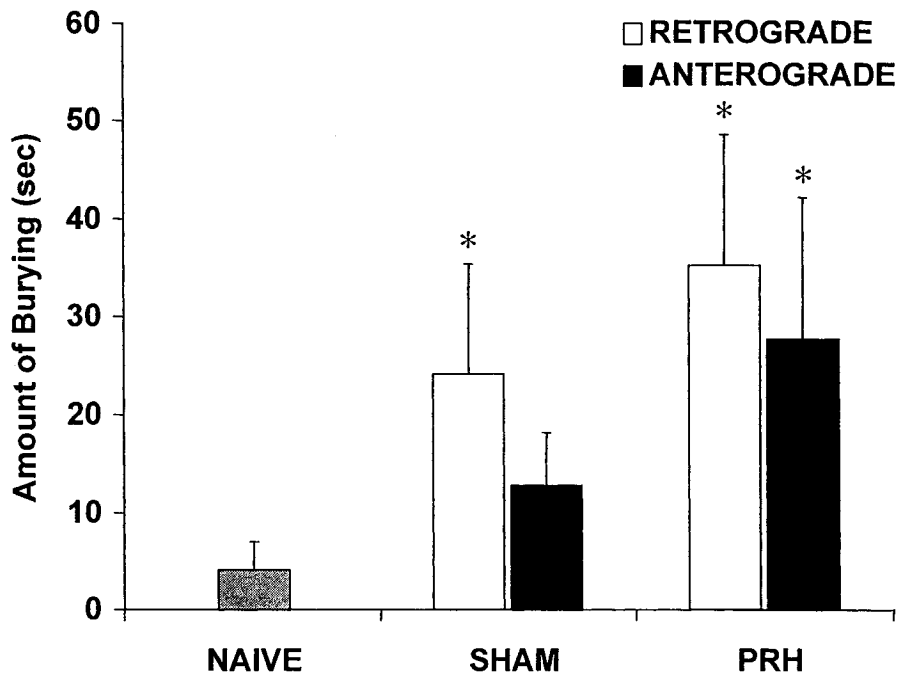

Figure 24. Mean (± *SEM*) amount of time spent burying during the retention test by NAÏVE, SHAM, and PRH rats in the RETROGRADE and ANTEROGRADE conditions (*$p < .05$ versus NAÏVE).

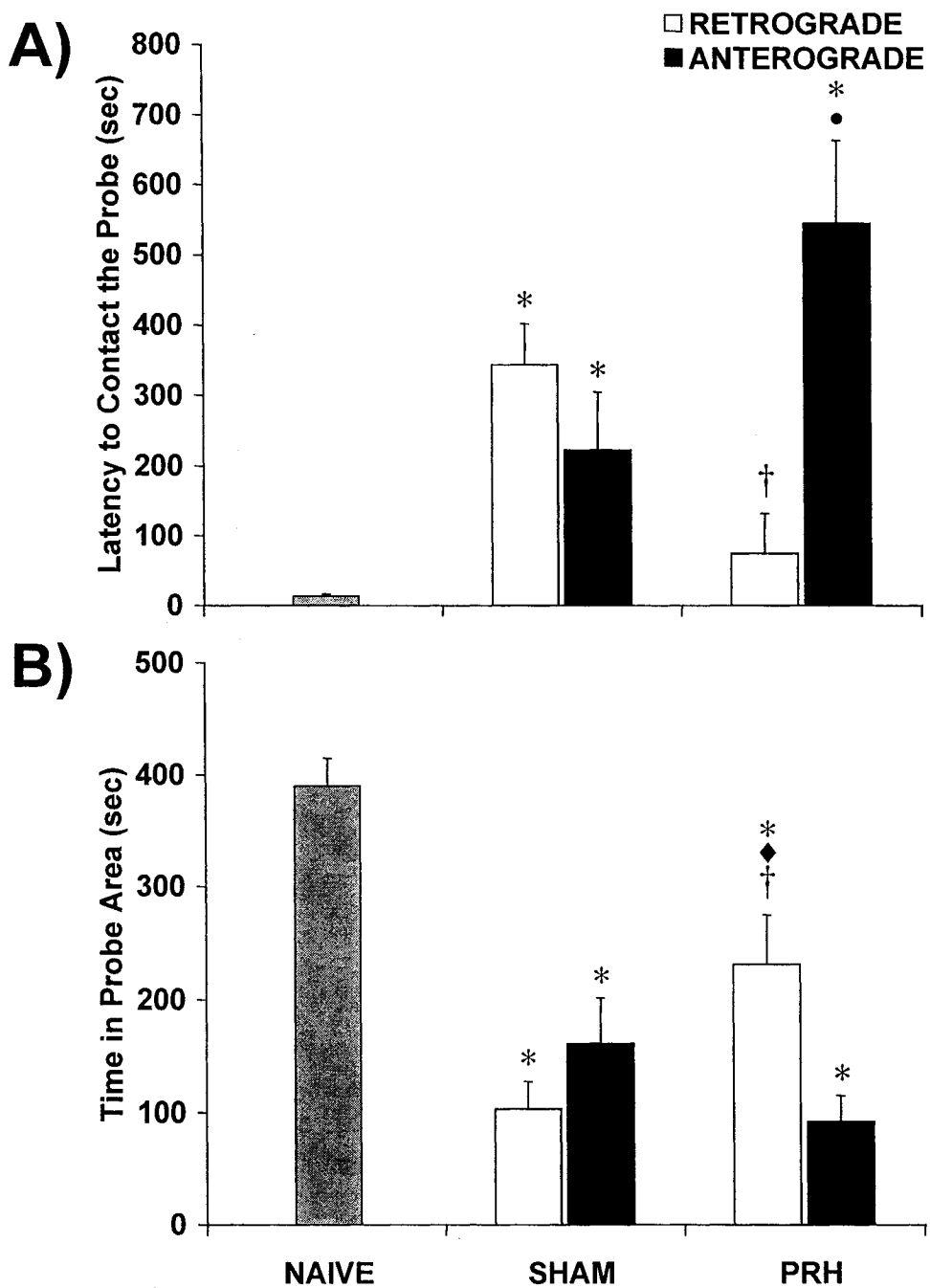

Figure 25. Mean (± *SEM*) **(A)** latency to contact the probe and **(B)** amount of time spent in the probe area of the chamber during the retention test for NAÏVE, SHAM and PRH rats in the RETROGRADE and ANTEROGRADE conditions (*$p < .05$ versus NAIVE; •$p < .05$ versus SHAM-ANTEROGRADE; ♦$p < .05$ versus SHAM-RETROGRADE; † $p < .05$ versus PRH-ANTEROGRADE).

RETROGRADE vs. NAÏVE, $U = 0.0, p < .001$; SHAM-ANTEROGRADE vs. NAÏVE, $U = 2.5, p < .001$; PRH-ANTEROGRADE vs. NAÏVE, $U = 0.0, p < .001$; PRH-RETROGRADE vs. NAIVE, $U = 46.5, p = .345$). The PRH-ANTEROGRADE rats had significantly longer latencies than SHAM-ANTEROGRADE rats, $U = 10.0, p < .05$. In contrast, the PRH-RETROGRADE rats had significantly shorter latencies than SHAM-RETROGRADE rats, $U = 8.0, p < .01$. The PRH-RETROGRADE rats also had significantly shorter latencies than PRH-ANTEROGRADE rats, $U = 2.0, p < .01$.

Figure 25B illustrates the amount of time spent in the half area of the chamber where the probe was located. All the groups that were exposed to the electrified probe during the acquisition session spent significantly less time in the probe area than NAÏVE rats (SHAM-RETROGRADE vs. NAÏVE, $U = 1.0, p < .001$; PRH-RETROGRADE vs. NAÏVE, $U = 15.0, p < .01$; SHAM-ANTEROGRADE vs. NAÏVE, $U = 7.0, p < .001$; PRH-ANTEROGRADE vs. NAIVE, $U = 0.0, p < .001$). In addition, the PRH-RETROGRADE rats spent significantly more time in the probe area than the SHAM-RETROGRADE rats, $U = 14.5, p < .05$, but no significant differences were found between PRH- and SHAM-ANTEROGRADE rats, $U = 53.0, p = .151$. The PRH-RETROGRADE rats also spent significantly more time in the probe area than the PRH-ANTEROGRADE rats, $U = 10.0, p < .05$.

3.3.3 DISCUSSION

The main finding from the current experiment suggests that memory for object-fear conditioning is not dependent on the PRH. The PRH-RETROGRADE rats buried significantly more than the NAÏVE rats and as much as the SHAM-RETROGRADE rats

during the retention test. Another important finding is that even after a 14-d retention interval, the PRH lesions did not cause anterograde amnesia because the PRH-ANTEROGRADE rats buried significantly more than NAÏVE rats and avoided the probe significantly more than NAÏVE and SHAM-ANTEROGRADE rats.

The absence of retrograde amnesia for object-fear conditioning is inconsistent with previous evidence implicating the PRH in memory for cued fear conditioning (Campeau & Davis, 1995a; Corodimas & LeDoux, 1995; Falls, Bakken, & Heldt, 1997; Rosen et al., 1992). The conflicting findings are most difficult to resolve since shock-probe fear-conditioning retention performance likely depends on the visual and olfactory features of the probe and that post-training PRH damage disrupts fear conditioning to cues specific to each of these sensory modalities.

However, in the studies reporting impaired cued fear conditioning following post-training PRH lesions the cues were neither tangible nor the source of the aversive stimulus, whereas in the current experiment the probe was both. These properties may have minimized the role of the PRH in memory for cued fear conditioning. Moreover, in contrast to the cue used in the current experiment, the studies reporting impairments did not use cues that involved the integration of multiple sensory features. It is possible that the combination of the various sensory properties of the probe might have made the cue more complex, which in turn would render the fear conditioning dependent on neural structures that are thought to be involved in processing multidimensional information, such as the HPC. The finding from Experiment 2 in which HPC lesions impaired object-fear conditioning supports this possibility.

It is also possible that the studies reporting retrograde amnesia for cued fear conditioning following PRH lesions are only reporting a behavioural deficit for the single fear response they assessed and preemptively concluded that it reflects a memory deficit. In the current experiment, several behaviours were assessed to index fear and memory, and only one was intact following the lesions. The PRH-RETROGRADE rats buried during the retention test, but contacted the probe more quickly and spend more time in the probe area than SHAM rats. This discrepancy between burying and avoidance responses suggests that PRH lesions may disrupt some fear behaviours while leaving others intact and emphasizes that the assessment of only one fear behaviour may lead to erroneous conclusions about memory for fear conditioning.

The evidence from Experiment 3 and 4 already suggested that PRH lesions did not cause anterograde amnesia for object-fear conditioning for a 1-d retention interval, but it was unclear whether memory would still be intact after a substantially longer retention interval. Several studies have shown that anterograde amnesia is more likely to be apparent when the retention interval between training and testing is long (Clark et al., 2001; Clark et al., 2000; Mumby et al., 1995; Vnek & Rothblat, 1996). Consequently, the PRH rats, in the present experiment were tested after a 14-d retention interval to maximize the chances of detecting any memory impairment. However, the pretraining PRH lesions did not impair burying nor avoidance of the probe, suggesting that memory for object-fear conditioning is intact for a lasting period of time following PRH lesions.

It is interesting that the PRH lesions in this experiment did not impair burying. In Experiment 3, rats with aspiration lesions of the PRH had a severe burying deficit. In contrast, rats with neurotoxic lesions of the PRH, in this experiment, successfully buried

during both the acquisition session and the retention test. Since the extent of PRH damage in both experiments was similar, the divergence in burying by PRH rats in the two experiments cannot be accounted for by differences in lesion size within the PRH. The divergent findings are most likely due to the method used to damage the PRH. It is likely that the fibers of passage within and/or surrounding the PRH are involved in burying. The findings of Experiment 4, however, clearly demonstrated that rats with aspiration lesions of the PRH are able to bury to the same extent as SHAM rats. Consequently, damage to the fibers of passage within and/or surrounding the PRH appears only to impair rather than block the block the expression of burying.

Although, pretraining neurotoxic and aspiration lesions had differential effects on the burying response, both lesion methods enhanced avoidance of the probe. Therefore, the contention, from Experiment 3, that the PRH is involved in mediating avoidance of an object associated with a fear-eliciting event is supported by the results of the current experiment. It is also noteworthy that in the current experiment the pre- and post-training PRH lesions had a dissociative effect on avoidance. During the retention test, PRH-ANTEROGRADE rats avoided the probe significantly more then the SHAM rats, whereas the PRH-RETROGRADE rats avoided it significantly less. Moreover, a significant difference was found between both PRH groups. The reason for this dissociation is unclear, but it strengthens the view that the PRH is involved in mediating avoidance in object-fear conditioning because lesions of the PRH reliably affect avoidance in the shock-probe fear-conditioning test.

In summary, the present findings clearly suggest that PRH is not necessary for memory for object-fear conditioning because neither pre- nor post-training lesions cause

amnesia. However, the findings suggest that the PRH is involved in mediating avoidance of an object associated with a fear-eliciting event.

3.4 SUMMARY AND IMPLICATIONS

A major part of the rationale for examining the role of the PRH in object-fear conditioning was that the structure is thought to be important for object recognition (Buffalo et al., 1999; Bussey et al., 1999; Bussey et al., 2002; Ennaceur & Aggleton, 1997; Gaffan & Murray, 1992; Glenn & Mumby, 1996; Meunier et al., 1993; Mumby et al., 2002; Mumby & Pinel, 1994; Murray & Bussey, 1999; Thornton et al., 1997; Zola-Morgan, Squire, Amaral et al., 1989). Hence, it was believed that damaging the PRH would impair object-recognition processes and in turn cause object-fear conditioning deficits. However, rats with extensive PRH damage, which typically cause object-recognition deficits (Glenn & Mumby, 1996), showed robust memory for an association between an object and a fear-eliciting event, suggesting that object-fear conditioning does not require object-recognition.

It seems paradoxical that an object can evoke fear because it was previously associated with a fear-eliciting event, and yet not be recognized. However, dissociations between responding to a previously encountered stimulus and recognizing it have often been reported in patients suffering from amnesia (Cohen & Squire, 1980; Squire, Cohen, & Zouzounis, 1984; Warrington & Weiskrantz, 1968). For instance, amnesic patients are often able to successfully perform a motor task on which they were previously trained without recalling ever having done the task before (Cohen & Squire, 1980; Squire et al., 1984). Such dissociations have led to the proposal that there are multiple parallel memory systems in the brain (Cohen & Squire, 1980; Squire, 1987). It is possible that damaging the PRH disrupted one memory system involved in object-fear conditioning, but left parallel systems that are also involved in object-fear conditioning functional.

The PRH was also considered potentially important for object-fear conditioning because several studies have reported impaired fear conditioning following damage to the PRH (Burwell et al., 2004; Campeau & Davis, 1995a; Herzog & Otto, 1997, 1998; Rosen et al., 1992). Based on these later studies it has even been proposed that the PRH is involved in learning and remembering sensory information, which is associated with the fear-eliciting event during fear conditioning (Burwell et al., 2004; Otto & Giardino, 2001). However, the experiments, in this chapter, clearly demonstrated that memory for object-fear conditioning is not dependent on the PRH, and thus fail to support this view.

An alternative belief is that the PRH may have a predominant role in the processing of fear-eliciting stimuli that guide the expression of fear responses. In this chapter, it was found that lesions of the PRH altered avoidance of an object associated with a fear-eliciting event and that it was dependent on the environmental conditions in which the object was encountered. Similar changes in expression of fear responses evoked by fear-eliciting stimuli have been reported in monkeys with rhinal cortex damage (Meunier & Bachevalier, 2002). In addition, the parahippocampal region, which includes the PRH, is activated in humans processing words of threatening valence (Isenberg et al., 1999). Thus, it is possible that the PRH may be responsible for processing and appraising stimuli involved in fear conditioning, but not a specialized role in establishing and retaining information about the association.

It has been speculated that the changes in fear responses following PRH damage are due to a disruption of the interaction between the PRH and AMY (Meunier & Bachevalier, 2002; Otto & Giardino, 2001). There are major projections between the two structures (Deacon et al., 1983; Ottersen, 1982; Shi & Davis, 2001) and it is well known

that the AMY is involved in the expression of fear (M. Davis, 1992; J. E. LeDoux, 2000; Maren, 2001b). Therefore, it is possible that PRH lesions changed the processing of the information that is sent to the AMY and in turn altered the fear responses that are expressed in object-fear conditioning.

However, object-fear conditioning does not appear to be dependent on the AMY. Damage to the AMY does not alter avoidance in the shock-probe fear-conditioning test (Lehmann et al., 2000, 2003; Treit & Menard, 1997; Treit, Pesold et al., 1993). Despite this evidence, it has been argued that other fear responses need to be assessed prior to concluding that the AMY is not required for object-fear conditioning (Maren, 2003b). Consequently, the experiments in the next chapter aim at more comprehensively evaluating the role of the AMY in shock-probe fear conditioning.

CHAPTER 4

THE AMY AND OBJECT-FEAR CONDITIONING

The experiments in the preceding two chapters examined the roles of the HPC and PRH in object-fear conditioning because both structures are involved in fear conditioning and object recognition. It was found that the HPC lesions, but not PRH lesions, caused amnesia for object-fear conditioning, and only retrograde amnesia. Although investigating the role of the latter two structures was warranted, the region that would usually be considered most critical for object-fear conditioning is the AMY because pre- and post-training AMY lesions characteristically cause severe and lasting fear conditioning impairments regardless of the type of stimuli that is associated with the fear-eliciting event (Campeau & Davis, 1995b; Corodimas & LeDoux, 1995; Cousens & Otto, 1998; Gale et al., 2004; Goosens & Maren, 2001; Maren, 1998, 1999a; Maren et al., 1996; Sananes & Davis, 1992).

However, two recent studies reported that AMY lesions do not affect memory for object-fear conditioning (Lehmann et al., 2000, 2003). Specifically, pre- and post-training AMY lesions did not disrupt avoidance of an object that was previously paired with a shock. In spite of these findings, the conclusion that the AMY is not necessary for object-fear conditioning has been debated (Maren, 2003b).

According to Maren (2003b), avoidance of an object associated with a shock is potentially a poor behavioural index of fear conditioning. This assertion is based on evidence suggesting that one may avoid a fear-eliciting stimulus without expressing other overt or covert responses that infer a general state of fear. Hence, rats with AMY damage, tested for object-fear conditioning, might remember to avoid the object, but not

experience any fear. Adding support to this argument is evidence from patients with AMY damage showing that they can explicitly report that a cue predicts the occurrence of an aversive stimulus, but do not show the typical increase in skin-conductance response, indicative of fear, when presented with the cue (Bechara, Damasio, Damasio, & Lee, 1999; Bechara et al., 1995).

It has been proposed that burying would be a better index of fear then avoidance in the shock-probe fear-conditioning test (Maren, 2003b). Thus, the goal of the experiments in this chapter was to elucidate whether the AMY is necessary for object-fear conditioning by assessing burying, as well as avoidance.

Experiment 6 was designed to assess whether pretraining AMY lesions would, at the very least, impair burying in the shock-probe fear-conditioning test. It was found that the lesions did not significantly affect burying, suggesting that pretraining AMY lesions did not disrupt memory or the expression of fear pertaining to object-fear conditioning.

The purpose of Experiment 7 was to extend the findings of Experiment 6 by assessing the effects of pretraining AMY lesions on changes in stress hormone release, which can be used to infer fear, in object-fear conditioning. Again, it was found that AMY lesions do not cause anterograde amnesia or disrupt fear of an object that was associated with an aversive stimulus because the lesions did not prevent or attenuate the increase in stress hormone release during the shock-probe fear-conditioning retention test.

The aim of Experiment 8 was to determine the effects of post-training AMY lesions on burying and avoidance in the shock-probe fear-conditioning tests. It was found that the

lesions impaired both behaviours during the retention test, suggesting that the AMY has an important role in memory for object-fear conditioning.

4.1 EXPERIMENT 6: The Effects of Pretraining Lesions of the AMY In the Shock-Probe Fear-Conditioning Test

It has been shown that pretraining inactivation of the AMY, with the sodium channel blocker tetrodotoxin, does not impair avoidance in the shock-probe fear-conditioning test (Lehmann et al., 2003). Such reversible lesions assure that the expression of behavioural responses on the retention test are not be affected by any performance deficits. However, to be successful, reversible lesions need to have lasted long enough to be in effect during the period when object-fear conditioning is dependent on the AMY. Because the precise period at which memory processes for object-fear conditioning may require the AMY are unknown, in the current experiment, electrolytic lesions of the AMY were used. This method causes permanent damage that would assuredly cover the period that necessitates the AMY, if indeed it is involved in object-fear conditioning. Also, to increase the chances of finding a memory deficit, a 14-d retention interval was used in the current experiment, which is substantially longer than the 4-d retention interval used in the Lehmann and colleagues study.

In addition, burying of an object that was previously associated with an aversive stimulus may be a more appropriate behavioural index of fear than avoidance (Maren, 2003b). Consequently, the effects of pretraining AMY lesions on burying and avoidance in the shock-probe fear-conditioning test were assessed. It was hypothesized that if the lesions disrupt fear conditioning, then they should impair burying on a retention test. Accordingly, a small shock-probe chamber was used in order to maximize the amount of burying expressed by the control rats and thereby reduce the chances of a floor effect.

4.1.1 METHODS

4.1.1.1 Subjects

Male Long-Evans rats (Charles River, Quebec; 300-350 g) served as subjects. Their housing conditions were the same as in Experiment 1 with the exception that they were provided with approximately 30 g of food daily.

4.1.1.2 Surgery

Rats were anesthetized with isoflurane (Janssen, Toronto, Ontario) in 0.8 L/min oxygen at 14.7 PSIA at 21°C (Benson Medical Industries, Markham, Ontario). The rats were then placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA) and a midline scalp incision was made to expose the top of the skull. Four holes were drilled into the skull and a bipolar stainless steel electrode, insulated with Teflon except for approximately 0.5 mm at the tip was lowered into the AMY (see Table 3 for coordinates). Electric current was then delivered (1.5 mA for 15-s) at each site sequentially. Following the lesions, the scalp incision was closed using wound clips and an antibiotic powder (Cicatrin, GlaxoWellcome) was applied to the wound. As the rats began awakening, they were given a dose of diazepam (.2 cc; 10 mg/ml, i.p.; Hoffman-La Roche, Mississauga, Ontario). The same surgical procedures were used for the SHAM rats, except that no damage was done to the skull or brain of these rats. All the rats were allowed to recover for a minimum of two weeks before beginning behavioural testing.

4.1.1.3 Behavioural Procedures

The shock-probe fear-conditioning test procedures were the same as those in Experiment 4 with the exception that the interval between acquisition and retention was 14 days.

TABLE 3. Coordinates relative to Bregma (in mm) for electrolytic lesions of the AMY

AP	ML	DV
-2.3	±4.8	-9.6
-3.3	±5.0	-9.8

4.1.1.4 Histology

The same procedures as in Experiment 1 were used.

4.1.1.5 Statistical Analyses

The same procedures as in Experiment 1 were used.

4.1.2 RESULTS

4.1.2.1 Histology

The location and extent of the smallest and largest AMY lesions are shown in Figure 26. Two rats were excluded from the experiment because they sustained more damage outside, than within, the AMY or less than 50% damage to the central nucleus and BLA in one hemisphere. The lesion rats that were retained had extensive bilateral damage to the BLA and central nucleus the two regions of the AMY that are thought to be important for memory and expression of fear conditioning (Maren, 2001b). However, minor sparing was found unilaterally in the BLA of 6 rats, unilaterally in the central nucleus and BLA of 1 rat, and bilaterally in both regions of 4 rats. It is important to point out that this sparing was predominantly in the most anterior and/or posterior portion of the AMY. Damage was found in the medial nucleus of the AMY in all lesion rats, but the extent of the damage was variable. Damage to the substantia innominata, nucleus basalis, caudate nucleus, globus pallidus, endopiriform nucleus, and to the piriform cortex was also found, in at least one hemisphere, of almost all the lesion rats. However, no damage was found in the PRH or ventral HPC, with the exception of 1 rat that sustained minor damage to the anterior PRH in one hemisphere.

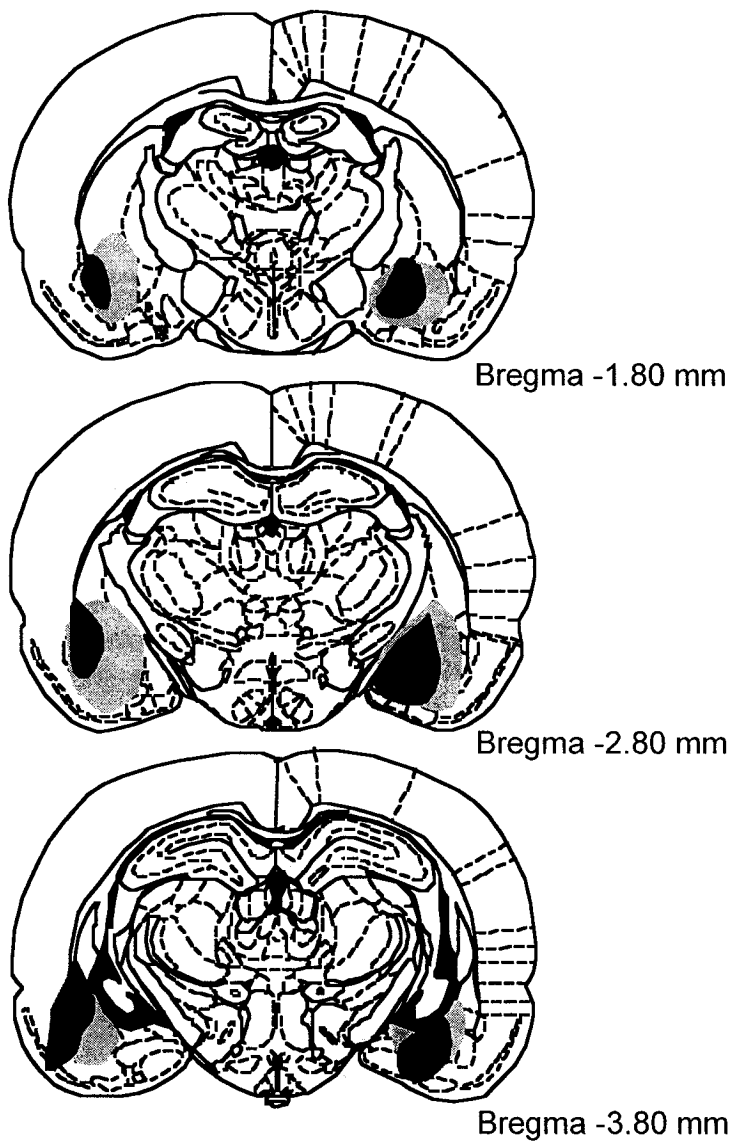

Figure 26. Illustrations of the smallest (dark grey) and largest (light gray) lesion observed bilaterally through the rostral and caudal extent of the AMY rats. Atlas plates are from Paxinos and Watson (1997).

4.1.2.2 Behavioural Results

The data from the two NAÏVE groups (SHAM and AMY) were pooled because their behavioural results on the acquisition session and retention test were not statistically different (see Appendix B). Consequently, the analytical comparisons of the data involved three groups. The NAÏVE group (n= 6), which included all the rats exposed to the non-electrified probe, and the two groups of rats exposed to the electrified probe: SHAM (n = 8) and AMY (n = 10).

4.1.2.2.1 ***Acquisition.*** Figure 27 shows the number of contact-induced shocks the rats received during the acquisition session. The AMY rats received significantly more than the SHAM rats, $U = 3.0, p < .001$. There was no significant difference in shock reactivity between the SHAM ($M = 2.06$, $SEM = 0.11$) and the AMY rats ($M = 1.93 \pm SEM = 0.07$), $U = 32.0, p = .235$.

Figure 28 shows the amount of time spent burying during the acquisition session. The rats exposed to the electrified probe buried significantly more than the NAÏVE rats (SHAM vs. NAÏVE, $U = 0.0, p < .001$; AMY vs. NAÏVE, $U = 0.0, p < .001$). No significant difference was found between the AMY and SHAM rats, $U = 34.0, p = .297$.

4.1.2.2.2 ***Retention.*** Figure 29 shows the amount of time spent burying on the retention test. The SHAM rats buried significantly more than the NAÏVE rats, $U = 6.0, p < .01$, as did the AMY rats, $U = 6.0, p < .01$. No significant difference was found between the AMY and SHAM rats, $U = 33.0, p = .266$.

Figure 30A illustrates the latency to initially contact the probe on the retention test. The groups that were exposed to the electrified probe during the acquisition session had significantly longer latencies than the NAÏVE rats (SHAM vs. NAÏVE, $U = 1.0, p < .01$;

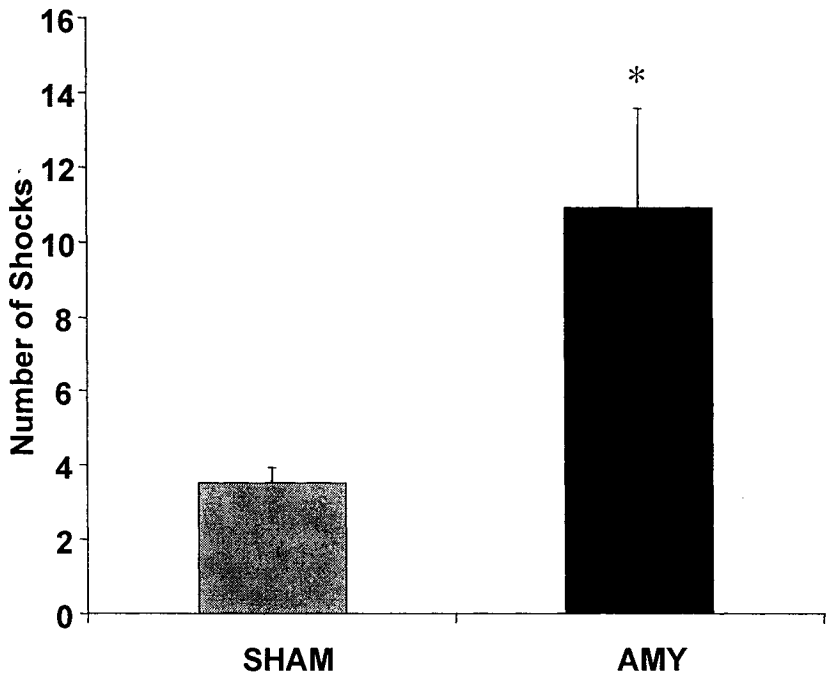

Figure 27. Mean (± *SEM*) number of contact-induced shocks received during the acquisition session by SHAM and AMY rats that were exposed to the electrified probe (*$p < .05$ versus SHAM).

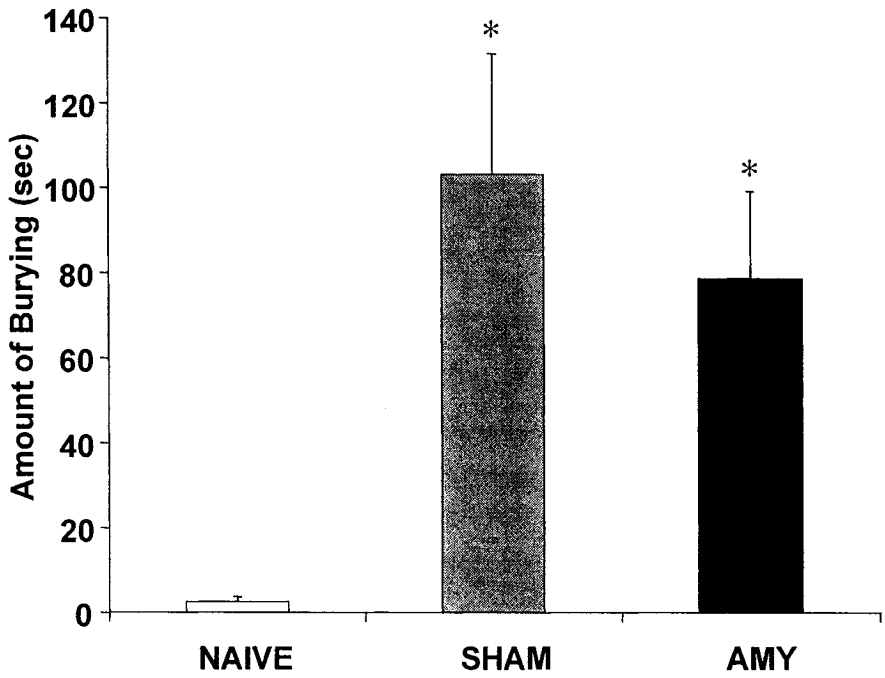

Figure 28. Mean (± *SEM*) amount of time spent burying during the acquisition session by NAÏVE, SHAM, and AMY rats (*$p < .05$ versus NAIVE).

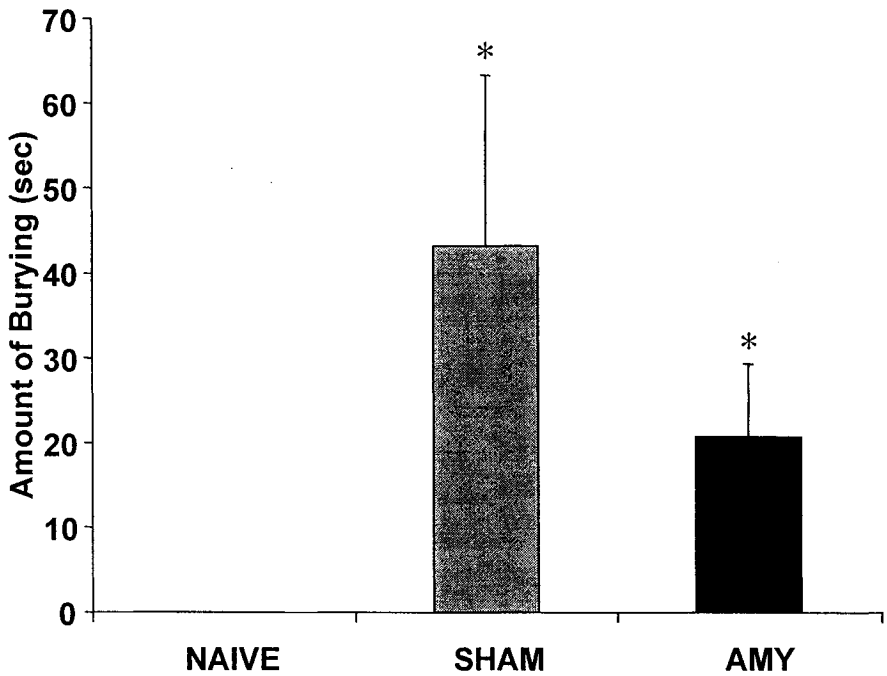

Figure 29. Mean (± *SEM*) amount of time spent burying during the retention test by NAÏVE, SHAM, and AMY rats (*$p < .05$ versus NAIVE).

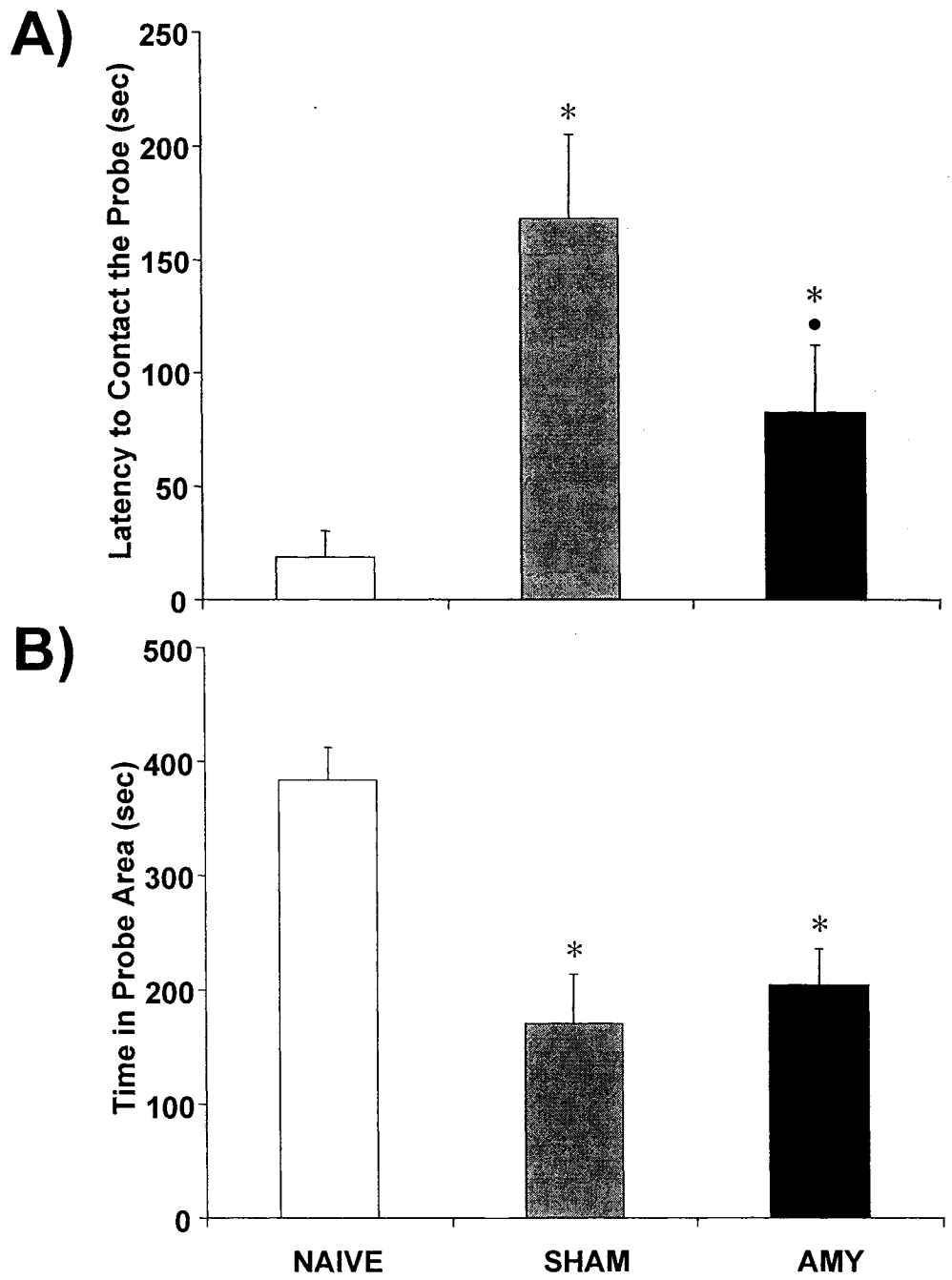

Figure 30. Mean (± *SEM*) **(A)** latency to contact the probe and **(B)** amount of time spent in the probe area of the chamber during the retention test for NAÏVE, SHAM, and AMY rats (*$p < .05$ versus NAIVE; • $p < .05$ versus SHAM).

AMY vs. NAÏVE, $U = 12.0, p < .05$). However, the AMY rats had significantly shorter latencies than the SHAM rats, $U = 21.0, p < .05$.

Figure 30B illustrates the amount of time spent in the half of the chamber where the probe was located. The SHAM and AMY rats spent significantly less time in the probe area than the NAÏVE rats (SHAM vs. NAÏVE, $U = 3.0, p < .01$; AMY vs. NAÏVE, $U = 2.0, p < .001$). No significant difference was found between the AMY and SHAM rats, $U = 34.0, p = .297$.

3.1.3 DISCUSSION

The current findings suggest that AMY damage does not cause anterograde amnesia for object-fear conditioning because the AMY rats buried significantly more than the NAÏVE rats and not significantly less than SHAM rats during the shock-probe fear-conditioning retention test. The AMY rats also spent less time in the probe area of the chamber than NAÏVE rats and as little as SHAM rats during the test. However, in spite of having greater latencies to contact the probe than the NAÏVE rats, the AMY rats contacted the probe more quickly than the SHAM rat, suggesting that the lesions impaired avoidance.

It is interesting that the AMY lesions reduced the latencies to contact the probe during the retention test without impairing burying. However, the AMY rats not only had an avoidance deficit during the retention test, but also during the acquisition session because they received significantly more contact-induced shocks than the SHAM rats. This latter finding suggests that the reduced latencies to contact the probe may simply

reflect the involvement of the AMY in avoidance processes that are independent of fear conditioning.

Indeed, there is compelling evidence suggesting that AMY lesions cause avoidance deficits in the shock-probe test that are not related to mnemonic processes. Lehmann and colleagues (2003) found that while the AMY was reversibly inactivated during a shock-probe acquisition session rats had impaired avoidance of the probe, but in a following retention test, when the AMY was intact again, the rats avoided the probe as much as the control rats. Thus, the AMY does not seem to be needed for acquiring the association between the probe and the shock, but is required to successfully avoid the fear-eliciting stimulus.

More importantly, it has been argued that burying is a more appropriate index of fear than avoidance in the shock-probe fear-conditioning test (Maren, 2003b). Avoidance, under certain conditions, might only reflect knowledge of the association between the probe independent of any fear. In contrast, it is believed that both knowledge of the association and fear are required to evoke and sustain burying in the shock-probe fear-conditioning test (Maren, 2003b; Treit & Menard, 1997; Treit, Pesold et al., 1993). Thus, the finding that the AMY rats successfully buried during the retention test implies that the lesions did not impair memory for the association between the probe and the shock, but also strongly suggests that the lesions did not reduce fear.

The current findings add to the increasing number of recent studies that have reported intact fear conditioning following AMY lesions (Cahill et al., 2000; Helmstetter, 1992b; Helmstetter & Bellgowan, 1994; 2000; Lehmann et al., 2003; Parent, Quirarte et al., 1995; Parent et al., 1992; Parent et al., 1994; Sutherland & McDonald, 1990;

Vanderwolf et al., 1988), but they also suggest that anterograde memory for fear conditioning may survive AMY damage for a lasting period of time. The longest retention interval in a study reporting intact fear conditioning following pretraining AMY lesions was 4-d (Lehmann et al., 2003). Yet, in the current experiment, the retention test was conducted 14-d after the acquisition session and the AMY lesions did not cause any memory or fear impairments.

It has been shown that rats with AMY lesions may overcome their fear conditioning deficits with extensive training (Maren, 1998, 1999a; Parent, Quirarte et al., 1995). For instance, Maren (1998) reported that AMY-damaged rats displayed as much freezing as control rats in a contextual fear-conditioning test when they received 10 times the usual amount of shocks that is needed for asymptotic performance in control rats (Maren, 1999a). This is an important issue to consider in the current experiment, since the AMY rats received significantly more contact-induced shocks than the SHAM rats. However, the number of shocks the AMY rats received in this experiment is only approximately three times more than that received by the SHAM rats and dramatically less than the 75 shocks that the AMY-damaged rats received in Maren's (1999a) study. Thus, the spared object-fear conditioning in AMY rats is unlikely the result of overtraining. Moreover, supporting this argument, Lehmann et al. (2003) demonstrated that, in the shock-probe fear-conditioning test, memory is intact even when rats with AMY lesions are limited to the same number of contact-induced shocks as control rats.

The absence of anterograde amnesia for object-fear conditioning following AMY lesions in this experiment is not likely due to incomplete lesions. All the lesion rats included in the experiment had substantial damage to the BLA, which is the region within

the AMY thought to be necessary for acquiring and storing fear-conditioning information (Fanselow & LeDoux, 1999; Gale et al., 2004). The AMY lesions included in the present experiment were also as large as those reported in other fear conditioning studies that have found fear conditioning impairments (Antoniadis & McDonald, 2001; M. Kim & Davis, 1993a; Maren, 1999a). In addition, the AMY rats received more contact-induced shocks during the acquisition session, suggesting that the lesions were effective in disrupting behaviour that is known to be dependent on the AMY in the shock-probe test.

In sum, pretraining AMY lesions do not reduce the amount of burying, which is considered to be a reliable measure of fear, evoked by an object associated with an aversive event. Thus, damage to the AMY is not sufficient to induce anterograde amnesia for object-fear conditioning.

4.2 EXPERIMENT 7: The AMY and ACTH In the Shock-Probe Fear-Conditioning Test

In the previous experiment, rats with pretraining AMY lesions buried as much as the SHAM rats and avoided the probe more than the NAÏVE rats during the retention test, suggesting that the AMY is not necessary for object-fear conditioning. However, it could be argued these responses reflect knowledge of the association between the probe and the shock rather than an emotional response to the probe.

In animals, including humans, emotional situations generally cause increases in stress hormone levels (see Anisman & Merali, 1999). For instance, rats exposed to a predator odour will have increased adrenocortropin hormone (ACTH) levels, suggesting that they are stressed and likely in general state of fear (Adamec, Kent, Anisman, Shallow, & Merali, 1998). Similarly, fear conditioning causes increases in stress hormone levels and these increases can be used as a covert physiological measure of memory and fear (Coover, Ursin, & Levine, 1973a, 1973b, 1974; Goldstein et al., 1996).

Consequently, the goal of this experiment was to assess whether pretraining AMY lesions prevent or reduce an increase in ACTH levels during the shock-probe fear-conditioning retention test. Specifically, this assessment allowed inferring whether AMY rats' are stressed by the probe and likely in a fearful state during the retention test.

4.2.1 METHODS

4.2.1.1 Subjects

Male Long-Evans rats (Charles River, Quebec; 300-350 g) served as subjects. Their housing conditions were the same as in Experiment 1 with the exception that they were provided with approximately 30 g of food daily.

4.2.1.2 Surgery

The procedure was the same as for Experiment 6 with the exception that some rats received neurotoxic AMY lesions. The procedure for the neurotoxic AMY lesions was similar to that of Experiment 1, but all rats were anesthetized with isoflurane (Janssen, Toronto, Ontario) in 0.8 L/min oxygen at 14.7 PSIA at 21°C (Benson Medical Industries, Markham, Ontario) and only received one infusion of 0.8 μl of NMDA at flow rate of 0.2 μl/min with an additional 4-m for diffusion in each hemisphere (2.3 mm posterior and 5.0 mm lateral to bregma and -6.7 mm ventral from dura).

4.2.1.3 Behavioural Procedures and Blood Sampling

4.2.1.3.1 ***Habituation.*** Rats were habituated to the shock-probe chamber with the three opaque walls that was used in Experiment 1 for 15-m on four consecutive days. This specific chamber was used because it allowed the experimenter to sample blood from the rats during the retention test without being seen and did not require a lid that would impede the sampling.

4.2.1.3.2 ***Catheter Implant.*** Within 24-h following completion of habituation, rats were anesthetized with isoflurane (Janssen, Toronto, Ontario) in 0.8 L/min oxygen at 14.7 PSIA at 21°C (Benson Medical Industries, Markham, Ontario), incised at the neck, and the left jugular vein was isolated. A 28 mm length of sylastic tubing (Dow Corning,

Midland, MI; outer-diameter = 1.19 mm., inner-diameter. = 0.64 mm) was then inserted into the jugular vein up to the atrium of the heart. Following the insertion, the tubing was secured in to position with suture thread, exteriorized between the rats' scapulae, and filled with heparinized sterile saline (50 U heparin/1ml). Finally, the incision was closed using wound clips and antibiotic powder (Cicatrin, GlaxoWellcome) was applied to the wound.

4.2.1.3.3 ***Acquisition.*** The day following catheter implantation, the rats were given the shock-probe acquisition session. The procedures were the same as those in Experiment 1 with the exception that only the opaque shock-probe chamber was used.

4.2.1.3.4 ***Retention.*** The morning after acquisition, each rats' silastic jugular catheter was connected to approximately 70 cm of PE-50 tubing, administered 0.05 ml of heparin (1000 U/ml), and were left undisturbed for a minimum of 2-h in their home-cage prior to sampling and testing. Blood sampling was initiated, for each rat, 5-min before the beginning of the retention test. Specifically, while the rats were still in their home-cage, blood was withdrawn (0.35-0.4 ml) through the extension tubing and replaced with heparinized saline (50 U/ml). The sampling procedure lasted approximately 2-min and did not appear to disturb the rats' behaviour. The rats were then transported into the opaque shock-probe chamber for the retention test. The procedures were the same as those used in Experiment 1 with the exception that a blood sample was taken immediately at the beginning, at MINUTE 5, and at MINUTE 15 of the test. The sampling procedure during the test followed the same procedure as that of the sample taken in the home-cage.

Immediately after being collected, each blood sample was refrigerated at 4°C. At the end of the test day, the samples were centrifuged for 4-min at 10,000 rpm and -5°C, after which plasma was stored at -80°C until ACTH analysis.

4.2.1.5 ACTH Analysis

Plasma ACTH concentrations were determined using a radioimmunoassay kit obtained from DiaSorin (Stillwater, MN). Each plasma sample was determined in duplicate. Intra-assay variability was 4% and nonspecific binding less than 5%.

4.2.1.5 Histology

The same procedures as in Experiment 1 were used.

4.2.1.6 Statistical Analyses

The same procedures as in Experiment 1 were used for analyzing the overt behavioural data. For the ACTH levels, a percent difference score (Δ ACTH) was calculated for each rat for the MINUTE 5 and MINUTE 15 samples from the one collected immediately at the beginning of the retention test. This permitted a more accurate isolation of the changes in ACTH due to the object-fear conditioning test. It was expected that if AMY lesions impair object-fear conditioning, then only the SHAM rats should show an increases in ACTH during the retention test. Accordingly, the Δ ACTH for each group at MINUTE 5 and MINUTE 15 were analyzed with one-sample t-tests to determine whether the changes were significantly greater than zero. In addition, one-tailed independent samples t-tests were conducted on the Δ ACTH at MINUTE 5 and MINUTE 15 to determine whether the SHAM and AMY rats had greater changes than the NAÏVE rats and whether the AMY rats had smaller changes than the SHAM rats.

4.2.2 RESULTS

4.2.2.1 Histology

The location and extent of the smallest and largest AMY lesions are shown in Figure 31. Five rats were excluded from the experiment because they sustained more damage outside, than within, the AMY or less than 50% damage to the central nucleus and BLA in one hemisphere. However, 1 lesion rat that was retained in the NAÏVE condition had less than 50% damage in one hemisphere. All the other lesion rats that were retained had extensive bilateral damage to the BLA and central nucleus and this damage was similar to that of the lesions found in Experiment 6 and previously published studies (Antoniadis & McDonald, 2001; Coover et al., 1973a; Goldstein et al., 1996; M. Kim & Davis, 1993a). Specifically, 8 rats had complete, or almost complete, ablation of the central nucleus and BLA, whereas 1 rat had unilateral sparing of the anterior portion of the central nucleus and BLA, as well as the lateral portion of the BLA. Damage was found in the medial nucleus of the AMY in all lesion rats, but the extent of the damage was variable. Damage to the substantia innominata, nucleus basalis, caudate nucleus, globus pallidus, endopiriform nucleus, and to the piriform cortex was found, in at least one hemisphere, of almost all the lesion rats. Two rats sustained minor damage to the EC and ventral HPC in one hemisphere. No damage was found in the PRH.

4.2.2.2 Behavioural Results

The data from the two NAÏVE groups (SHAM and AMY) were pooled because their overt behavioural results on the acquisition session and retention test were similar and the ACTH results were not statistically different (see Appendix B). Consequently, the analytical comparisons of the data involved three groups. The NAÏVE group (n= 7),

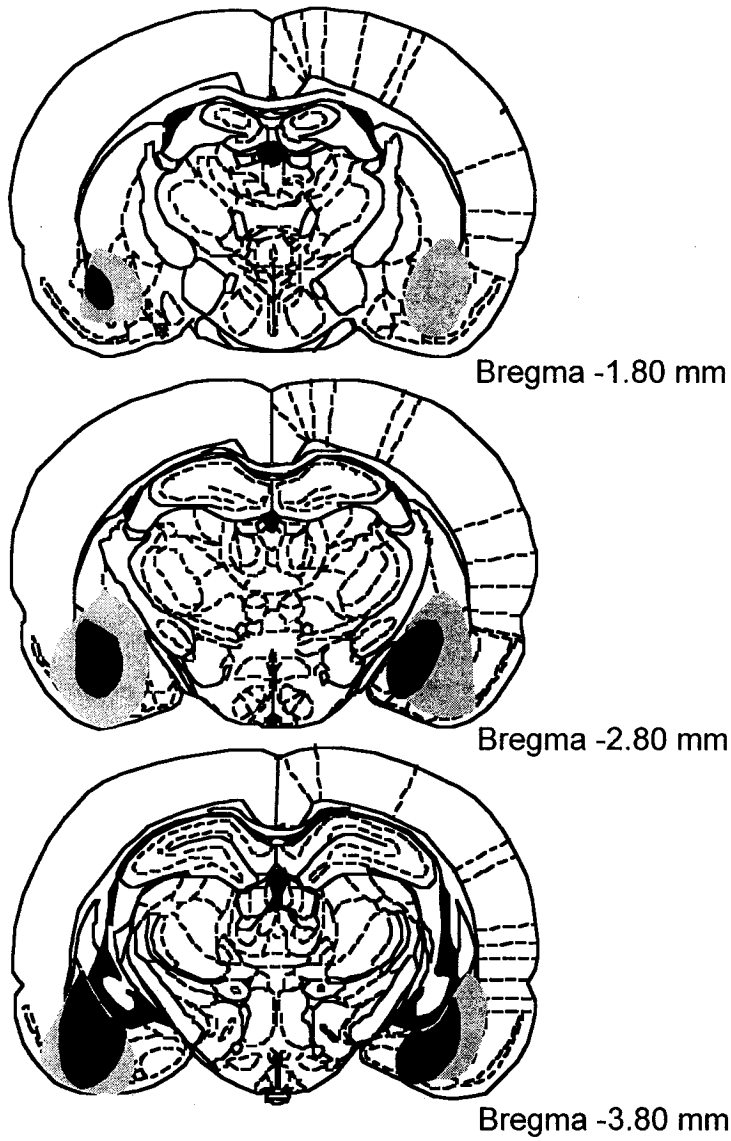

Figure 31. Illustrations of the smallest (dark gray) and largest (light gray) lesion observed bilaterally through the rostral and caudal extent of the AMY rats. Atlas plates are from Paxinos and Watson (1997).

which included all the rats exposed to the non-electrified probe, and the two groups of rats exposed to the electrified probe: SHAM (n = 7) and AMY (n = 7).

4.2.2.2.1 ***Acquisition.*** Figure 32 shows the number of contact-induced shocks the rats received during the acquisition session. The AMY rats received significantly more shocks than the SHAM rats, $U = 5.5, p < .01$. There was no significant difference in shock reactivity between the SHAM ($M = 2.37$, $SEM = 0.21$) and the AMY rats ($M = 2.29 \pm SEM = 0.19$), $U = 23.5, p = .448$.

Figure 33 shows the amount of time spent burying during the acquisition session. The rats exposed to the electrified probe buried significantly more than the NAÏVE rats (SHAM vs. NAÏVE, $U = 0.0, p < .001$; AMY vs. NAÏVE, $U = 0.0, p < .001$). No significant difference was found between the AMY and SHAM rats, $U = 22.5, p = .399$.

4.2.2.2.2 ***Retention.*** Figure 34 shows the amount of time spent burying on the retention test. The SHAM rats buried significantly more than the NAÏVE rats, $U = 1.0, p < .001$, as did the AMY rats, $U = 12.5, p = .05$. However, the AMY rats buried significantly less than the SHAM rats, $U = 10.0, p < .05$.

Figure 35A illustrates the latency to initially contact the probe on the retention test. The groups that were exposed to the electrified probe during the acquisition session had significantly longer latencies than the NAÏVE rats (SHAM vs. NAÏVE, $U = 0.0, p < .001$; AMY vs. NAÏVE, $U = 11.0, p < .05$). However, the AMY rats had significantly shorter latencies than the SHAM rats, $U = 7.5.0, p < .05$.

Figure 35B illustrates the amount of time spent in the half of the chamber where the probe was located. The SHAM and AMY rats spent significantly less time in the probe area than the NAÏVE rats (SHAM vs. NAÏVE, $U = 0.0, p < .001$; AMY vs. NAÏVE, $U =$

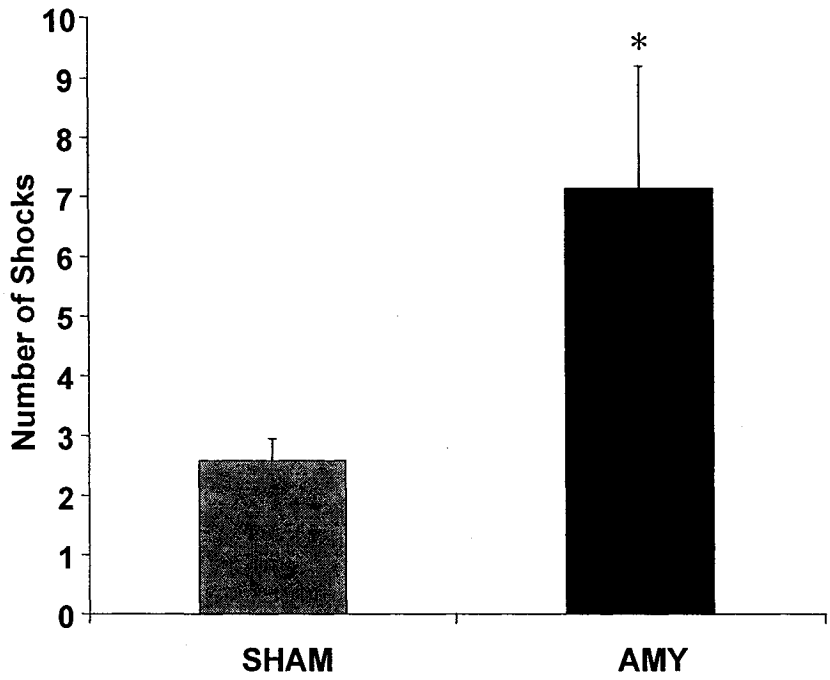

Figure 32. Mean (± *SEM*) number of contact-induced shocks received during the acquisition session by SHAM and AMY rats that were exposed to the electrified probe (*$p < .05$).

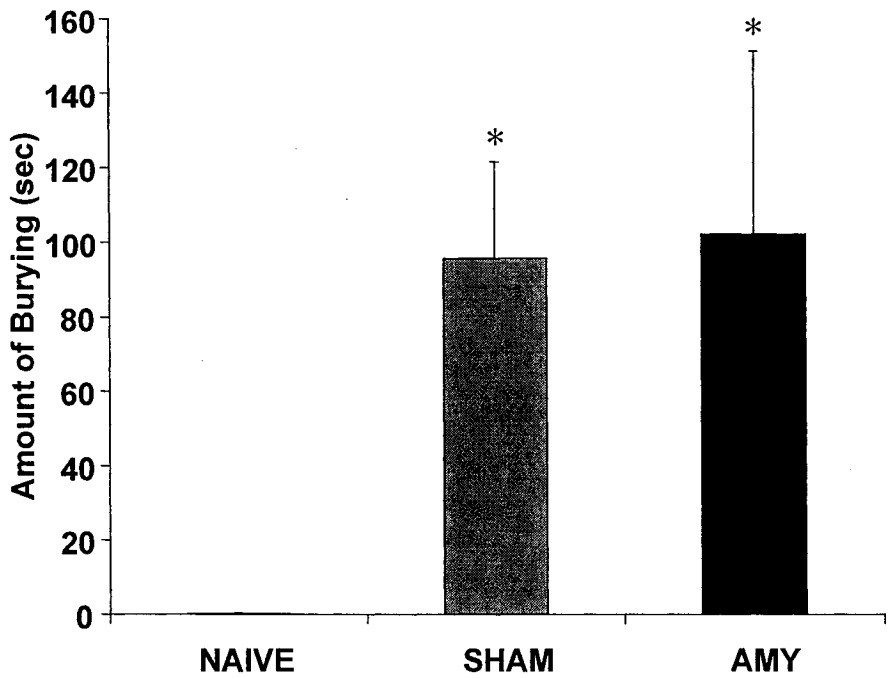

Figure 33. Mean (± *SEM*) amount of time spent burying during the acquisition session by the NAÏVE, SHAM, and AMY rats (*$p < .05$ versus NAIVE).

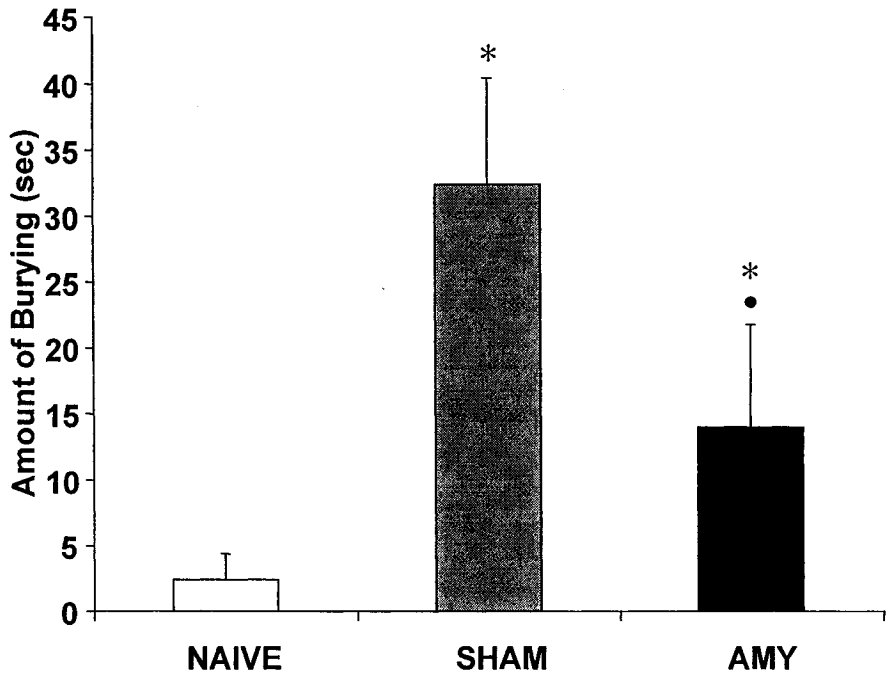

Figure 34. Mean (± *SEM*) amount of time spent burying during the retention test by NAÏVE, SHAM, and AMY rats (*$p < .05$ versus NAIVE; • $p < .05$ versus SHAM).

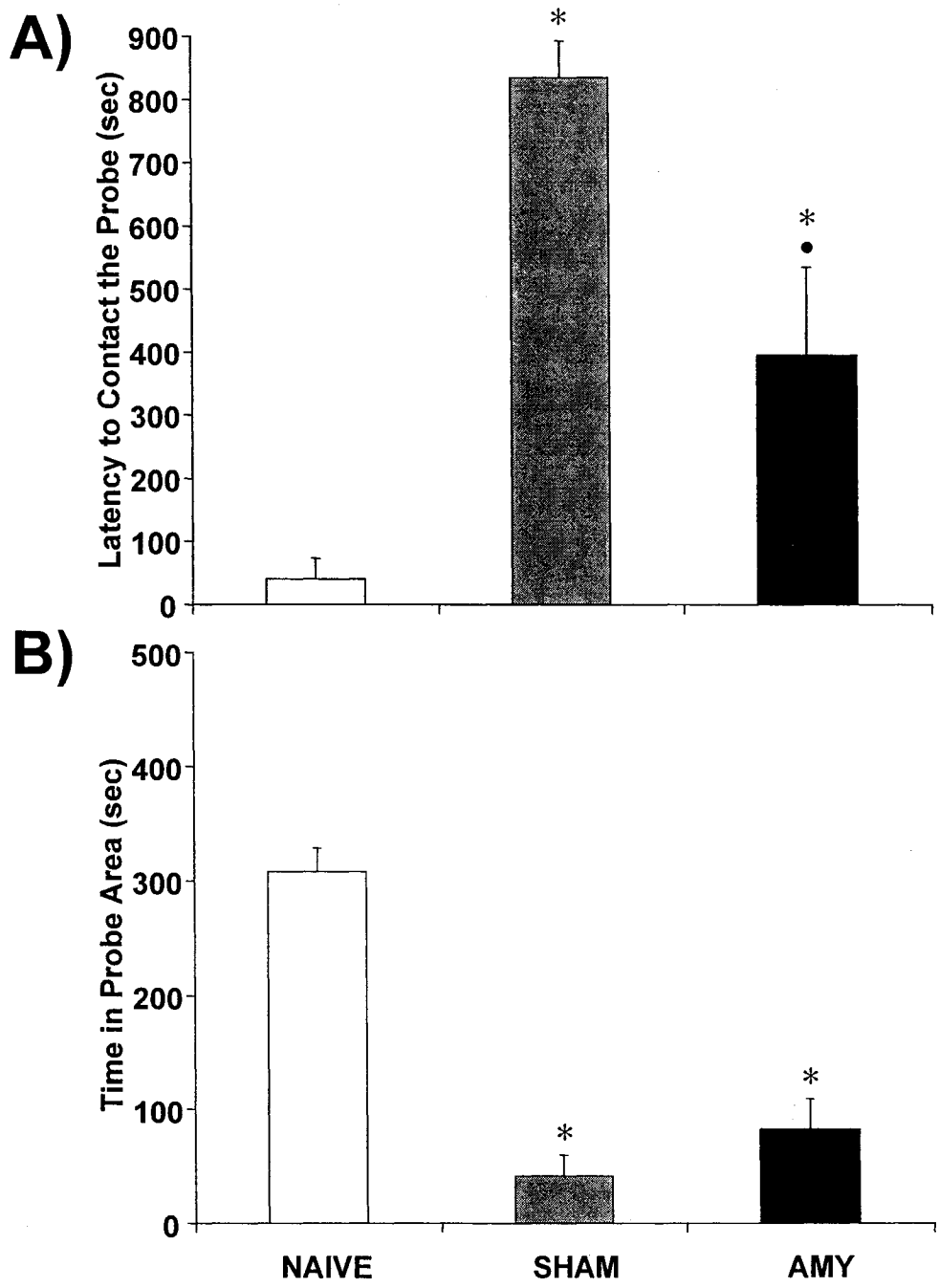

Figure 35. Mean (± *SEM*) (**A**) latency to contact the probe and (**B**) amount of time spent in the probe area of the chamber during the retention test for NAÏVE, SHAM, and AMY rats (*$p < .05$ versus NAIVE; • $p < .05$ versus SHAM).

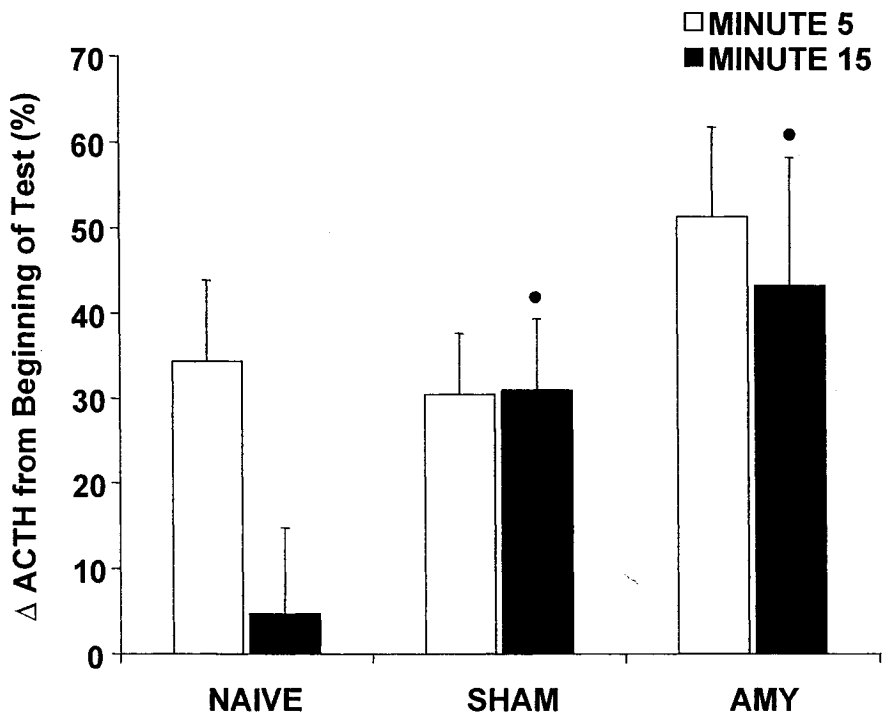

Figure 36. Mean (± *SEM*) percent change from the beginning of the shock-probe fear-conditioning retention test in ACTH plasma levels at MINUTE 5 and MINUTE 15 of the test for NAÏVE, SHAM, and AMY rats (• $p < .05$ versus NAÏVE-MINUTE 15).

0.0, $p < .001$). No significant difference was found between the AMY and SHAM rats, $U = 14.0, p = .09$.

Figure 36 shows percent change from the beginning of the shock-probe fear-conditioning retention test in ACTH plasma levels at MINUTE 5 and MINUTE 15 of the test. Each group had a significant increase in ACTH at MINUTE 5 of the retention test (NAÏVE, $t_{(5)} = 3.57, p < .01$; SHAM, $t_{(6)} = 4.25, p < .01$; AMY, $t_{(6)} = 4.89, p < .01$). However, at MINUTE 15 only the rats that had previously been shocked had a significant increased in ACTH levels (NAÏVE, $t_{(6)} = 0.46, p < .331$; SHAM, $t_{(6)} = 3.68, p < .01$; AMY, $t_{(6)} = 2.89, p < .05$). No significant differences were found between the groups at MINUTE 5 (SHAM vs. NAÏVE, $t_{(11)} = 0.33, p = .376$; AMY vs. NAIVE, $t_{(11)} = 1.18, p = .132$; AMY vs. SHAM, $t_{(12)} = 1.64, p = .064$). But, the SHAM and AMY rats had significantly greater ACTH increases than NAÏVE rats at MINUTE 15 (SHAM vs. NAÏVE, $t_{(12)} = 2.00, p < .05$; AMY vs. NAIVE, $t_{(12)} = 2.14, p < .05$), whereas no significant difference was found between the groups that had previously been shocked (AMY vs. SHAM, $t_{(12)} = 0.72, p = .244$).

4.2.3 DISCUSSION

Pretraining AMY lesions did not prevent or blunt the increase in ACTH plasma levels during the shock-probe fear-conditioning retention test, suggesting that damaging the AMY is not sufficient to cause anterograde amnesia nor disrupt experiencing stress in object-fear conditioning. Specifically, at the end of the retention test (MINUTE 15) AMY rats had significantly higher levels of ACTH than at the beginning of the test. Moreover, the change in ACTH levels at that time point in the AMY rats was greater than that of the

NAÏVE rats, and as big as that of the SHAM rats. In addition, the AMY rats buried and avoided the probe significantly more than the NAÏVE rats. However, the AMY rats did not express these overt behaviours to the same extent as the SHAM rats.

Interestingly, the increase in ACTH plasma levels during the shock-probe fear-conditioning retention test was small. Typically, a fear-eliciting stimulus in control rats increases ACTH levels by several folds (Maier, Ryan, Barksdale, & Kalin, 1986; Merali, Kent, Michaud, McIntyre, & Anisman, 2001). For example, rats exposed to cat odour have shown increases beyond 200% in ACTH plasma levels (Adamec et al., 1998). Yet, in the shock-probe fear-conditioning test the ACTH levels only increased by approximately 50% in the SHAM rats, and only at the end of the test was the increase greater than that of rats that did not undergo fear conditioning.

A possible reason for the small increase ACTH secretion is that during the test the rats had control over experiencing the aversive stimulus because contacting the probe was dependent on their behaviour. Indeed, it has been demonstrated that having control over a stressor, such as shock, may reduce the extent to which stress hormones increase (H. Davis et al., 1977; Dess, Linwick, Patterson, Overmier, & Levine, 1983; Swenson & Vogel, 1983). For instance, during a test session were all rats received shock, rats that were previously trained to escape it had smaller changes in plasma corticosterone levels than rats that had not been trained to escape the shock (H. Davis et al., 1977). Nevertheless, the amount of change in ACTH plasma levels is not the important factor, but rather whether the fear conditioning induced a change and it did in both the SHAM and AMY rats.

Although the AMY lesions did not affect plasma ACTH levels, the expression of overt fear behaviours were impaired in the current experiment. Contrary to the findings of Experiment 7, the AMY rats buried significantly less than the SHAM rats. They also avoided the probe less than the SHAM rats, which is inconsistent with Lehmann et al. (2003) findings. However, the SHAM rats in this experiment seemed to have avoided contacting the probe for twice as long as any other SHAM group included in this thesis. The SHAM rats in this experiment also had a higher burying mean than any other SHAM group that was tested in the same size chamber as the one used in this experiment. Thus, it is possible that the blood sampling affected the SHAM rats' overt behaviours more than the AMY rats during the retention test, which could account for the differences in overt behaviours.

In sum, the ACTH findings indicate that pretraining AMY lesions do not disrupt the stress response in the shock-probe fear-conditioning test and extend the findings of the previous experiment and those of Lehmann et al. (2003) suggesting that the AMY is not necessary for memory for object-fear conditioning even when the delay between acquisition and test is as long as 14-d.

4.3 EXPERIMENT 8: The Effects of Post-Training Lesions of the AMY In the Shock-Probe Fear-Conditioning Test

The previous two experiments failed to find anterograde amnesia for object-fear conditioning following AMY lesions. However, it remains possible that AMY lesions would cause retrograde amnesia for an association between an object and a fear-eliciting event.

Lehmann et al. (2000) tested this hypothesis and found that post-training AMY lesions induced 4-d after shock-probe fear conditioning did not disrupt avoidance of the probe on the retention test, suggesting that the AMY is not necessary for memory for object fear conditioning. However, as discussed earlier in this chapter, avoidance may not be the most reliable index of fear conditioning following AMY lesions. Thus, the current experiment aimed at examining the effects of post-training AMY lesions on burying and avoidance of an object that was paired with a shock.

The results of one study suggest that the AMY has a temporary role in memory for fear conditioning. Liang and colleagues (1982) found that damaging the AMY 2-d after training impaired avoidance of a compartment associated with a shock, but that damage induced 10-d after training did not. This latter finding raises the possibility that the 4-d interval between training and the induction of the AMY lesions, in Lehmann et al. (2000) study, was too long to cause any memory deficits. Thus, in order to examine the possibility that the AMY has a temporary role in object-fear conditioning, the present experiment assessed the effects of AMY lesions induced 1-d and 14-d following training in the shock-probe fear-conditioning test.

4.3.1 METHODS

4.3.1.1 Subjects

Male Long-Evans rats (Charles River, Quebec; 300-350 g) served as subjects. Their housing conditions were the same as in Experiment 1 with the exception that they were provided with approximately 30 g of food daily.

4.3.1.2 Surgery

The surgical procedures were identical to those in Experiment 6.

4.3.1.3 Behavioural Procedures

The procedures were the same as those in Experiment 2 with the exception that the small shock-probe chamber (see Experiment 4) was used and the rats were not habituated to the chamber.

4.3.1.4 Histology

The same procedures as in Experiment 1 were used.

4.3.1.5 Statistical Analyses

The same procedures as in Experiment 1 were used.

4.3.2 RESULTS

4.3.2.1 Histology

The location and extent of the largest and smallest AMY lesions in the REMOTE and RECENT condition are shown in Figure 37. Five rats were excluded from the experiment because they sustained more damage outside, than within, the AMY or less than 50% damage to the central nucleus and BLA in one hemisphere. The lesion rats that were retained had substantial bilateral damage to the BLA and central nucleus, the

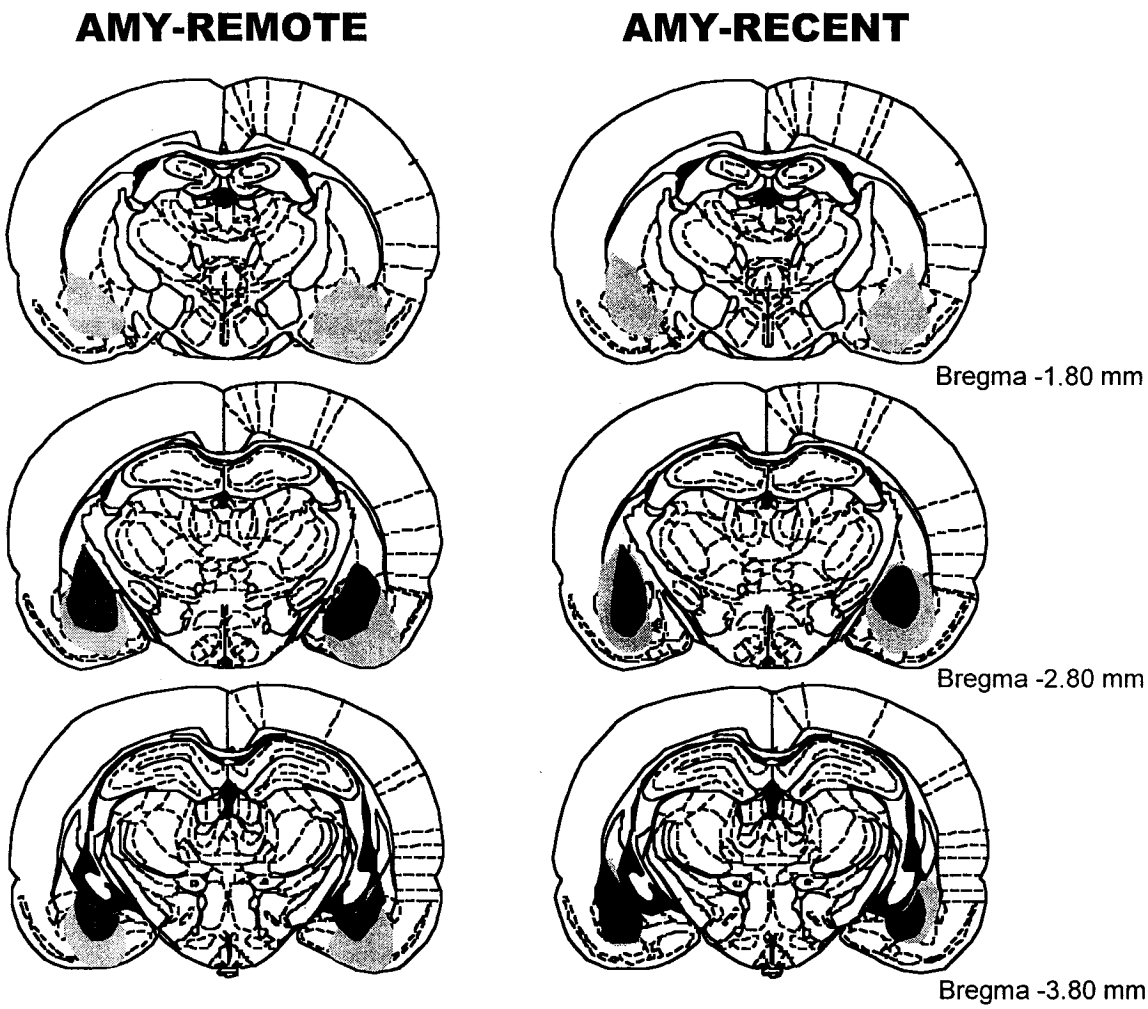

Figure 37. Illustrations of the smallest (dark grey) and largest (light gray) lesion observed bilaterally through the rostral and caudal extent of the AMY rats in the REMOTE and RECENT conditions. Atlas plates are from Paxinos and Watson (1997).

regions of the AMY typically implicated in memory or expression of fear conditioning (Maren, 2001b). However, some sparing was found unilaterally in the BLA of 4 rats, unilaterally in the central nucleus and BLA of 2 rats, and bilaterally in both regions of 6 rats. This sparing was predominantly in the anterior portion of the AMY. Damage was found in the medial nucleus of the AMY in all lesion rats, but the extent of the damage was variable. Damage to the substantia innominata, nucleus basalis, caudate nucleus, globus pallidus, endopiriform nucleus, and to the piriform cortex was also found, in at least one hemisphere, of almost all the lesion rats. The PRH, HPC, and EC were typically spared, except in 4 rats that sustained very minor damage to these regions.

4.3.2.2 Behavioural Results

The data from the four NAÏVE control groups (SHAM or AMY and REMOTE or RECENT) were pooled because their behavioural results on the acquisition session and retention test were not statistically different (see Appendix B). Consequently, the analytical comparisons of the data involved five groups. The NAÏVE group (n= 13), which included all the rats exposed to the non-electrified probe, and the four groups of rats exposed to the electrified probe: SHAM-REMOTE (n = 9), SHAM-RECENT (n = 8), AMY-REMOTE (n = 8), and AMY-RECENT (n = 6).

4.3.2.2.1 ***Acquisition.*** Figure 38 shows the number of contact-induced shocks the rats received during the acquisition session. The AMY rats received a similar number of contact-induced shocks as SHAM rats (AMY-REMOTE vs. SHAM-REMOTE, $U = 19.5$, $p = .465$; AMY-RECENT vs. SHAM-RECENT, $U = 49.0$, $p = .469$).

There was no significant difference in shock reactivity between SHAM-REMOTE rats ($M = 2.26$, $SEM = 0.13$) and AMY-REMOTE rats ($M = 2.18$, $SEM = 0.13$), $U = 17.0$,

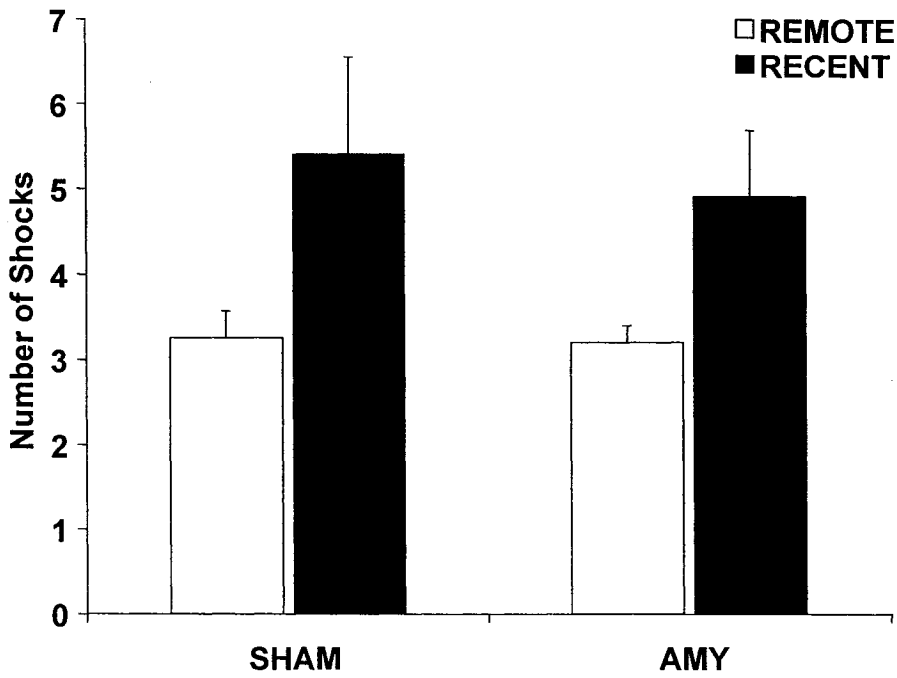

Figure 38. Mean (± *SEM*) number of contact-induced shocks received during the acquisition session by SHAM and AMY rats in the REMOTE and RECENT memory test conditions that were exposed to the electrified probe.

$p = .325$, nor between SHAM-RECENT ($M = 2.18$, $SEM = 0.14$) and AMY-RECENT rats ($M = 1.80$, $SEM = 0.08$), $U = 33.0, p = .097$.

Figure 39 shows the amount of time spent burying during the acquisition session. The rats that were exposed to the electrified probe buried more than the NAÏVE rats (SHAM-REMOTE vs. NAÏVE, $U = 4.0, p < .001$; SHAM-RECENT vs. NAÏVE, $U = 2.0, p < .001$; AMY-REMOTE vs. NAIVE, $U = 4.5, p < .01$; AMY-RECENT vs. NAIVE, $U = 0.0, p < .001$). No differences were found between the AMY rats the SHAM rats (AMY-REMOTE vs. SHAM-REMOTE, $U = 20.0, p = .500$; AMY-RECENT vs. SHAM-RECENT, $U = 40.5, p = .237$).

4.3.2.2.2 *Retention.* Figure 40 shows the amount of time spent burying during the retention test. The rats exposed to the electrified probe during the acquisition session buried significantly more than the NAÏVE rats (SHAM-REMOTE vs. NAÏVE, $U = 6.5, p < .001$; SHAM-RECENT vs. NAÏVE, $U = 12.0, p < .001$; AMY-REMOTE vs. NAÏVE, $U = 14.0, p < .05$; AMY-RECENT vs. NAÏVE, $U = 26.5, p < .01$). However, the AMY rats buried significantly less than their respective SHAM rats (AMY-REMOTE vs. SHAM-REMOTE, $U = 7.0, p < .05$; AMY-RECENT vs. SHAM-RECENT, $U = 17.5, p < .01$). Moreover, the interval between conditioning and surgery did not influence the amount of burying on the retention test since no significant differences were found between SHAM-REMOTE and -RECENT, $U = 40.0, p = .500$, nor between AMY-REMOTE and -RECENT, $U = 24.5, p = .475$.

Figure 41A illustrates the latency to initially contact the probe on the retention test. The SHAM rats had significantly longer latencies than NAÏVE rats (SHAM-REMOTE vs. NAÏVE, $U = 0.0, p < .001$; SHAM-RECENT vs. NAÏVE, $U = 0.0, p < .001$). The

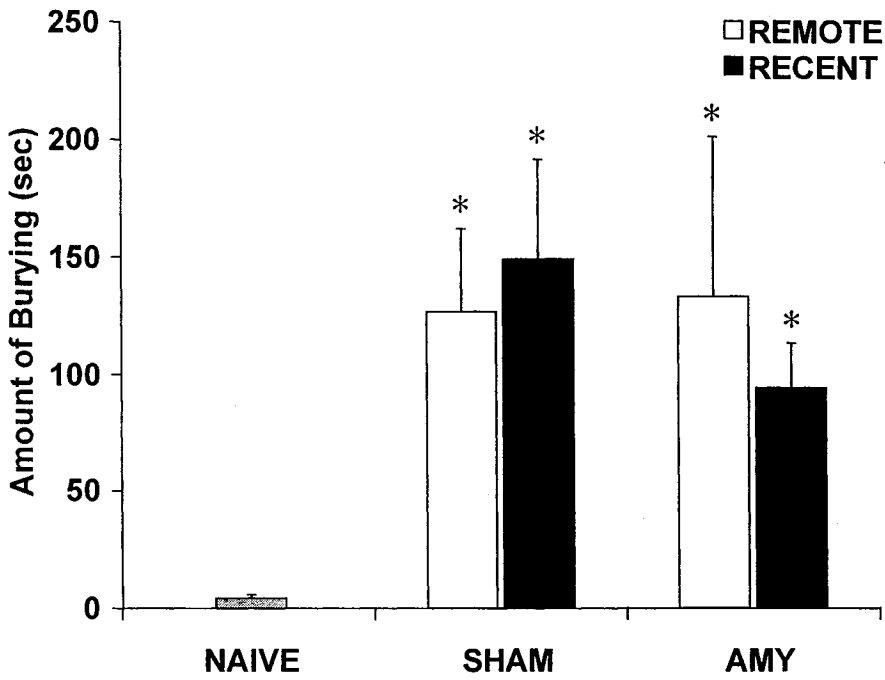

Figure 39. Mean (± *SEM*) amount of time spent burying during the acquisition session by NAÏVE, SHAM, and AMY rats in the REMOTE and RECENT conditions (*$p < .05$ versus NAIVE).

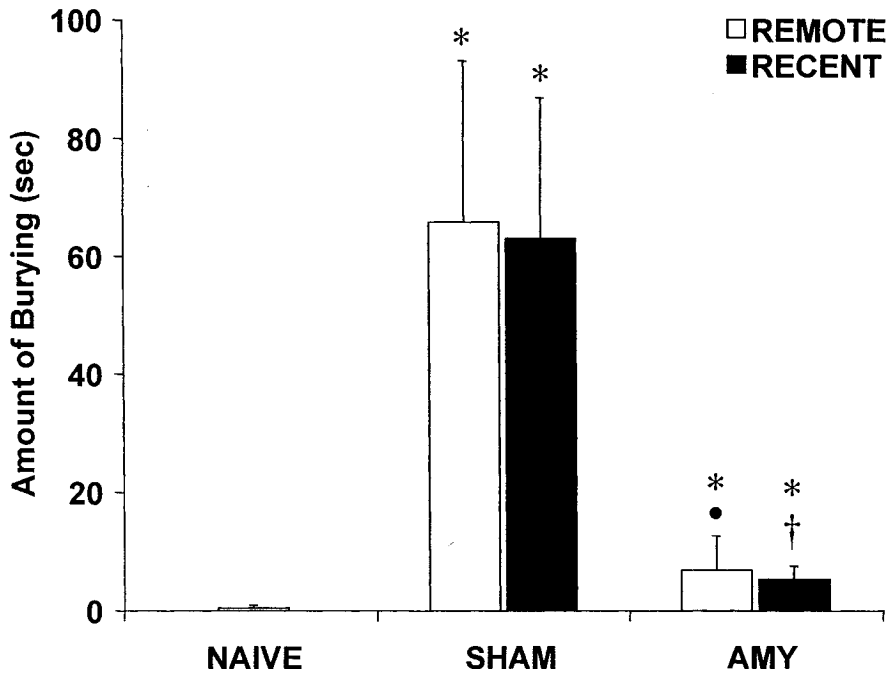

Figure 40. Mean (± *SEM*) amount of time spent burying during the retention test by NAÏVE, SHAM, and AMY rats in the REMOTE and RECENT conditions (*$p < .05$ versus NAÏVE; •$p < .05$ versus SHAM-REMOTE; † $p < .05$ versus SHAM-RECENT).

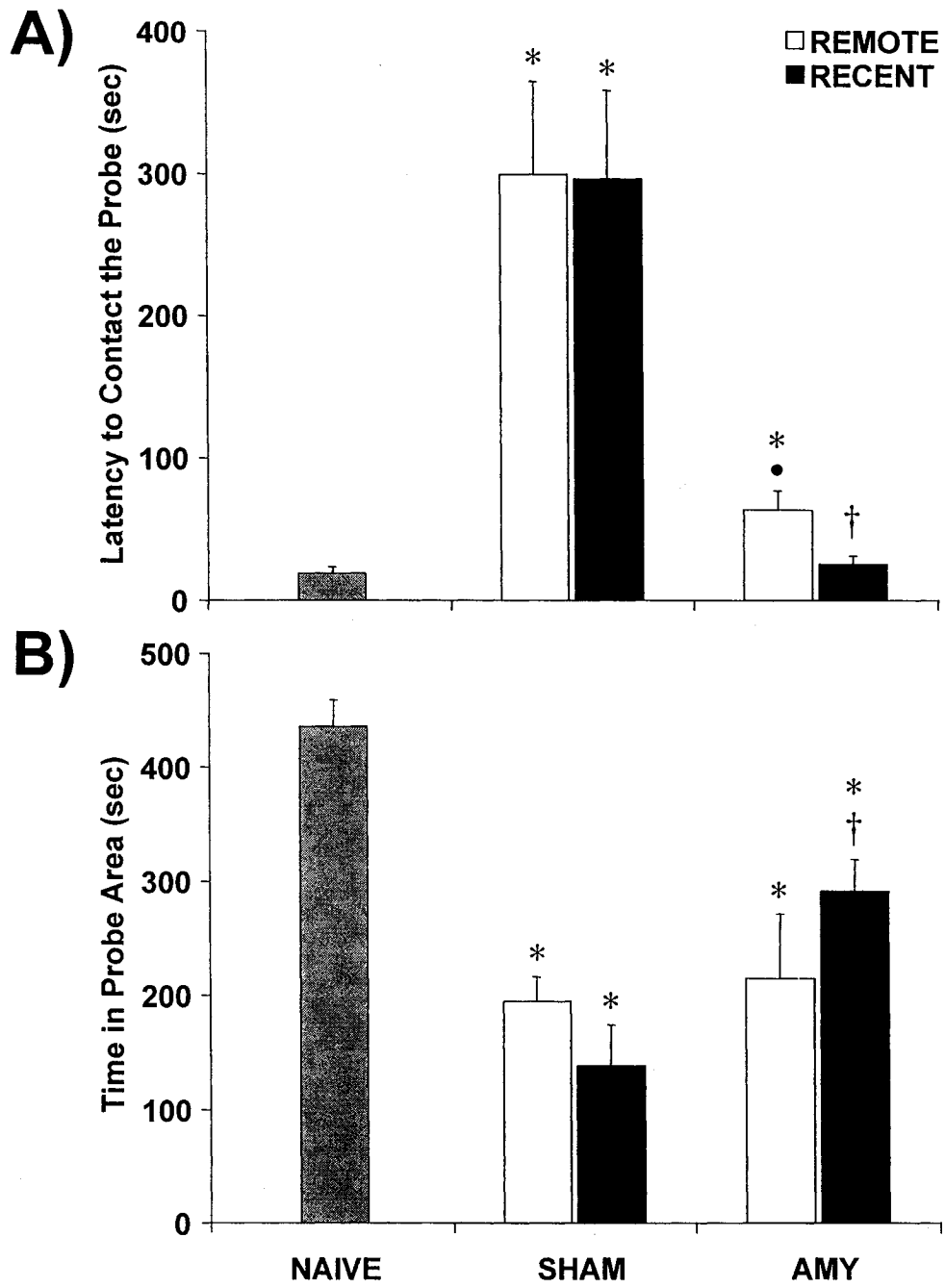

Figure 41. Mean (± *SEM*) **(A)** latency to contact the probe and **(B)** amount of time spent in the probe area of the chamber during the retention test for NAÏVE, SHAM and AMY rats in the REMOTE and RECENT conditions (*$p < .05$ versus NAÏVE; •$p < .05$ versus SHAM-REMOTE; † $p < .05$ versus SHAM-RECENT).

AMY-REMOTE rats also had significantly longer latencies than NAÏVE rats, $U = 6.0, p < .01$, but not the AMY-RECENT rats, $U = 40.5, p = .154$. Both groups of AMY rats were significantly faster in contacting the probe than their respective group of SHAM rats (AMY-REMOTE vs. SHAM-REMOTE, $U = 1.0, p < .01$; AMY-RECENT vs. SHAM-RECENT, $U = 0.0, p < .001$). In addition, the interval between conditioning and surgery did not have a significant effect on the latencies to contact the probe for the SHAM rats (SHAM-REMOTE vs. SHAM-RECENT, $U = 40.0, p = .500$), but it did for the AMY rats (AMY-REMOTE vs. AMY-RECENT, $U = 7.0, p < .05$).

Figure 41B illustrates the amount of time spent in the half of the chamber where the probe was located. The SHAM and AMY rats spent significantly less time in the probe area than the NAÏVE rats (SHAM-REMOTE vs. NAÏVE, $U = 0.0, p < .001$; SHAM-RECENT vs. NAÏVE, $U = 2.0, p < .001$; AMY-REMOTE vs. NAÏVE, $U = 8.0, p < .05$; AMY-RECENT vs. NAÏVE, $U = 10.0, p < .001$). The amount of time spent in the probe area did not differ between AMY and SHAM rats from the REMOTE condition, $U = 18.0, p = .385$. However, the AMY-RECENT avoided the probe area significantly less than the SHAM-RECENT rats, $U = 14.0, p < .01$. The interval between conditioning and surgery did not affect the amount of time spent in the probe area for SHAM or AMY rats. Specifically, no significant difference was found between the SHAM-REMOTE rats and -RECENT rats, $U = 22.0, p = .055$, nor between the AMY-REMOTE and -RECENT rats, $t_{(13)} = -1.379, p = .096$). For the latter analysis, a discrepancy was found between the results of the non-parametric test and the parametric test. Given that the homogeneity of variance assumption was not violated for these data, a one-tailed independent-sample t-test was used and considered to be the most appropriate test.

4.3.3 DISCUSSION

The findings of this experiment suggest that AMY lesions cause severe and lasting retrograde amnesia in the shock-probe fear-conditioning test. Specifically, regardless of whether they were in the REMOTE or RECENT condition, AMY rats buried significantly less and contacted the probe more quickly than the SHAM rats. However, the lesions did not completely eliminate object-fear conditioning. The AMY rats in both the REMOTE and RECENT conditions buried significantly more and spent significantly less time in the probe area of the chamber than the NAÏVE rats. In addition, the AMY-REMOTE rats had significantly longer latencies to contact the probe than the NAÏVE rats, though this difference was far from being as great as that between the SHAM and NAÏVE rats.

These results support the findings of numerous studies that found retrograde amnesia for fear conditioning. However, they are inconsistent with Lehmann and colleagues' (2000) findings that post-training AMY lesions do not disrupt avoidance in the shock-probe fear-conditioning test. A possible explanation for the discrepancy in avoidance results between this and Lehmann et al.'s experiment is that the neurotoxic lesions in the latter study likely spared fibers of passage within and/or surrounding the AMY that are necessary for shock-probe avoidance conditioning, whereas the electrolytic lesions in the current experiment these destroyed fibers of passage. Further research addressing this possibility, however, is needed.

Liang et al. (1982) found that AMY lesions induced 10-d, but not 2-d, after inhibitory avoidance did not impair memory, raising the possibility that the AMY might be temporarily involved in object-fear conditioning. Yet, the present experiment failed to

find compelling evidence supporting this hypothesis. Whether the AMY lesions were induced 1-d or 14-d following acquisition the burying and avoidance deficits compared to the SHAM rats were similar. Moreover, no significant differences were found between the two AMY groups on the three behaviours used as indices of memory and fear during the retention tests. The absence of temporal gradient is unlikely due to too short of an interval between acquisition and the induction of the lesions, since the it was as long as the longest interval in the Liang et al.'s study.

However, the lasting involvement of the AMY in memory for object-fear conditioning is consistent with the results of several studies that have a failed to find temporally-graded retrograde amnesia following AMY damage in other fear conditioning tests than inhibitory avoidance (M. Kim & Davis, 1993b; Lee et al., 1996; Maren et al., 1996). For instance, recently, it was found that damaging the AMY as long as 16 months after auditory and contextual fear conditioning still caused retrograde amnesia (Gale et al., 2004). Thus, the majority of the evidence suggests that the AMY has a long lasting, if not permanent, role in memory for fear conditioning.

4.4 SUMMARY AND IMPLICATIONS

It is commonly believed that the AMY is necessary for memory for fear conditioning to all types of stimuli (Campeau & Davis, 1995b; Corodimas & LeDoux, 1995; Cousens & Otto, 1998; Gale et al., 2004; Goosens & Maren, 2001; Maren, 1998, 1999a; Maren et al., 1996; Sananes & Davis, 1992). Thus, it was considered essential to examine the role of the AMY in object-fear conditioning and it was found in the current chapter that damage to the AMY causes retrograde, but not anterograde, amnesia for an association between an object and a fear-eliciting event.

The retrograde amnesia for object-fear conditioning following AMY lesions suggests that the structure is "normally" involved in acquiring and retaining object-fear conditioning information. Yet, the absence of anterograde amnesia in Experiment 6 and 7 suggests that other brain structures subsume memory for this information in the event of AMY damage.

This is not the first instance in which pre- and post-training AMY lesions had dissociable effects on memory for fear conditioning (M. Kim & Davis, 1993a; Maren, 1998, 1999a). However, it is the first time that this dissociation is found in rats that did not receive extensive training (i.e., number of shocks) and have permanent AMY damage. It is also the sole instance to demonstrate that rats with pretraining AMY lesions are able to retain fear-conditioning information for a lasting period of time.

Importantly, in this chapter, there is compelling evidence that object-fear conditioning in rats with pretraining AMY lesions indeed involves the expression of fear. In Experiment 6 the AMY rats buried and in Experiment 7 the AMY rats had an increase in ACTH plasma levels greater than rats that did not undergo fear conditioning. These

latter responses are considered to be reliable indices of fear and thus suggest that the AMY rats not only remembered the association, but were also in a general state of fear.

CHAPTER 5

GENERAL DISCUSSION

The purpose of this thesis was to examine the role of three MTL structures, the HPC, PRH, and AMY in memory for object-fear conditioning. It was hypothesized that pre- and post-training lesions to these structures would cause amnesia for an association between an object and a fear-eliciting event because each is implicated in object recognition and/or fear conditioning. The main findings of the experiments included in this thesis are summarized in Table 4.

Amnesia for object-fear conditioning was only found following post-training lesions of the HPC or AMY. Post-training lesions of the PRH did not impair memory for the association between the object and the fear-eliciting event nor did pretraining lesions of either three structures. Yet, pre-training lesions of the HPC impaired contextual fear conditioning, suggesting that the HPC is involved in memory for an association between a context and a fear-eliciting event. Thus, memory for object-fear conditioning is dependent on the HPC and AMY, but not the PRH.

Although pre- and post-training lesions of the PRH did not impair memory for object-fear conditioning, the lesions affected avoidance of the object associated with the fear-eliciting event. Indeed, pretraining PRH lesions reliably enhanced avoidance, whereas post-training lesions impaired avoidance during the object-fear conditioning test. The PRH lesions also impaired burying elicited by the object associated with the fear-eliciting event, but this occurred only following pretraining aspiration lesions and this effect was dependent on the size of the testing chamber.

TABLE 4. Summary of the findings of Chapter 2,3, and 4

			Results			
	Structure	**Lesion**	**Burying**	**Avoidance**	**ACTH**	**Memory**
Anterograde Test						
	HPC	Neurotoxic	Impaired	Impaired	-	Contextual Impairment
	PRH	Aspiration	Impaired	Enhanced	-	Intact
		Neurotoxic	Spared	Enhanced	-	Intact
	PORH	Aspiration	Spared	Spared	-	Intact
	AMY	Electrolytic	Spared	Impaired	Spared	Intact
Retrograde Test						
	HPC	Neurotoxic	Impaired	Impaired	-	Impaired
	PRH	Neurotoxic	Spared	Impaired	-	Intact
	AMY	Electrolytic	Impaired	Impaired	-	Impaired

5.1 THEORETICAL IMPLICATIONS

The findings of this thesis have several important theoretical implications. The findings directly relate to theory about the neural basis of cued fear conditioning, and also contextual fear conditioning. In addition, the findings may be discussed within the framework of the neural basis of object recognition. Thus, this section describes the conceptual contributions of this thesis to each of these topics.

5.1.1 Cued Fear Conditioning

Learning and remembering an association between a discrete stimulus and fear-eliciting event has been known, for many years, to be dependent on the MTL (R. J. Blanchard & Fial, 1968; Brady et al., 1954; Delacour & Borst, 1972; Grossman et al., 1975; Horvath, 1963; Weiskrantz, 1956). Recent research, however, has tried to determine the specific contributions of the various MTL structures to cued fear conditioning.

It is predominantly believed that, within the MTL, the AMY is necessary for acquiring and retaining the emotional properties of the fear-eliciting event so that it can be associated with cues related to the event, such as sounds, odours, and discrete visual stimuli (M. Davis, 1997; Fanselow & Gale, 2003; J. LeDoux, 2003; Maren, 2001b; Otto & Giardino, 2001). The retrograde amnesia for object-fear conditioning following AMY lesions in Experiment 8 supports this position.

It is impossible to precisely determine whether the AMY lesions strictly caused amnesia for the emotional aspects of the event. However, it is unlikely that the lesions disrupted memory for the object because AMY lesions do not impair object recognition (Mumby et al., 1995; Murray & Mishkin, 1998; Zola-Morgan, Squire et al., 1989a). It is

also unlikely that the lesions impaired memory of the association between the object and the shock because AMY lesions do not impair object-reward associations, such as memory for an association between an object and a food (Mumby et al., 1995; Zola-Morgan, Squire et al., 1989a). Arguably, food is an appetitive stimulus and thus may involve different brain circuitry than an aversive stimulus like shock, but the fact remains that AMY lesions do not disrupt the ability to remember associations between objects and stimuli that have motivational properties. Consequently, the AMY lesions in the current thesis most likely disrupted object-fear conditioning because they caused retrograde amnesia for the fear properties of the event, which is consistent with previous theory on the role of the AMY in cued fear conditioning (M. Davis, 1997; Fanselow & LeDoux, 1999; J. LeDoux, 1998, 2003; Maren, 2001b, 2003a).

However, it is important to consider that the retrograde amnesia after damage to the AMY was found following electrolytic lesions, which does not solely damage the cell bodies within the structure. Actually, neurotoxic lesions of the AMY, which are more likely of only destroying cell bodies (Jarrard, 2002; Jarrard & Meldrum, 1993), do not cause retrograde amnesia for object-fear conditioning (Lehmann et al., 2000). Thus, it remains possible that the AMY lesion-induced amnesia in this thesis does not accurately reflect the role of the AMY in object-fear conditioning, but rather the role of collateral damage to fibers of passage that normally participate in memory processes related to object-fear conditioning. Future research should directly compare the effects of electrolytic and neurotoxic AMY lesion on memory for object-fear conditioning to clearly delineate its role.

Perhaps the AMY is not as essential for cued fear conditioning as asserted in theory. Indeed, the absence of anterograde amnesia for object-fear conditioning after AMY lesions, in Experiment 6 and 7, is in agreement with this view. Alternative roles in fear conditioning have been suggested for the AMY (Cahill et al., 2000; Cahill et al., 1999; Lehmann et al., 2003; McGaugh, 2004). For instance, McGaugh (2004) proposed that the AMY is involved in modulating the consolidation of memories for the fear-eliciting event being processed in other structures. Unfortunately, the experiments in Chapter 4 that examined the role of the AMY in object-fear conditioning did not directly assess this possibility and it should be investigated in future research. However, the absence of anterograde amnesia following AMY lesions suggests that it is unlikely that the AMY modulates consolidation of object-fear conditioning and that McGaugh's theory is not supported in this instance.

Another view suggests that the AMY is involved in certain fear conditioning mechanisms and not others. In attempt to reconcile previous discrepant findings between object-fear conditioning and other types of cued fear conditioning following AMY lesions Lehmann and colleagues (2000; 2003) suggested that AMY lesions may impair the expression of specific fear behaviour such as freezing, and spare others such as avoidance and burying. If this contention were true, however, then the pre- and post-training AMY lesions in the current thesis would not have had dissociable effects on memory. The lesions in both instances would have equally disrupted or spared avoidance and burying.

A more appropriate view would be that, similar to the multiple parallel memory systems theory proposed by White and McDonald (2002), there might be parallel systems

involved in fear conditioning. Such, that if one system is impaired, then another may "take-over" the conditioning. This latter proposal likely accounts best for the dissociable effects of pre- and post-training AMY lesions on memory for object-fear conditioning in this thesis. More specifically, object-fear conditioning would normally involve an AMY-based system, such that damaging this system after the information was acquired would cause retrograde amnesia. In contrast, damage to AMY-based system before object-fear conditioning would enable another neural circuit to subsume the conditioning.

Within the MTL, the PRH is also proposed to be a structure critically involved in cued fear conditioning because it would be implicated in the processing and retention of the specific cues that are associated with the emotional properties of the event that are located in the AMY (Campeau & Davis, 1995a; Otto & Giardino, 2001). The anatomical connectivity of the PRH with the unimodal and polymodal sensory areas and with the AMY (Burwell, 2000; Burwell & Amaral, 1998a; Deacon et al., 1983; Miller & Vogt, 1984; Shi & Davis, 2001) supports this contention, as does empirical evidence from several lesion studies (Campeau & Davis, 1995a; Herzog & Otto, 1998; Otto et al., 2000; Rosen et al., 1992). However, the findings in the current thesis suggest that the PRH is not as important as believed for cued fear conditioning and that the PRH's proposed role needs to be reconsidered.

Although the findings in Chapter 3 suggest that memory for object-fear conditioning is not impaired following PRH damage, they do indicate that lesions of the PRH had a marked effect on behaviour during the retention test. Specifically, pretraining PRH lesions enhanced avoidance of the probe, whereas post-training lesions impaired avoidance of the probe while leaving memory intact. Thus, the PRH seems to have an

important role in processing cued fear conditioning information, but does not appear necessary for acquiring and remembering that information. Perhaps the PRH is only necessary for integrating the features of the cue and of transmitting this information to other structures in order to evoke the appropriate responses to cope with the fear-eliciting cue.

The assertion that the HPC, the largest MTL structure, is not necessary for cued fear conditioning has recently been revised (Bast et al., 2001a). The HPC, and in particular its ventral region, is now considered to be an important structure in neural models of cued fear conditioning holt (Bast et al., 2001b; Maren, 1999b; Maren & Holt, 2004; Richmond et al., 1999). Although its specific contributions are unclear, it is suggested that the HPC is involved because of its reciprocal connections with the AMY (Bast et al., 2001a; French et al., 2003; Maren & Holt, 2004; A. J. McDonald & Mascagni, 1997; Pikkarainen et al., 1999; Pitkanen et al., 2002). The retrograde amnesia for object-fear conditioning findings following HPC lesions is consistent with this new claim.

It is again impossible to identify what information was lost following the HPC lesions. Consistent with previous object recognition findings (Gaskin et al., 2003), it may be that the HPC lesions disrupted memory for the object. However, HPC object recognition processes are believed to be dependent on inputs from the PRH and lesions to this latter structure typically impair object-recognition (Lavenex & Amaral, 2000; Squire et al., 2004). Yet, the PRH lesions did not cause amnesia for object-fear conditioning, suggesting that object-fear condition is not dependent on object recognition. Accordingly, it is unlikely that the object-fear conditioning amnesia following post-training HPC lesion can be explained by recognition deficits.

It is more likely that the HPC lesions impaired the expression of fear. Indeed, the impaired burying during the acquisition session in HPC-damaged rats in Experiment 1 suggests that the lesions reduced unconditioned fear, which necessarily undermines any fear conditioning deficit. This reduction in unconditioned fear is possibly due to a disruption of the HPC-AMY connectivity. However, contrary to HPC lesions, AMY lesions do not impair unconditioned burying (Treit & Menard, 1997; Treit, Pesold et al., 1993). For example, pretraining lesions of the AMY in Experiment 6 and 7 did not reduce the amount of burying during the acquisition session. The dissociable effects of HPC and AMY lesions on burying suggest that each have distinct roles in fear. More importantly, it suggests that the retrograde amnesia following HPC lesions is not attributable to a disruption in AMY inputs. The latter claim also supports the previous argument that there are several parallel neural systems involved in fear conditioning.

5.1.2 Contextual Fear Conditioning

In addition to being implicated in cued fear conditioning, the HPC, PRH, and AMY are also considered to be critically involved in contextual fear conditioning. The AMY is believed to be necessary for acquiring and retaining the emotional features of a fear-eliciting event, so that they can be associated with the context in which the event occurred (Fanselow & Gale, 2003; Maren, 2001b). On the other hand, the HPC is claimed to be involved in learning and remembering the configuration of elemental stimuli that compose the context, so that it can be associated with the emotional properties of the event that are thought to be located in the AMY (Fanselow & Gale, 2003; Maren, 2001b; Rudy & O'Reilly, 2001). Likewise, it is proposed that the PRH plays a role in acquiring

and retaining configural information, which is associated with the emotional features of the event processed in the AMY (Burwell et al., 2004).

In Experiment 1, the HPC lesions impaired fear conditioning in the congruent context, which involved, in part, learning and remembering that a given configuration of elemental stimuli is associated with a fear-eliciting event. However, the HPC lesions had no impairing effects on fear conditioning when the rats were tested in the incongruent context, which had never been associated with the shock. Combined, these findings suggest that the HPC is necessary for contextual fear conditioning and supports the common view of HPC function in fear conditioning (Anagnostaras et al., 2001; Fanselow, 2000; Maren, 2001b; Rudy & O'Reilly, 2001).

The findings from some of the other experiments, in spite of not being explicitly designed to assess contextual fear conditioning, also add to our understanding of the neural basis of contextual fear conditioning. For instance, the burying impairment induced by aspiration lesions in Chapter 3 was dependent on features of the testing context. Specifically, the PRH aspiration lesions almost eliminated burying in the large shock-probe chamber, but did not significantly reduce burying in the small chamber. These latter findings suggest that the PRH may be involved in appraising contextual information that guides behaviour. A caveat to this possibility is that neurotoxic lesions did not affect burying in the large chamber in Experiment 5. This raises the possibility that the aspiration lesions produced collateral damage to surrounding structures and/or pathways of the PRH, which would account for the changes in burying.

The absence of anterograde and retrograde amnesia following neurotoxic PRH lesions in the shock-probe fear-conditioning test suggests that neither object-fear

conditioning nor contextual fear conditioning were affected. The evidence from Experiment 1 suggests that impairments in contextual fear conditioning leads to impaired performance during the shock-probe fear-conditioning retention test. Accordingly, if PRH lesions had impaired contextual fear conditioning, then the PRH-damaged rats would not have performed as well as the SHAM rats during the retention test. Thus, the findings from Chapter 3 suggest that, contrary to current views (Burwell et al., 2004), the PRH is not critical for contextual fear conditioning and the integration of configural stimuli.

For the same reasons, the absence of anterograde amnesia following AMY lesions in Chapter 4 suggests that the AMY is not necessary for contextual fear conditioning and is inconsistent with its commonly believed functions. However, several other studies have also found spared contextual fear conditioning after pretraining AMY lesions, suggesting that present theory is likely incorrect. Unfortunately the specific conditions that lead to impaired or spared memory for contextual fear conditioning are unknown.

5.1.3 Object Recognition

The known contributions of the HPC and PRH to object recognition (Buffalo et al., 1999; Gaskin et al., 2003; Glenn & Mumby, 1996; Mumby et al., 2002; Mumby & Pinel, 1994; Zola et al., 2000) were a major part of the rationale for investigating the role of the HPC and PRH in object-fear conditioning. Yet, lesions of the PRH never caused memory deficits for object-fear conditioning and neither did pretraining HPC lesions. It is important to point out that these lack of deficits do not necessarily imply that the lesions did not affect object recognition. There are several accounts of dissociations between recognizing a previously encountered stimulus and responding to it in amnesic patients

(Cohen & Squire, 1980; Squire et al., 1984; Warrington & Weiskrantz, 1968). Consequently, the findings of this thesis have little to contribute to theory focusing on the neural basis of object recognition.

Nonetheless, modification of the shock-probe fear-conditioning test may lead to new methods for assessing the role of the PRH and HPC in object recognition. In the current version of the test, the rats are only presented with one object and are not required to discriminate between two or more objects like it is in other object recognition tests (Ennaceur & Delacour, 1988; Mumby, Pinel, & Wood, 1990). Thus, it possible that object recognition deficits would be detected in HPC and PRH rats if they were presented with several different objects during the shock-probe retention test and required to discriminate the one that was associated with shock.

5.2 THE DISSOCIABLE EFFECTS OF PRE- AND POST-TRAINING LESIONS ON MEMORY

The absence of anterograde amnesia following lesions to a brain structure simply suggests that the structure is **not necessary** for the organism to learn and remember the information. Under no circumstances does it indicate whether the structure is normally involved in mnemonic functions. Accordingly, the intact memory findings following pretraining lesions, in this thesis, are of little value on their own because it is impossible to make inferences about the contributions of these structures to object-fear conditioning in the undamaged brain.

In contrast, any evidence of retrograde amnesia after damage to a given structure implies that it is **normally involved** in mnemonic function. Hence, the retrograde

amnesia induced by the HPC and AMY lesions suggest that both structures normally contribute to memory for object-fear conditioning.

The dissociations between anterograde and retrograde amnesia following HPC and AMY lesions, in the current thesis, suggest that more than one neural system may underlie object-fear conditioning. The absence of anterograde amnesia after damage to the HPC or AMY suggests that another neural system supports the conditioning in event of damage to either the structure. Future research should pinpoint each of these systems and determine whether they work in parallel.

5.3 IMPORTANCE OF THE LESION METHOD

The different effects of various lesions methods on memory is a recurring finding in this thesis, as in many published studies (Campeau & Davis, 1995a; Fanselow, 2000; Mumby & Glenn, 2000; Thornton et al., 1997). For example, in Chapter 3, aspiration lesions of the PRH impaired burying, whereas neurotoxic lesions did not. Similarly, it was found in Experiment 8 that electrolytic lesion of the AMY caused retrograde amnesia for object-fear conditioning; whereas Lehmann and colleagues (2000) found that neurotoxic AMY lesions did not.

Through out the thesis, the discrepant findings caused by the various lesion methods have been attributed to damage or lack of damage to the fibers of passage within and/or surrounding the targeted structure. Indeed, this explanation seems the most parsimonious because it applies to all instances included in this thesis.

However, there is evidence that lesion methods that equally damage fibers of passage may have different effects on memory and behaviour (Glenn & Mumby, 1996,

1998; Glenn et al., 2003; Liu & Bilkey, 1998; Mumby & Glenn, 2000; Wiig & Bilkey, 1994). For instance, Glenn and colleagues (2000) found that electrolytic, but not aspiration, lesions of the PRH impair memory in the water maze task. Moreover, research from this group showed that the induction of aspiration, electrolytic, and neurotoxic lesions of the PRH results in different patterns of expression of the immediate early gene c-fos in the brain (Glenn, Woodside, & Mumby, 2002). These latter findings suggest that each lesions method has different neural consequences that go beyond the fibers-of-passage explanation. The specific consequences of the different expression of c-fos following each lesion method are still unknown. Nonetheless, one should consider the possibility that other factors than damage or lack of damage to fibers of passage may account for discrepant findings after damaging a structure with different methods.

5.4 SUBSTANTIATION OF THE SHOCK-PROBE FEAR-CONDITIONING TEST

The shock-probe test has not been extensively used for fear conditioning and has occasionally been subject to criticism. Nonetheless, there is compelling evidence, including some from the current thesis that supports the contention that shock-probe fear conditioning is a reliable fear-conditioning test. This section discusses some of the issues that have been debated and highlights the findings from the thesis that substantiate the test.

5.4.1 Shock-Probe Fear Conditioning: Instrumental or Pavlovian?

It is important to consider that the studies that have found cued fear conditioning impairments following HPC, PRH, or AMY lesions usually involved Pavlovian conditioning procedures, which differ from the conditioning procedures in the shock-

probe fear-conditioning test (see Maren, 2003b). In shock-probe fear conditioning the shock is dependent on the rat's behaviour, whereas it is not in the typical Pavlovian fear-conditioning tasks. Consequently, it is believed that shock-probe fear conditioning involves both Pavlovian conditioning and instrumental conditioning (Lehmann et al., 2000, 2003; Maren, 2003b). In shock-probe fear conditioning, the association between the probe and the shock remains similar, for instance, to the association between the tone and the shock that is learned during Pavlovian auditory fear conditioning. However, the rats may also learn to avoid the probe through instrumental contingencies. Given that Pavlovian and instrumental conditioning may occur simultaneously in the shock-probe fear-conditioning test, it is possible that the experiments reporting an absence of a cued fear conditioning impairment following lesions in the current thesis may be due to the sparing of one or both types of conditioning. In contrast, the findings that post-training HPC and AMY lesions impair shock-probe fear conditioning suggests that both types of conditioning were affected.

5.4.2 Evidence that Fear Is Involved

An important question is whether or not the burying and avoidance of the probe observed during the shock-probe retention test reflects fear regardless of the type of conditioning that supports the responses. There are a number of studies that suggest that fear is not necessary to maintain avoidance responding (Kamin, Brimer, & Black, 1963; Mineka, 1985; Mineka & Gino, 1980; Mineka & Hendersen, 1985; Solomon & Wynne, 1954; Starr & Mineka, 1977). However, the dissociation between fear and avoidance only occurs after extensive training, whereas fear seems necessary and is always expressed during the early stages of avoidance (Bechara et al., 1999; Kamin et al., 1963; Mineka,

1979; Mineka & Gino, 1980; Mineka & Hendersen, 1985; Solomon & Wynne, 1954; Starr & Mineka, 1977). During shock-probe fear conditioning, the rats learn to avoid the probe very quickly and with minimal training. Moreover, there is no opportunity for extinction of the conditioned fear between the acquisition session and the retention test because the rats are not exposed to the probe or the context during the retention interval. Consequently, shock-probe avoidance likely involves the expression of fear.

Additionally, in Experiment 1 the SHAM rats tended to avoid the probe more in the context that involved contextual fear conditioning (i.e., congruent condition) than the one that did not (i.e., incongruent condition), suggesting that the probe avoidance response is a measure of fear. Given that the conditioning pertaining solely to the probe should remain the same in the congruent and incongruent contexts, the differences in avoidance is likely due to the differences in fear generated by the testing contexts. This modulation of the avoidance of the probe by the context implies that the avoidance response in the shock-probe test reflects fear and that avoidance has some validity as a measure of fear conditioning. This also implies that the instances in which HPC-, PRH-, and AMY-damaged rats avoided of the probe during the retention test likely reflected sparing of fear conditioning to the probe.

It has also been suggested that burying may not be a valid measure of fear in the shock-probe test because rats bury in instances that do not involve fear, such as nesting (Fanselow et al., 1987). However, the experiments in this thesis always, with the exception of Experiment 4, included rats that never received shock, and in each one the SHAM rats buried significantly more than the NAÏVE rats. This finding strongly suggests that the association between the probe and the shock accounts for the burying

and doubtful that it is an expression of nesting behaviour. It is more likely that the burying reflects the rats engaging in a response that would create a barrier between them and the fear-eliciting object. This view has previously been proposed as the reason for which rats and other rodents bury the entrance of their burrow when a predator is present (Moser & Tait, 1983). Thus, the burying during the shock-probe fear-conditioning retention test is likely an index of fear similar to that expressed when rats are threatened by a predator.

Between avoidance and burying in the shock-probe fear-conditioning test, it has been suggested that burying is a better measure of fear (Maren, 2003b). Yet, the evidence in this thesis does not support that claim. If burying were a better and more sensitive measure of fear than avoidance, than in the cases of a fear-conditioning deficit in the current thesis, burying would have been more affected by the lesions than avoidance. However, there were instances in which avoidance was more affected by the lesions than burying. For example, in Experiment 8, in which AMY lesions caused retrograde amnesia, the AMY rats buried significantly more than the NAÏVE rats, although they did not avoid contacting the probe more than the NAÏVE rats. Consequently, the belief that burying is a better measure of fear than avoidance in the shock-probe fear-conditioning test is not supported and it is probably best to consider both responses to be equally indicative of fear conditioning.

5.4.3 Benefits of Assessing Several Behaviours

A major advantage of the shock-probe fear-conditioning test is that several behaviours may be assessed easily and non-obtrusively, which may lead to a more comprehensive evaluation of memory. Unfortunately, the assessment of a single

behaviour, which is presently the most common occurrence in the field of fear conditioning, may result in incorrect conclusions about whether a structure is involved in memory. A good example from this thesis is in Experiment 5, post-training neurotoxic lesions of the PRH severely impaired avoidance of the probe, but burying remained intact. If avoidance were the sole behaviour assessed, then one would necessarily have reached the conclusion that memory for object-fear conditioning was impaired. However, by assessing both burying and avoidance, it is evident that the PRH lesions did not impair fear conditioning. Thus, brain damage may result in dissociable effects on fear behaviours and only the assessment of several leads to more accurate conclusion.

Several studies have found similar dissociations in fear conditioning tests (McNish et al., 1997; Sutherland & McDonald, 1990; Vanderwolf et al., 1988; Vazdarjanova & McGaugh, 1998). For instance, in one study, although lesions of the AMY in rats impaired freezing to a context that was paired with shock, they did not block avoidance of this same context (Vazdarjanova & McGaugh, 1998). Likewise, in another study, HPC damage disrupted freezing to a context associated with shock, while fear-potentiated startle elicited by the context remained intact (McNish et al., 1997).

In sum, fear conditioning elicits several fear responses and even though one of them may be affected by damage to a given structure, it does not necessarily imply that memory is impaired. Using the shock-probe fear-conditioning test reduces the possibility of wrongfully concluding a memory impairment following damage to a structure because several behaviours are expressed at once during the test and each may be quantified.

5.5 CONCLUSION

The findings from the experiments in this thesis suggest that the MTL is involved in object-fear conditioning. Specifically, damage to the HPC or AMY caused retrograde amnesia for object-fear conditioning. However, not every MTL structure contributes to memory for object-fear conditioning because neither pre- nor post-training PRH damage caused memory impairments.

The findings, however, suggest that the PRH contributes to the expression of fear conditioning responses. Indeed, pretraining neurotoxic PRH lesions enhanced avoidance, whereas post-training lesions reduced avoidance. In addition, aspiration lesions of the PRH impaired burying and this effect was context dependent. Therefore, structures within the MTL that are not involved in the mnemonic processes pertaining to object-fear conditioning may still be involved processes that guide fear conditioning responses, such as appraising stimuli.

The dissociable effects of pre- and post-training HPC and AMY lesions on memory for object-fear conditioning also suggest that more than one memory system can subsume object-fear conditioning. Moreover, the absence of anterograde and retrograde amnesia following PRH lesions and the absence of anterograde amnesia following HPC and AMY lesions contrasts with previously published cued fear conditioning findings. These conflicting findings suggest that object-fear conditioning involves different neural circuits and memory systems than other types of cued fear conditioning.

In sum, the present thesis provides new evidence about the neural basis of fear conditioning and the supports the notion that neural structures differentially contribute to memory and the expression of fear conditioning.

REFERENCES

Adamec, R., Kent, P., Anisman, H., Shallow, T., & Merali, Z. (1998). Neural plasticity, neuropeptides and anxiety in animals--implications for understanding and treating affective disorder following traumatic stress in humans. *Neurosci Biobehav Rev, 23*(2), 301-318.

Aggleton, J. P., Blindt, H. S., & Rawlins, J. N. (1989). Effects of amygdaloid and amygdaloid-hippocampal lesions on object recognition and spatial working memory in rats. *Behav Neurosci, 103*(5), 962-974.

Aggleton, J. P., Keen, S., Warburton, E. C., & Bussey, T. J. (1997). Extensive cytotoxic lesions involving both the rhinal cortices and area TE impair recognition but spare spatial alternation in the rat. *Brain Res Bull, 43*(3), 279-287.

Alvarez, P., Zola-Morgan, S., & Squire, L. R. (1995). Damage limited to the hippocampal region produces long-lasting memory impairment in monkeys. *J Neurosci, 15*(5 Pt 2), 3796-3807.

Amaral, D. G. (1999). Introduction: what is where in the medial temporal lobe? *Hippocampus, 9*(1), 1-6.

Amaral, D. G., & Cowan, W. M. (1980). Subcortical afferents to the hippocampal formation in the monkey. *J Comp Neurol, 189*(4), 573-591.

Amaral, D. G., & Price, J. L. (1984). Amygdalo-cortical projections in the monkey (Macaca fascicularis). *J Comp Neurol, 230*(4), 465-496.

Amaral, D. G., Veazey, R. B., & Cowan, W. M. (1982). Some observations on hypothalamo-amygdaloid connections in the monkey. *Brain Res, 252*(1), 13-27.

Anagnostaras, S. G., Gale, G. D., & Fanselow, M. S. (2001). Hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus, 11*(1), 8-17.

Anagnostaras, S. G., Maren, S., & Fanselow, M. S. (1999). Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. *J Neurosci, 19*(3), 1106-1114.

Anisman, H., & Merali, Z. (1999). Understanding stress: characteristics and caveats. *Alcohol Res Health, 23*(4), 241-249.

Antoniadis, E. A., & McDonald, R. J. (2001). Amygdala, hippocampus, and unconditioned fear. *Exp Brain Res, 138*(2), 200-209.

Bachevalier, J., & Mishkin, M. (1989). Mnemonic and neuropathological effects of occluding the posterior cerebral artery in Macaca mulatta. *Neuropsychologia, 27*(1), 83-105.

Bachevalier, J., Parkinson, J. K., & Mishkin, M. (1985). Visual recognition in monkeys: effects of separate vs. combined transection of fornix and amygdalofugal pathways. *Exp Brain Res, 57*(3), 554-561.

Bast, T., Zhang, W. N., & Feldon, J. (2001a). Hippocampus and classical fear conditioning. *Hippocampus, 11*(6), 828-831.

Bast, T., Zhang, W. N., & Feldon, J. (2001b). The ventral hippocampus and fear conditioning in rats. Different anterograde amnesias of fear after tetrodotoxin inactivation and infusion of the GABA(A) agonist muscimol. *Exp Brain Res, 139*(1), 39-52.

Baxter, M. G., & Murray, E. A. (2001). Opposite relationship of hippocampal and rhinal cortex damage to delayed nonmatching-to-sample deficits in monkeys. *Hippocampus, 11*(1), 61-71.

Bechara, A., Damasio, H., Damasio, A. R., & Lee, G. P. (1999). Different contributions of the human amygdala and ventromedial prefrontal cortex to decision-making. *J Neurosci, 19*(13), 5473-5481.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science, 269*(5227), 1115-1118.

Beckstead, R. M., Domesick, V. B., & Nauta, W. J. (1979). Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res, 175*(2), 191-217.

Berk, M. L., & Finkelstein, J. A. (1981). Afferent projections to the preoptic area and hypothalamic regions in the rat brain. *Neuroscience, 6*(8), 1601-1624.

Bermudez-Rattoni, F., Introini-Collison, I., Coleman-Mesches, K., & McGaugh, J. L. (1997). Insular cortex and amygdala lesions induced after aversive training impair retention: effects of degree of training. *Neurobiol Learn Mem, 67*(1), 57-63.

Blanchard, D. C., & Blanchard, R. J. (1972). Innate and conditioned reactions to threats in rats with amygdaloid lesions. *J Comp Physiol Psychol, 81*, 281-290.

Blanchard, D. C., Blanchard, R. J., Lee, E. M. C., & Fukunaga, K. K. (1977). Movement arrest and the hippocampus. *Physiol Psychol, 5*, 331-335.

Blanchard, R. J., Dielman, T. E., & Blanchard, D. C. (1968a). Postshock crouching: Familiarity with the stimulus situation. *Psychon Sci, 10*, 371-372.

Blanchard, R. J., Dielman, T. E., & Blanchard, D. C. (1968b). Prolonged aftereffects of a single foot shock. *Psychono Sci, 10*, 327-328.

Blanchard, R. J., & Fial, R. A. (1968). Effects of limbic lesions on passive avoidance and reactivity to shock in the albino rat. *J Comp Physiol Psychol, 66*, 606-612.

Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature, 361*(6407), 31-39.

Brady, J. V., Schreiner, L., Geller, I., & Kling, A. (1954). Subcortical mechanisms in emotional behavior; the effect of rhinencephalic injury upon the acquisition and

retention of a conditioned avoidance response in cats. *J Comp Physiol Psychol, 47*(3), 179-186.

Brown, M. W., & Xiang, J. Z. (1998). Recognition memory: neuronal substrates of the judgement of prior occurrence. *Prog Neurobiol, 55*(2), 149-189.

Bucci, D. J., Phillips, R. G., & Burwell, R. D. (2000). Contributions of postrhinal and perirhinal cortex to contextual information processing. *Behav Neurosci, 114*(5), 882-894.

Bucci, D. J., Saddoris, M. P., & Burwell, R. D. (2002). Contextual fear discrimination is impaired by damage to the postrhinal or perirhinal cortex. *Behav Neurosci, 116*(3), 479-488.

Buffalo, E. A., Ramus, S. J., Clark, R. E., Teng, E., Squire, L. R., & Zola, S. M. (1999). Dissociation between the effects of damage to perirhinal cortex and area TE. *Learn Mem, 6*(6), 572-599.

Burns, L. H., Annett, L., Kelley, A. E., Everitt, B. J., & Robbins, T. W. (1996). Effects of lesions to amygdala, ventral subiculum, medial prefrontal cortex, and nucleus accumbens on the reaction to novelty: implication for limbic-striatal interactions. *Behav Neurosci, 110*(1), 60-73.

Burwell, R. D. (2000). The parahippocampal region: corticocortical connectivity. *Ann N Y Acad Sci, 911*, 25-42.

Burwell, R. D., & Amaral, D. G. (1998a). Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *J Comp Neurol, 398*(2), 179-205.

Burwell, R. D., & Amaral, D. G. (1998b). Perirhinal and postrhinal cortices of the rat: interconnectivity and connections with the entorhinal cortex. *J Comp Neurol, 391*(3), 293-321.

Burwell, R. D., Saddoris, M. P., Bucci, D. J., & Wiig, K. A. (2004). Corticohippocampal contributions to spatial and contextual learning. *J Neurosci, 24*(15), 3826-3836.

Burwell, R. D., Witter, M. P., & Amaral, D. G. (1995). Perirhinal and postrhinal cortices of the rat: a review of the neuroanatomical literature and comparison with findings from the monkey brain. *Hippocampus, 5*(5), 390-408.

Bussey, T. J., Duck, J., Muir, J. L., & Aggleton, J. P. (2000). Distinct patterns of behavioural impairments resulting from fornix transection or neurotoxic lesions of the perirhinal and postrhinal cortices in the rat. *Behav Brain Res, 111*(1-2), 187-202.

Bussey, T. J., Muir, J. L., & Aggleton, J. P. (1999). Functionally dissociating aspects of event memory: the effects of combined perirhinal and postrhinal cortex lesions on object and place memory in the rat. *J Neurosci, 19*(1), 495-502.

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2002). Perirhinal cortex resolves feature ambiguity in complex visual discriminations. *Eur J Neurosci, 15*(2), 365-374.

Bussey, T. J., Saksida, L. M., & Murray, E. A. (2003). Impairments in visual discrimination after perirhinal cortex lesions: testing 'declarative' vs. 'perceptual-mnemonic' views of perirhinal cortex function. *Eur J Neurosci, 17*(3), 649-660.

Cahill, L., Vazdarjanova, A., & Setlow, B. (2000). The basolateral amygdala complex is involved with, but is not necessary for, rapid acquisition of Pavlovian 'fear conditioning'. *Eur J Neurosci, 12*(8), 3044-3050.

Cahill, L., Weinberger, N. M., Roozendaal, B., & McGaugh, J. L. (1999). Is the amygdala a locus of "conditioned fear"? Some questions and caveats. *Neuron, 23*(2), 227-228.

Campeau, S., & Davis, M. (1995a). Involvement of subcortical and cortical afferents to the lateral nucleus of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J Neurosci, 15*(3 Pt 2), 2312-2327.

Campeau, S., & Davis, M. (1995b). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated

startle in rats trained concurrently with auditory and visual conditioned stimuli. *J Neurosci, 15*(3 Pt 2), 2301-2311.

Clark, R. E., West, A. N., Zola, S. M., & Squire, L. R. (2001). Rats with lesions of the hippocampus are impaired on the delayed nonmatching-to-sample task. *Hippocampus, 11*(2), 176-186.

Clark, R. E., Zola, S. M., & Squire, L. R. (2000). Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci, 20*(23), 8853-8860.

Clugnet, M. C., & LeDoux, J. E. (1990). Synaptic plasticity in fear conditioning circuits: induction of LTP in the lateral nucleus of the amygdala by stimulation of the medial geniculate body. *J Neurosci, 10*(8), 2818-2824.

Cohen, N. J., & Squire, L. R. (1980). Preserved learning and retention of pattern-analyzing skill in amnesia: dissociation of knowing how and knowing that. *Science, 210*(4466), 207-210.

Conde, F., Maire-Lepoivre, E., Audinat, E., & Crepel, F. (1995). Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. *J Comp Neurol, 352*(4), 567-593.

Coover, G. D., Ursin, H., & Levine, S. (1973a). Corticosterone and avoidance in rats with basolateral amygdala lesions. *J Comp Physiol Psychol, 85*(1), 111-122.

Coover, G. D., Ursin, H., & Levine, S. (1973b). Plasma-corticosterone levels during active-avoidance learning in rats. *J Comp Physiol Psychol, 82*(1), 170-174.

Coover, G. D., Ursin, H., & Levine, S. (1974). Corticosterone levels during avoidance learning in rats with cingulate lesions suggest an instrumental reinforcement deficit. *J Comp Physiol Psychol, 87*(5), 970-977.

Corodimas, K. P., & LeDoux, J. E. (1995). Disruptive effects of posttraining perirhinal cortex lesions on conditioned fear: contributions of contextual cues. *Behav Neurosci, 109*(4), 613-619.

Cousens, G., & Otto, T. (1998). Both pre- and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. *Behav Neurosci, 112*(5), 1092-1103.

Davis, H., Porter, J. W., Livingstone, J., Herrmann, T., MacFadden, L., & Levine, S. (1977). Pituitary-adrenal acivity and leverpress shock escape behavior. *Physiol Psychol, 5*(3), 280-284.

Davis, M. (1992). The role of the amygdala in fear and anxiety. *Annu Rev Neurosci, 15*, 353-375.

Davis, M. (1997). Neurobiology of fear responses: the role of the amygdala. *J Neuropsychiatry Clin Neurosci, 9*(3), 382-402.

Deacon, T. W., Eichenbaum, H., Rosenberg, P., & Eckmann, K. W. (1983). Afferent connections of the perirhinal cortex in the rat. *J Comp Neurol, 220*(2), 168-190.

Degroot, A., & Treit, D. (2002). Dorsal and ventral hippocampal cholinergic systems modulate anxiety in the plus-maze and shock-probe tests. *Brain Res, 949*(1-2), 60-70.

Degroot, A., & Treit, D. (2004). Anxiety is functionally segregated within the septo-hippocampal system. *Brain Res, 1001*(1-2), 60-71.

Delacour, J., & Borst, A. (1972). Failure to find homology in rat, cat, and monkey for functions of a subcortical structure in avoidance conditioning. *J Comp Physiol Psychol, 80*(3), 458-468.

Dess, N. K., Linwick, D., Patterson, J., Overmier, J. B., & Levine, S. (1983). Immediate and proactive effects of controllability and predictability on plasma cortisol responses to shocks in dogs. *Behav Neurosci, 97*(6), 1005-1016.

DiCara, L. V., Braun, J. J., & Pappas, B. A. (1970). Classical conditioning and instrumental learning of cardiac and gastrointestinal responses following removal of neocortex in the rat. *J Comp Physiol Psychol, 73*(2), 208-216.

Dong, H. W., Petrovich, G. D., & Swanson, L. W. (2001). Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Res Brain Res Rev, 38*(1-2), 192-246.

Douglas, R. J., & Isaacson, R. L. (1964). Hippocampal lesions and activity. *Psychon Sci, 1*, 187-188.

Dunn, L. T., & Everitt, B. J. (1988). Double dissociations of the effects of amygdala and insular cortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. *Behav Neurosci, 102*(1), 3-23.

Ennaceur, A., & Aggleton, J. P. (1997). The effects of neurotoxic lesions of the perirhinal cortex combined to fornix transection on object recognition memory in the rat. *Behav Brain Res, 88*(2), 181-193.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res, 31*(1), 47-59.

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Exp Brain Res, 113*(3), 509-519.

Fahy, F. L., Riches, I. P., & Brown, M. W. (1993). Neuronal activity related to visual recognition memory: long-term memory and the encoding of recency and familiarity information in the primate anterior and medial inferior temporal and rhinal cortex. *Exp Brain Res, 96*(3), 457-472.

Fallon, J. H., & Moore, R. Y. (1978a). Catecholamine innervation of the basal forebrain. III. Olfactory bulb, anterior olfactory nuclei, olfactory tubercle and piriform cortex. *J Comp Neurol, 180*(3), 533-544.

Fallon, J. H., & Moore, R. Y. (1978b). Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. *J Comp Neurol, 180*(3), 545-580.

Falls, W. A., Bakken, K. T., & Heldt, S. A. (1997). Lesions of the perirhinal cortex interfere with conditioned excitation but not with conditioned inhibition of fear. *Behav Neurosci, 111*(3), 476-486.

Fanselow, M. S. (1980). Conditioned and unconditional components of post-shock freezing. *Pavlov J Biol Sci, 15*(4), 177-182.

Fanselow, M. S. (2000). Contextual fear, gestalt memories, and the hippocampus. *Behav Brain Res, 110*(1-2), 73-81.

Fanselow, M. S., & Gale, G. D. (2003). The amygdala, fear, and memory. *Ann N Y Acad Sci, 985*, 125-134.

Fanselow, M. S., & LeDoux, J. E. (1999). Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron, 23*(2), 229-232.

Fanselow, M. S., Sigmundi, R. A., & Williams, J. L. (1987). Response selection and the hierarchical organization of species-specific defense reactions: The relationship between freezing, flight, and defensive burying. *Psychol Rec, 37*, 381-386.

Farr, S. A., Banks, W. A., La Scola, M. E., Flood, J. F., & Morley, J. E. (2000). Permanent and temporary inactivation of the hippocampus impairs T-maze footshock avoidance acquisition and retention. *Brain Res, 872*(1-2), 242-249.

File, S. E., Zangrossi, H., Jr., Viana, M., & Graeff, F. G. (1993). Trial 2 in the elevated plus-maze: a different form of fear? *Psychopharmacology (Berl), 111*(4), 491-494.

Fonberg, E. (1965). Effect of partial destruction of the amygdaloid complex on the emotional-defensive behaviour of dogs. *Bull Acad Pol Sci Biol, 13*(7), 429-432.

Frankland, P. W., Cestari, V., Filipkowski, R. K., McDonald, R. J., & Silva, A. J. (1998). The dorsal hippocampus is essential for context discrimination but not for contextual conditioning. *Behav Neurosci, 112*(4), 863-874.

French, S. J., Hailstone, J. C., & Totterdell, S. (2003). Basolateral amygdala efferents to the ventral subiculum preferentially innervate pyramidal cell dendritic spines. *Brain Res, 981*(1-2), 160-167.

Friedman, D. P., Murray, E. A., O'Neill, J. B., & Mishkin, M. (1986). Cortical connections of the somatosensory fields of the lateral sulcus of macaques: evidence for a corticolimbic pathway for touch. *J Comp Neurol, 252*(3), 323-347.

Gaffan, D. (1974). Recognition impaired and association intact in the memory of monkeys after transection of the fornix. *J Comp Physiol Psychol, 86*(6), 1100-1109.

Gaffan, D., & Murray, E. A. (1992). Monkeys (Macaca fascicularis) with rhinal cortex ablations succeed in object discrimination learning despite 24-hr intertrial intervals and fail at matching to sample despite double sample presentations. *Behav Neurosci, 106*(1), 30-38.

Gaffan, D., Parker, A., & Easton, A. (2001). Dense amnesia in the monkey after transection of fornix, amygdala and anterior temporal stem. *Neuropsychologia, 39*(1), 51-70.

Gale, G. D., Anagnostaras, S. G., Godsil, B. P., Mitchell, S., Nozawa, T., Sage, J. R., et al. (2004). Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats. *J Neurosci, 24*(15), 3810-3815.

Gaskin, S., Tremblay, A., & Mumby, D. G. (2003). Retrograde and anterograde object recognition in rats with hippocampal lesions. *Hippocampus, 13*(8), 962-969.

Glenn, M. J., & Mumby, D. G. (1996). Place- and object-recognition deficits following lesions of the hippocampus or perirhinal cortex in rats: a double dissociation. *Soc Neurosci Abstract, 22*, 1120.

Glenn, M. J., & Mumby, D. G. (1998). Place memory is intact in rats with perirhinal cortex lesions. *Behav Neurosci, 112*(6), 1353-1365.

Glenn, M. J., Nesbitt, C., & Mumby, D. G. (2003). Perirhinal cortex lesions produce variable patterns of retrograde amnesia in rats. *Behav Brain Res, 141*(2), 183-193.

Glenn, M. J., Woodside, B., & Mumby, D. G. (2002). Electrolytic, but not aspiration, lesions of the perirhinal cortex in rats increase fos activation in the cortex. *Soc Neurosci Abstract, 478.3*.

Goldstein, L. E., Rasmusson, A. M., Bunney, B. S., & Roth, R. H. (1996). Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. *J Neurosci, 16*(15), 4787-4798.

Goosens, K. A., & Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learn Mem, 8*(3), 148-155.

Grossman, S. P., Grossman, L., & Walsh, L. (1975). Functional organization of the rat amygdala with respect to avoidance behavior. *J Comp Physiol Psychol, 88*(2), 829-850.

Guldin, W. O., & Markowitsch, H. J. (1983). Cortical and thalamic afferent connections of the insular and adjacent cortex of the rat. *J Comp Neurol, 215*(2), 135-153.

Helmstetter, F. J. (1992a). The amygdala is essential for the expression of conditional hypoalgesia. *Behav Neurosci, 106*(3), 518-528.

Helmstetter, F. J. (1992b). Contribution of the amygdala to learning and performance of conditional fear. *Physiol Behav, 51*(6), 1271-1276.

Helmstetter, F. J., & Bellgowan, P. S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behav Neurosci, 108*(5), 1005-1009.

Herkenham, M. (1978). The connections of the nucleus reuniens thalami: evidence for a direct thalamo-hippocampal pathway in the rat. *J Comp Neurol, 177*(4), 589-610.

Herzog, C., & Otto, T. (1997). Odor-guided fear conditioning in rats: 2. Lesions of the anterior perirhinal cortex disrupt fear conditioned to the explicit conditioned stimulus but not to the training context. *Behav Neurosci, 111*(6), 1265-1272.

Herzog, C., & Otto, T. (1998). Contributions of anterior perirhinal cortex to olfactory and contextual fear conditioning. *Neuroreport, 9*(8), 1855-1859.

Horvath, F. E. (1963). Effects of basolateral amygdalectomy on three types of avoidance behavior in cats. *J Comp Physiol Psychol, 56*, 380-389.

Isenberg, N., Silbersweig, D., Engelien, A., Emmerich, S., Malavade, K., Beattie, B., et al. (1999). Linguistic threat activates the human amygdala. *Proc Natl Acad Sci U S A, 96*(18), 10456-10459.

Ishikawa, A., & Nakamura, S. (2003). Convergence and interaction of hippocampal and amygdalar projections within the prefrontal cortex in the rat. *J Neurosci, 23*(31), 9987-9995.

Jarrard, L. E. (2002). Use of excitotoxins to lesion the hippocampus: update. *Hippocampus, 12*(3), 405-414.

Jarrard, L. E., & Meldrum, B. S. (1993). Selective excitotoxic pathology in the rat hippocampus. *Neuropathol Appl Neurobiol, 19*(5), 381-389.

Jay, T. M., Glowinski, J., & Thierry, A. M. (1989). Selectivity of the hippocampal projection to the prelimbic area of the prefrontal cortex in the rat. *Brain Res, 505*(2), 337-340.

Kamin, L. J., Brimer, C. J., & Black, A. H. (1963). Conditioned suppression as a monitor of fear of the CS in the course of avoidance training. *J Comp Physiol Psychol, 56*, 497-501.

Kelley, A. E., Domesick, V. B., & Nauta, W. J. (1982). The amygdalostriatal projection in the rat--an anatomical study by anterograde and retrograde tracing methods. *Neuroscience, 7*(3), 615-630.

Kemble, E. D., Blanchard, D. C., & Blanchard, R. J. (1990). Effects of regional amygdaloid lesions on flight and defensive behaviors of wild black rats (Rattus rattus). *Physiol Behav, 48*(1), 1-5.

Kesner, R. P., Berman, R. F., & Tardif, R. (1992). Place and taste aversion learning: role of basal forebrain, parietal cortex, and amygdala. *Brain Res Bull, 29*(3-4), 345-353.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256*(5057), 675-677.

Kim, J. J., Rison, R. A., & Fanselow, M. S. (1993). Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. *Behav Neurosci, 107*(6), 1093-1098.

Kim, M., & Davis, M. (1993a). Electrolytic lesions of the amygdala block acquisition and expression of fear-potentiated startle even with extensive training but do not prevent reacquisition. *Behav Neurosci, 107*(4), 580-595.

Kim, M., & Davis, M. (1993b). Lack of a temporal gradient of retrograde amnesia in rats with amygdala lesions assessed with the fear-potentiated startle paradigm. *Behav Neurosci, 107*(6), 1088-1092.

King, F. A. (1958). Effects of septal and amygdaloid lesions on emotional behavior and conditioned avoidance responses in the rat. *J Nerv Ment Dis, 126*(1), 57-63.

Kluver, H., & Bucy, P. C. (1937). "Psychic blindness" and other symptoms following temporal lobectomy in Rhesus monkeys. *A J Physiol, 119*, 352-353.

Koo, J. W., Han, J. S., & Kim, J. J. (2004). Selective neurotoxic lesions of basolateral and central nuclei of the amygdala produce differential effects on fear conditioning. *J Neurosci, 24*(35), 7654-7662.

Kopchia, K. L., Altman, H. J., & Commissaris, R. L. (1992). Effects of lesions of the central nucleus of the amygdala on anxiety-like behaviors in the rat. *Pharmacol Biochem Behav, 43*(2), 453-461.

Korte, S. M., Korte-Bouws, G. A., Bohus, B., & Koob, G. F. (1994). Effect of corticotropin-releasing factor antagonist on behavioral and neuroendocrine responses during exposure to defensive burying paradigm in rats. *Physiol Behav, 56*(1), 115-120.

Kosel, K. C., Van Hoesen, G. W., & Rosene, D. L. (1982). Non-hippocampal cortical projections from the entorhinal cortex in the rat and rhesus monkey. *Brain Res, 244*(2), 201-213.

Kosel, K. C., Van Hoesen, G. W., & Rosene, D. L. (1983). A direct projection from the perirhinal cortex (area 35) to the subiculum in the rat. *Brain Res, 269*(2), 347-351.

Kosel, K. C., Van Hoesen, G. W., & West, J. R. (1981). Olfactory bulb projections to the parahippocampal area of the rat. *J Comp Neurol, 198*(3), 467-482.

Krettek, J. E., & Price, J. L. (1977a). Projections from the amygdaloid complex and adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. *J Comp Neurol, 172*(4), 723-752.

Krettek, J. E., & Price, J. L. (1977b). Projections from the amygdaloid complex to the cerebral cortex and thalamus in the rat and cat. *J Comp Neurol, 172*(4), 687-722.

LaBar, K. S., & LeDoux, J. E. (1996). Partial disruption of fear conditioning in rats with unilateral amygdala damage: correspondence with unilateral temporal lobectomy in humans. *Behav Neurosci, 110*(5), 991-997.

Lavenex, P., & Amaral, D. G. (2000). Hippocampal-neocortical interaction: a hierarchy of associativity. *Hippocampus, 10*(4), 420-430.

Lavenex, P., Suzuki, W. A., & Amaral, D. G. (2002). Perirhinal and parahippocampal cortices of the macaque monkey: projections to the neocortex. *J Comp Neurol, 447*(4), 394-420.

LeDoux, J. (1998). Fear and the brain: where have we been, and where are we going? *Biol Psychiatry, 44*(12), 1229-1238.

LeDoux, J. (2003). The emotional brain, fear, and the amygdala. *Cell Mol Neurobiol, 23*(4-5), 727-738.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annu Rev Neurosci, 23*, 155-184.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990). The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. *J Neurosci, 10*(4), 1062-1069.

Lee, Y., Walker, D., & Davis, M. (1996). Lack of a temporal gradient of retrograde amnesia following NMDA-induced lesions of the basolateral amygdala assessed with the fear-potentiated startle paradigm. *Behav Neurosci, 110*(4), 836-839.

Lehmann, H., Treit, D., & Parent, M. B. (2000). Amygdala lesions do not impair shock-probe avoidance retention performance. *Behav Neurosci, 114*(1), 107-116.

Lehmann, H., Treit, D., & Parent, M. B. (2003). Spared anterograde memory for shock-probe fear conditioning after inactivation of the amygdala. *Learn Mem, 10*(4), 261-269.

Liang, K. C., McGaugh, J. L., Martinez, J. L., Jr., Jensen, R. A., Vasquez, B. J., & Messing, R. B. (1982). Post-training amygdaloid lesions impair retention of an inhibitory avoidance response. *Behav Brain Res, 4*(3), 237-249.

Liu, P., & Bilkey, D. K. (1998). Perirhinal cortex contributions to performance in the Morris water maze. *Behav Neurosci, 112*(2), 304-315.

Mahut, H., Moss, M., & Zola-Morgan, S. (1981). Retention deficits after combined amygdalo-hippocampal and selective hippocampal resections in the monkey. *Neuropsychologia, 19*(2), 201-225.

Mahut, H., Zola-Morgan, S., & Moss, M. (1982). Hippocampal resections impair associative learning and recognition memory in the monkey. *J Neurosci, 2*(9), 1214-1220.

Maier, S. F., Ryan, S. M., Barksdale, C. M., & Kalin, N. H. (1986). Stressor controllability and the pituitary-adrenal system. *Behav Neurosci, 100*(5), 669-674.

Maren, S. (1998). Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. *J Neurosci, 18*(8), 3088-3097.

Maren, S. (1999a). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *J Neurosci, 19*(19), 8696-8703.

Maren, S. (1999b). Neurotoxic or electrolytic lesions of the ventral subiculum produce deficits in the acquisition and expression of Pavlovian fear conditioning in rats. *Behav Neurosci, 113*(2), 283-290.

Maren, S. (2000). Auditory fear conditioning increases CS-elicited spike firing in lateral amygdala neurons even after extensive overtraining. *Eur J Neurosci, 12*(11), 4047-4054.

Maren, S. (2001a). Is there savings for pavlovian fear conditioning after neurotoxic basolateral amygdala lesions in rats? *Neurobiol Learn Mem, 76*(3), 268-283.

Maren, S. (2001b). Neurobiology of Pavlovian fear conditioning. *Annu Rev Neurosci, 24*, 897-931.

Maren, S. (2003a). The amygdala, synaptic plasticity, and fear memory. *Ann N Y Acad Sci, 985*, 106-113.

Maren, S. (2003b). What the amygdala does and doesn't do in aversive learning. *Learn Mem, 10*(5), 306-308.

Maren, S., Aharonov, G., & Fanselow, M. S. (1996). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient. *Behav Neurosci, 110*(4), 718-726.

Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavlovian fear conditioning in rats. *Behav Brain Res, 88*(2), 261-274.

Maren, S., & Fanselow, M. S. (1996). The amygdala and fear conditioning: has the nut been cracked? *Neuron, 16*(2), 237-240.

Maren, S., & Fanselow, M. S. (1997). Electrolytic lesions of the fimbria/fornix, dorsal hippocampus, or entorhinal cortex produce anterograde deficits in contextual fear conditioning in rats. *Neurobiol Learn Mem, 67*(2), 142-149.

Maren, S., & Holt, W. G. (2004). Hippocampus and Pavlovian fear conditioning in rats: muscimol infusions into the ventral, but not dorsal, hippocampus impair the acquisition of conditional freezing to an auditory conditional stimulus. *Behav Neurosci, 118*(1), 97-110.

Matus-Amat, P., Higgins, E. A., Barrientos, R. M., & Rudy, J. W. (2004). The role of the dorsal hippocampus in the acquisition and retrieval of context memory representations. *J Neurosci, 24*(10), 2431-2439.

McDonald, A. J. (2003). Is there an amygdala and how far does it extend? An anatomical perspective. *Ann N Y Acad Sci, 985*, 1-21.

McDonald, A. J., & Mascagni, F. (1997). Projections of the lateral entorhinal cortex to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience, 77*(2), 445-459.

McDonald, R. J., & White, N. M. (1993). A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. *Behav Neurosci, 107*(1), 3-22.

McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu Rev Neurosci, 27*, 1-28.

McKernan, M. G., & Shinnick-Gallagher, P. (1997). Fear conditioning induces a lasting potentiation of synaptic currents in vitro. *Nature, 390*(6660), 607-611.

McNish, K. A., Gewirtz, J. C., & Davis, M. (1997). Evidence of contextual fear after lesions of the hippocampus: a disruption of freezing but not fear-potentiated startle. *J Neurosci, 17*(23), 9353-9360.

Menard, J., & Treit, D. (1999). Effects of centrally administered anxiolytic compounds in animal models of anxiety. *Neurosci Biobehav Rev, 23*(4), 591-613.

Merali, Z., Kent, P., Michaud, D., McIntyre, D., & Anisman, H. (2001). Differential impact of predator or immobilization stressors on central corticotropin-releasing hormone and bombesin-like peptides in Fast and Slow seizing rat. *Brain Res, 906*(1-2), 60-73.

Meunier, M., & Bachevalier, J. (2002). Comparison of emotional responses in monkeys with rhinal cortex or amygdala lesions. *Emotion, 2*(2), 147-161.

Meunier, M., Bachevalier, J., Mishkin, M., & Murray, E. A. (1993). Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *J Neurosci, 13*(12), 5418-5432.

Meunier, M., Bachevalier, J., Murray, E. A., Malkova, L., & Mishkin, M. (1999). Effects of aspiration versus neurotoxic lesions of the amygdala on emotional responses in monkeys. *Eur J Neurosci, 11*(12), 4403-4418.

Miller, M. W., & Vogt, B. A. (1984). Direct connections of rat visual cortex with sensory, motor, and association cortices. *J Comp Neurol, 226*(2), 184-202.

Mineka, S. (1979). The role of fear in theories of avoidance learning, flooding, and extinction. *Psychol Bull, 86*, 985-1010.

Mineka, S. (1985). The frightful complexity of the origins of fears. In F. R. Brush & J. B. Overmeir (Eds.), *Affect, Conditioning, and Cognition.* (pp. 55-73). Hillsdale, NJ: Erlbaum.

Mineka, S., & Gino, A. (1980). Dissociation between conditioned emotional response and extended avoidance performance. *Learn Motiv, 11*, 476-502.

Mineka, S., & Hendersen, R. W. (1985). Controllability and predictability in acquired motivation. *Annu Rev Psychol, 36*, 495-529.

Mishkin, M. (1978). Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus. *Nature, 273*(5660), 297-298.

Moser, C. G., & Tait, R. W. (1983). Environmental control of multiple defensive responses in a conditioned burying paradigm. *J Comp Psychol, 97*(4), 338-352.

Muller, J., Corodimas, K. P., Fridel, Z., & LeDoux, J. E. (1997). Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. *Behav Neurosci, 111*(4), 683-691.

Mumby, D. G. (2001). Perspectives on object-recognition memory following hippocampal damage: lessons from studies in rats. *Behav Brain Res, 127*(1-2), 159-181.

Mumby, D. G., Astur, R. S., Weisend, M. P., & Sutherland, R. J. (1999). Retrograde amnesia and selective damage to the hippocampal formation: memory for places and object discriminations. *Behav Brain Res, 106*(1-2), 97-107.

Mumby, D. G., & Glenn, M. J. (2000). Anterograde and retrograde memory for object discriminations and places in rats with perirhinal cortex lesions. *Behav Brain Res, 114*(1-2), 119-134.

Mumby, D. G., Glenn, M. J., Nesbitt, C., & Kyriazis, D. A. (2002). Dissociation in retrograde memory for object discriminations and object recognition in rats with perirhinal cortex damage. *Behav Brain Res, 132*(2), 215-226.

Mumby, D. G., & Pinel, J. P. (1994). Rhinal cortex lesions and object recognition in rats. *Behav Neurosci, 108*(1), 11-18.

Mumby, D. G., Pinel, J. P., Kornecook, T. J., Shen, M. J., & Redila, V. A. (1995). Memory deficits follwoing lesions of the hippocampus or amygdala in rats: Assessment by an object-memory test battery. *Psychobiology, 23*, 26-36.

Mumby, D. G., Pinel, J. P., & Wood, E. R. (1990). Nonrecurring-items delayed nonmatching-to-sample in rats: a new paradigm for testing non-spatial working memory. *Psychobiology, 18*, 321-326.

Murray, E. A., & Bussey, T. J. (1999). Perceptual-mnemonic functions of the perirhinal cortex. *Trends Cogn Sci, 3*(4), 142-151.

Murray, E. A., Bussey, T. J., Hampton, R. R., & Saksida, L. M. (2000). The parahippocampal region and object identification. *Ann N Y Acad Sci, 911*, 166-174.

Murray, E. A., & Mishkin, M. (1983). Severe tactual memory deficits in monkeys after combined removal of the amygdala and hippocampus. *Brain Res, 270*(2), 340-344.

Murray, E. A., & Mishkin, M. (1984). Severe tactual as well as visual memory deficits follow combined removal of the amygdala and hippocampus in monkeys. *J Neurosci, 4*(10), 2565-2580.

Murray, E. A., & Mishkin, M. (1998). Object recognition and location memory in monkeys with excitotoxic lesions of the amygdala and hippocampus. *J Neurosci, 18*(16), 6568-6582.

Nadel, L., & Moscovitch, M. (1997). Memory consolidation, retrograde amnesia and the hippocampal complex. *Curr Opin Neurobiol, 7*(2), 217-227.

Neff, W. D., Diamond, I. T., & Casseday, J. H. (1975). Behavioral studies of auditory discrimination: central nervous system. In W. D. Keidel & W. D. Neff (Eds.), *Handbook of sensory physiology* (Vol. 2, pp. 307-400). Berlin: Springer.

Nemanic, S., Alvarado, M. C., & Bachevalier, J. (2004). The hippocampal/parahippocampal regions and recognition memory: insights from visual paired comparison versus object-delayed nonmatching in monkeys. *J Neurosci, 24*(8), 2013-2026.

O'Reilly, R. C., & Rudy, J. W. (2001). Conjunctive representations in learning and memory: principles of cortical and hippocampal function. *Psychol Rev, 108*(2), 311-345.

Ottersen, O. P. (1980). Afferent connections to the amygdaloid complex of the rat and cat: II. Afferents from the hypothalamus and the basal telencephalon. *J Comp Neurol, 194*(1), 267-289.

Ottersen, O. P. (1981). Afferent connections to the amygdaloid complex of the rat with some observations in the cat. III. Afferents from the lower brain stem. *J Comp Neurol, 202*(3), 335-356.

Ottersen, O. P. (1982). Connections of the amygdala of the rat. IV: Corticoamygdaloid and intraamygdaloid connections as studied with axonal transport of horseradish peroxidase. *J Comp Neurol, 205*(1), 30-48.

Ottersen, O. P., & Ben-Ari, Y. (1979). Afferent connections to the amygdaloid complex of the rat and cat. I. Projections from the thalamus. *J Comp Neurol, 187*(2), 401-424.

Otto, T., Cousens, G., & Herzog, C. (2000). Behavioral and neuropsychological foundations of olfactory fear conditioning. *Behav Brain Res, 110*(1-2), 119-128.

Otto, T., & Eichenbaum, H. (1992). Complementary roles of the orbital prefrontal cortex and the perirhinal-entorhinal cortices in an odor-guided delayed-nonmatching-to-sample task. *Behav Neurosci, 106*(5), 762-775.

Otto, T., & Giardino, N. D. (2001). Pavlovian conditioning of emotional responses to olfactory and contextual stimuli: a potential model for the development and expression of chemical intolerance. *Ann N Y Acad Sci, 933*, 291-309.

Otto, T., Schottler, F., Staubli, U., Eichenbaum, H., & Lynch, G. (1991). Hippocampus and olfactory discrimination learning: effects of entorhinal cortex lesions on olfactory learning and memory in a successive-cue, go-no-go task. *Behav Neurosci, 105*(1), 111-119.

Parent, M. B., Avila, E., & McGaugh, J. L. (1995). Footshock facilitates the expression of aversively motivated memory in rats given post-training amygdala basolateral complex lesions. *Brain Res, 676*(2), 235-244.

Parent, M. B., & McGaugh, J. L. (1994). Posttraining infusion of lidocaine into the amygdala basolateral complex impairs retention of inhibitory avoidance training. *Brain Res, 661*(1-2), 97-103.

Parent, M. B., Quirarte, G. L., Cahill, L., & McGaugh, J. L. (1995). Spared retention of inhibitory avoidance learning after posttraining amygdala lesions. *Behav Neurosci, 109*(4), 803-807.

Parent, M. B., Tomaz, C., & McGaugh, J. L. (1992). Increased training in an aversively motivated task attenuates the memory-impairing effects of posttraining N-methyl-D-aspartate-induced amygdala lesions. *Behav Neurosci, 106*(5), 789-797.

Parent, M. B., West, M., & McGaugh, J. L. (1994). Memory of rats with amygdala lesions induced 30 days after footshock-motivated escape training reflects degree of original training. *Behav Neurosci, 108*(6), 1080-1087.

Paxinos, G. (1995). *The rat nervous system* (2nd ed.). San Diego: Academic Press.

Paxinos, G., & Watson, C. (1997). *The Rat Brain in Stereotaxic Coordinates* (Compact 3rd ed.). San Diego, CA: Academic Press.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods, 14*(3), 149-167.

Pellow, S., & File, S. E. (1986). Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacol Biochem Behav, 24*(3), 525-529.

Pesold, C., & Treit, D. (1994). The septum and amygdala differentially mediate the anxiolytic effects of benzodiazepines. *Brain Res, 638*(1-2), 295-301.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci, 106*(2), 274-285.

Phillips, R. G., & LeDoux, J. E. (1994). Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. *Learn Mem, 1*(1), 34-44.

Phillips, R. G., & LeDoux, J. E. (1995). Lesions of the fornix but not the entorhinal or perirhinal cortex interfere with contextual fear conditioning. *J Neurosci, 15*(7 Pt 2), 5308-5315.

Pikkarainen, M., Ronkko, S., Savander, V., Insausti, R., & Pitkanen, A. (1999). Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. *J Comp Neurol, 403*(2), 229-260.

Pinel, J. P., Symons, L. A., Christensen, B. K., & Tees, R. C. (1989). Development of defensive burying in Rattus norvegicus: experience and defensive responses. *J Comp Psychol, 103*(4), 359-365.

Pinel, J. P., & Treit, D. (1978). Burying as a defensive response in rats. *J Comp Physiol Psychol, 92*, 708-712.

Pinel, J. P., Treit, D., Ladak, F., & MacLennan, A. J. (1980). Conditioned defensive burying in rats free to escape. *Anim Learn Behav, 35*, 31-44.

Pinel, J. P., Treit, D., & Wilkie, D. M. (1980). Stimulus control of defensive burying in the rat. *Learn Motiv, 8*, 447-451.

Pitkanen, A., & Amaral, D. G. (1998). Organization of the intrinsic connections of the monkey amygdaloid complex: projections originating in the lateral nucleus. *J Comp Neurol, 398*(3), 431-458.

Pitkanen, A., Kelly, J. L., & Amaral, D. G. (2002). Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the entorhinal cortex in the macaque monkey. *Hippocampus, 12*(2), 186-205.

Pitkanen, A., Pikkarainen, M., Nurminen, N., & Ylinen, A. (2000). Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. *Ann N Y Acad Sci, 911*, 369-391.

Poremba, A., & Gabriel, M. (1999). Amygdala neurons mediate acquisition but not maintenance of instrumental avoidance behavior in rabbits. *J Neurosci, 19*(21), 9635-9641.

Quirk, G. J., Armony, J. L., & LeDoux, J. E. (1997). Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. *Neuron, 19*(3), 613-624.

Quirk, G. J., Repa, C., & LeDoux, J. E. (1995). Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. *Neuron, 15*(5), 1029-1039.

Renner, M. J., Bennett, A. J., & White, J. C. (1992). Age and sex as factors influencing spontaneous exploration and object investigation by preadult rats (Rattus norvegicus). *J Comp Psychol, 106*(3), 217-227.

Renner, M. J., & Seltzer, C. P. (1991). Molar characteristics of exploratory and investigatory behavior in the rat (Rattus norvegicus). *J Comp Psychol, 105*(4), 326-339.

Richmond, M. A., Yee, B. K., Pouzet, B., Veenman, L., Rawlins, J. N., Feldon, J., et al. (1999). Dissociating context and space within the hippocampus: effects of complete, dorsal, and ventral excitotoxic hippocampal lesions on conditioned freezing and spatial learning. *Behav Neurosci, 113*(6), 1189-1203.

Robinson, E. (1963). Effect of Amygdalectomy on Fear-Motivated Behavior in Rats. *J Comp Physiol Psychol, 56*, 814-820.

Rogan, M. T., Staubli, U. V., & LeDoux, J. E. (1997). Fear conditioning induces associative long-term potentiation in the amygdala. *Nature, 390*(6660), 604-607.

Romanski, L. M., & LeDoux, J. E. (1992a). Bilateral destruction of neocortical and perirhinal projection targets of the acoustic thalamus does not disrupt auditory fear conditioning. *Neurosci Lett, 142*(2), 228-232.

Romanski, L. M., & LeDoux, J. E. (1992b). Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning. *J Neurosci, 12*(11), 4501-4509.

Roozendaal, B., Oldenburger, W. P., Strubbe, J. H., Koolhaas, J. M., & Bohus, B. (1990). The central amygdala is involved in the conditioned but not in the meal-induced cephalic insulin response in the rat. *Neurosci Lett, 116*(1-2), 210-215.

Rosen, J. B., Fanselow, M. S., Young, S. L., Sitcoske, M., & Maren, S. (1998). Immediate-early gene expression in the amygdala following footshock stress and contextual fear conditioning. *Brain Res, 796*(1-2), 132-142.

Rosen, J. B., Hitchcock, J. M., Miserendino, M. J., Falls, W. A., Campeau, S., & Davis, M. (1992). Lesions of the perirhinal cortex but not of the frontal, medial prefrontal, visual, or insular cortex block fear-potentiated startle using a visual conditioned stimulus. *J Neurosci, 12*(12), 4624-4633.

Rudy, J. W. (1993). Contextual conditioning and auditory cue conditioning dissociate during development. *Behav Neurosci, 107*(5), 887-891.

Rudy, J. W., Barrientos, R. M., & O'Reilly, R. C. (2002). Hippocampal formation supports conditioning to memory of a context. *Behav Neurosci, 116*(4), 530-538.

Rudy, J. W., & O'Reilly, R. C. (1999). Contextual fear conditioning, conjunctive representations, pattern completion, and the hippocampus. *Behav Neurosci, 113*(5), 867-880.

Rudy, J. W., & O'Reilly, R. C. (2001). Conjunctive representations, the hippocampus, and contextual fear conditioning. *Cogn Affect Behav Neurosci, 1*(1), 66-82.

Sacchetti, B., Lorenzini, C. A., Baldi, E., Tassoni, G., & Bucherelli, C. (1999). Auditory thalamus, dorsal hippocampus, basolateral amygdala, and perirhinal cortex role in the consolidation of conditioned freezing to context and to acoustic conditioned stimulus in the rat. *J Neurosci, 19*(21), 9570-9578.

Sananes, C. B., & Davis, M. (1992). N-methyl-D-aspartate lesions of the lateral and basolateral nuclei of the amygdala block fear-potentiated startle and shock sensitization of startle. *Behav Neurosci, 106*(1), 72-80.

Saper, C. B. (1982). Convergence of autonomic and limbic connections in the insular cortex of the rat. *J Comp Neurol, 210*(2), 163-173.

Saunders, R. C., Murray, E. A., & Mishkin, M. (1984). Further evidence that amygdala and hippocampus contribute equally to recognition memory. *Neuropsychologia, 22*(6), 785-796.

Selden, N. R., Everitt, B. J., Jarrard, L. E., & Robbins, T. W. (1991). Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. *Neuroscience, 42*(2), 335-350.

Shah, A. A., & Treit, D. (2004). Infusions of midazolam into the medial prefrontal cortex produce anxiolytic effects in the elevated plus-maze and shock-probe burying tests. *Brain Res, 996*(1), 31-40.

Shi, C., & Davis, M. (2001). Visual pathways involved in fear conditioning measured with fear-potentiated startle: behavioral and anatomic studies. *J Neurosci, 21*(24), 9844-9855.

Simon, H., Le Moal, M., & Calas, A. (1979). Efferents and afferents of the ventral tegmental-A10 region studied after local injection of [3H]leucine and horseradish peroxidase. *Brain Res, 178*(1), 17-40.

Solomon, R. L., & Wynne, L. C. (1954). Traumatic avoidance learning: the principles of anxiety conservation and partial irreversibility. *Psychol Rev, 61*(6), 353-385.

Squire, L. R. (1987). *Memory and brain.* New York: Oxford University Press.

Squire, L. R., & Alvarez, P. (1995). Retrograde amnesia and memory consolidation: a neurobiological perspective. *Curr Opin Neurobiol, 5*(2), 169-177.

Squire, L. R., Clark, R. E., & Knowlton, B. J. (2001). Retrograde amnesia. *Hippocampus, 11*(1), 50-55.

Squire, L. R., Cohen, N. J., & Zouzounis, J. A. (1984). Preserved memory in retrograde amnesia: sparing of a recently acquired skill. *Neuropsychologia, 22*(2), 145-152.

Squire, L. R., Stark, C. E., & Clark, R. E. (2004). The medial temporal lobe. *Annu Rev Neurosci, 27*, 279-306.

Squire, L. R., & Zola-Morgan, S. (1988). Memory: brain systems and behavior. *Trends Neurosci, 11*(4), 170-175.

Squire, L. R., Zola-Morgan, S., & Chen, K. S. (1988). Human amnesia and animal models of amnesia: performance of amnesic patients on tests designed for the monkey. *Behav Neurosci, 102*(2), 210-221.

Starr, M. D., & Mineka, S. (1977). Determinants of fear over the course of avoidance learning. *Learn Motiv, 8*, 332-350.

Stefanacci, L., Suzuki, W. A., & Amaral, D. G. (1996). Organization of connections between the amygdaloid complex and the perirhinal and parahippocampal cortices in macaque monkeys. *J Comp Neurol, 375*(4), 552-582.

Steward, O. (1976). Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat. *J Comp Neurol, 167*(3), 285-314.

Steward, O., & Scoville, S. A. (1976). Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. *J Comp Neurol, 169*(3), 347-370.

Sutherland, R. J., & McDonald, R. J. (1990). Hippocampus, amygdala, and memory deficits in rats. *Behav Brain Res, 37*(1), 57-79.

Sutherland, R. J., McDonald, R. J., Hill, C. R., & Rudy, J. W. (1989). Damage to the hippocampal formation in rats selectively impairs the ability to learn cue relationships. *Behav Neural Biol, 52*(3), 331-356.

Suzuki, W. A., & Amaral, D. G. (2003). Where are the perirhinal and parahippocampal cortices? A historical overview of the nomenclature and boundaries applied to the primate medial temporal lobe. *Neuroscience, 120*(4), 893-906.

Suzuki, W. A., & Eichenbaum, H. (2000). The neurophysiology of memory. *Ann N Y Acad Sci, 911*, 175-191.

Swanson, L. W. (2003). The amygdala and its place in the cerebral hemisphere. *Ann N Y Acad Sci, 985*, 174-184.

Swenson, R. M., & Vogel, W. H. (1983). Plasma Catecholamine and corticosterone as well as brain catecholamine changes during coping in rats exposed to stressful footshock. *Pharmacol Biochem Behav, 18*(5), 689-693.

Tassoni, G., Lorenzini, C. A., Baldi, E., Sacchetti, B., & Bucherelli, C. (1999). A peculiar pattern of temporal involvement of rat perirhinal cortex in memory processing. *Behav Neurosci, 113*(6), 1161-1169.

Terlecki, L. J., Pinel, J. P., & Treit, D. (1979). Conditioned and unconditioned defensive burying in the rat. *Learn Motiv, 10*, 337-350.

Thompson, C. I. (1981). Learning in rhesus monkeys after amygdalectomy in infancy or adulthood. *Behav Brain Res, 2*(1), 81-101.

Thornton, J. A., Rothblat, L. A., & Murray, E. A. (1997). Rhinal cortex removal produces amnesia for preoperatively learned discrimination problems but fails to disrupt postoperative acquisition and retention in rhesus monkeys. *J Neurosci, 17*(21), 8536-8549.

Treit, D. (1985). The inhibitory effect of diazepam on defensive burying: anxiolytic vs. analgesic effects. *Pharmacol Biochem Behav, 22*(1), 47-52.

Treit, D. (1990). A comparison of anxiolytic and nonanxiolytic agents in the shock-probe/burying test for anxiolytics. *Pharmacol Biochem Behav, 36*(1), 203-205.

Treit, D., & Fundytus, M. (1988). A comparison of buspirone and chlordiazepoxide in the shock-probe/burying test for anxiolytics. *Pharmacol Biochem Behav, 30*(4), 1071-1075.

Treit, D., & Menard, J. (1997). Dissociations among the anxiolytic effects of septal, hippocampal, and amygdaloid lesions. *Behav Neurosci, 111*(3), 653-658.

Treit, D., Menard, J., & Royan, C. (1993). Anxiogenic stimuli in the elevated plus-maze. *Pharmacol Biochem Behav, 44*(2), 463-469.

Treit, D., Pesold, C., & Rotzinger, S. (1993). Dissociating the anti-fear effects of septal and amygdaloid lesions using two pharmacologically validated models of rat anxiety. *Behav Neurosci, 107*(5), 770-785.

Treit, D., Pinel, J. P., & Fibiger, H. C. (1981). Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. *Pharmacol Biochem Behav, 15*(4), 619-626.

Tsuda, A., Ida, Y., & Tanaka, M. (1988). The contrasting eefects of diazepam and yohimbine on conditioned defensive burying in rats. *Psychobiology, 16*(3), 213-217.

Vanderwolf, C. H., Kelly, M. E., Kraemer, P., & Streather, A. (1988). Are emotion and motivation localized in the limbic system and nucleus accumbens? *Behav Brain Res, 27*(1), 45-58.

Vazdarjanova, A., Cahill, L., & McGaugh, J. L. (2001). Disrupting basolateral amygdala function impairs unconditioned freezing and avoidance in rats. *Eur J Neurosci, 14*(4), 709-718.

Vazdarjanova, A., & McGaugh, J. L. (1998). Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. *Proc Natl Acad Sci U S A, 95*(25), 15003-15007.

Veening, J. G., Swanson, L. W., & Sawchenko, P. E. (1984). The organization of projections from the central nucleus of the amygdala to brainstem sites involved in central autonomic regulation: a combined retrograde transport-immunohistochemical study. *Brain Res, 303*(2), 337-357.

Vnek, N., & Rothblat, L. A. (1996). The hippocampus and long-term object memory in the rat. *J Neurosci, 16*(8), 2780-2787.

von Bohlen und Halbach, O., & Albrecht, D. (2002). Reciprocal connections of the hippocampal area CA1, the lateral nucleus of the amygdala and cortical areas in a combined horizontal slice preparation. *Neurosci Res, 44*(1), 91-100.

Walker, D. L., Cassella, J. V., Lee, Y., De Lima, T. C., & Davis, M. (1997). Opposing roles of the amygdala and dorsolateral periaqueductal gray in fear-potentiated startle. *Neurosci Biobehav Rev, 21*(6), 743-753.

Wan, H., Aggleton, J. P., & Brown, M. W. (1999). Different contributions of the hippocampus and perirhinal cortex to recognition memory. *J Neurosci, 19*(3), 1142-1148.

Warrington, E. K., & Weiskrantz, L. (1968). New method of testing long-term retention with special reference to amnesic patients. *Nature, 217*(132), 972-974.

Watkins, L. R., Wiertelak, E. P., & Maier, S. F. (1993). The amygdala is necessary for the expression of conditioned but not unconditioned analgesia. *Behav Neurosci, 107*(2), 402-405.

Weiskrantz, L. (1956). Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *J Comp Physiol Psychol, 49*, 381-391.

Weller, K. L., & Smith, D. A. (1982). Afferent connections to the bed nucleus of the stria terminalis. *Brain Res, 232*(2), 255-270.

White, N. M., & McDonald, R. J. (2002). Multiple parallel memory systems in the brain of the rat. *Neurobiol Learn Mem, 77*(2), 125-184.

Wiig, K. A., & Bilkey, D. K. (1994). The effects of perirhinal cortical lesions on spatial reference memory in the rat. *Behav Brain Res, 63*(1), 101-109.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (1999). Functional inactivation of the amygdala before but not after auditory fear conditioning prevents memory formation. *J Neurosci, 19*(24), RC48.

Witter, M. P., & Amaral, D. G. (1991). Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. *J Comp Neurol, 307*(3), 437-459.

Wyss, J. M. (1981). An autoradiographic study of the efferent connections of the entorhinal cortex in the rat. *J Comp Neurol, 199*(4), 495-512.

Zola, S. M., Squire, L. R., Teng, E., Stefanacci, L., Buffalo, E. A., & Clark, R. E. (2000). Impaired recognition memory in monkeys after damage limited to the hippocampal region. *J Neurosci, 20*(1), 451-463.

Zola-Morgan, S., & Squire, L. R. (1985). Medial temporal lesions in monkeys impair memory on a variety of tasks sensitive to human amnesia. *Behav Neurosci, 99*(1), 22-34.

Zola-Morgan, S., Squire, L. R., & Amaral, D. G. (1989a). Lesions of the amygdala that spare adjacent cortical regions do not impair memory or exacerbate the impairment following lesions of the hippocampal formation. *J Neurosci, 9*(6), 1922-1936.

Zola-Morgan, S., Squire, L. R., & Amaral, D. G. (1989b). Lesions of the hippocampal formation but not lesions of the fornix or the mammillary nuclei produce long-lasting memory impairment in monkeys. *J Neurosci, 9*(3), 898-913.

Zola-Morgan, S., Squire, L. R., Amaral, D. G., & Suzuki, W. A. (1989). Lesions of perirhinal and parahippocampal cortex that spare the amygdala and hippocampal formation produce severe memory impairment. *J Neurosci, 9*(12), 4355-4370.

Zola-Morgan, S., Squire, L. R., & Mishkin, M. (1982). The neuroanatomy of amnesia: amygdala-hippocampus versus temporal stem. *Science, 218*(4579), 1337-1339.

APPENDIX A

Immobility Results

1.1 AIM OF APPENDIX A

The purpose of this appendix is to summarize the results and findings of the immobility data from each experiment. Immobility was used as an inverse measure of activity on the retention test rather than a measure of memory. Nonetheless, immobility may be of importance for interpreting the results of the behaviours that were used as indices of memory and fear. Increased or decreased activity levels resulting from the lesions could potentially influence the performance of the rats during the retention test, and in particular, the avoidance results. Thus, it was essential to measure immobility, analyze the data, and determine whether it was a influential factor for performance on the retention test.

Similar to the data for the other overt behaviours, the immobility data for all experiments were analyzed with non-parametric tests because homogeneity of variance or normality of the distribution were usually violated. However, given that there were no specific predictions about the effects of the lesions on immobility, an omnibus test was conducted using a Kruskal-Wallis test to analyze the data. If a significant difference was found, then it was followed by appropriate post-hoc pair-wise comparisons using two-tailed Mann-Whitney U tests.

1.2 IMMOBILITY RESULTS FROM EXPERIMENT 1

Experiment 1 examined the effects of pretraining HPC lesions in the shock-probe fear-conditioning test. Figure A-1 shows the amount of time the rats spent immobile during the retention test. No significant difference among the groups was found ($X^2_{(4)} = 5.455, p = .244$), suggesting that immobility did not influence the results in Experiment 1.

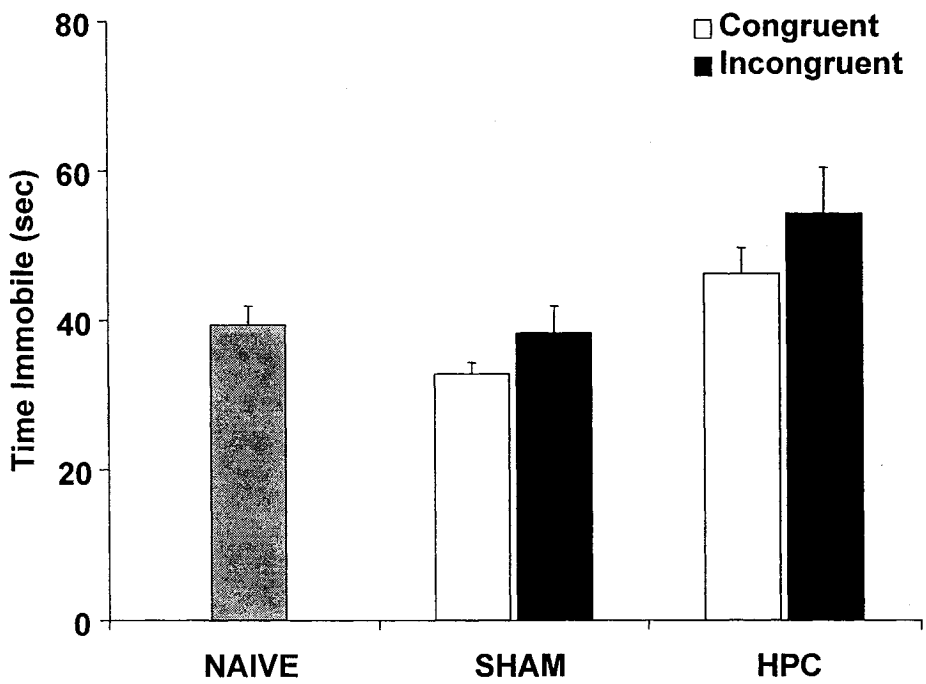

Figure A-1. Mean (± *SEM*) amount of time spent immobile during the retention tests by NAÏVE, SHAM, and HPC rats.

1.3 IMMOBILITY RESULTS FROM EXPERIMENT 2

Experiment 2 examined the effects of post-training HPC lesions in the shock-probe fear-conditioning test. Figure A-2 shows the amount of time the rats spent immobile during the retention test. A significant difference among the groups was found ($X^2_{(4)} = 18.55, p < .001$). Pair-wise comparisons indicated that the SHAM-REMOTE and HPC-REMOTE rats spent more time immobile than the NAÏVE rats (all $ps < 0.05$), suggesting that the two groups were less active than the NAÏVE rats. This difference, however, cannot account for the HPC-REMOTE rats' avoidance and burying impairments during the retention test, since it would be expected that increased, and not decreased, activity levels would likely lead to impairments.

In contrast, the HPC-RECENT rats spent significantly less time immobile than the NAÏVE, SHAM-RECENT, and HPC-REMOTE rats (all $ps < 0.05$). These latter results suggest that the HPC-RECENT rats were more active than the other three groups and that it may be a factor accounting for part of the avoidance and/or burying deficits on the retention test. However, the HPC-RECENT rats spent less time in the probe area than the NAÏVE rats, suggesting that despite greater activity levels they were still able to direct their behaviour. Thus, it is unlikely that increased activity is at the basis of the impairments on the other behavioural measures of the retention test. More importantly, the HPC-REMOTE rats, similarly to the HPC-RECENT rats, had impaired burying and avoidance on the test, even though they were not more active. These latter findings, more convincingly, suggest that the decreased immobility of the HPC-RECENT rats did not affect avoidance and burying.

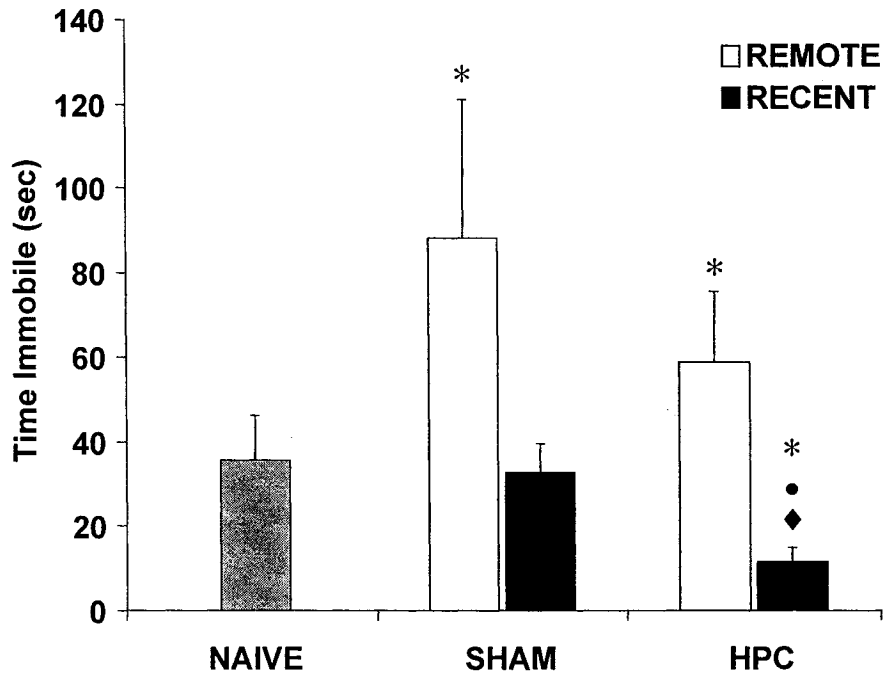

Figure A-2. Mean (± *SEM*) amount of time spent immobile during the retention tests by NAÏVE, SHAM, and HPC rats in the REMOTE and RECENT conditions (*$p < .05$ versus NAIVE; •$p < .05$ versus SHAM-RECENT; ♦$p < .05$ versus HPC-REMOTE).

1.4 IMMOBILITY RESULTS FROM EXPERIMENT 3

Experiment 3 examined the effects of pretraining aspiration lesions of the PRH and PORH in the shock-probe fear-conditioning test. Figure A-3 shows the amount of time the rats spent immobile during the retention test. A significant difference among the groups was found ($X^2_{(3)} = 12.035, p < .01$). Specifically, pair-wise comparisons indicated that the SHAM and PRH rats spent more time immobile than the NAÏVE rats (all *ps* < 0.05). Although these findings suggest that the SHAM and PRH rats were less active than the NAÏVE rats, they do not importantly contribute to the mnemonic findings in this experiment because immobility between the PRH and SHAM rats did not significantly differ.

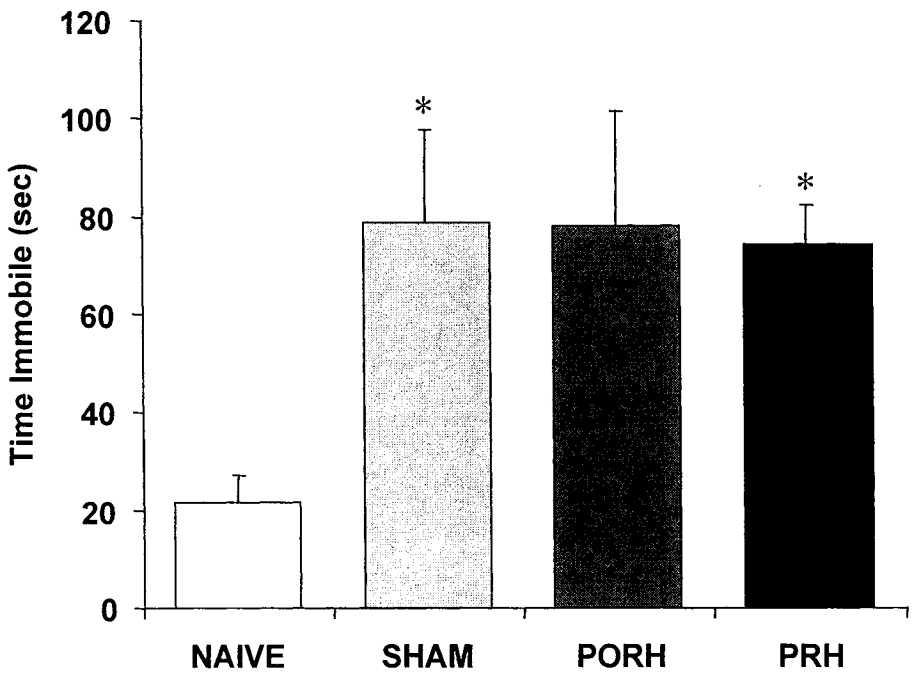

Figure A-3. Mean (± *SEM*) amount of time spent immobile during the retention tests by NAÏVE, SHAM, PORH, and PRH rats (*$p < .05$ versus NAÏVE).

1.5 IMMOBILITY RESULTS FROM EXPERIMENT 4

Experiment 4 examined the effects of PRH aspiration lesions on burying in the small shock-probe chamber. Figure A-4 shows the amount of time the rats spent immobile during the retention test. No significant difference between the groups was found ($U = 18.5, p = .721$), suggesting that immobility did not influence the results in Experiment 4.

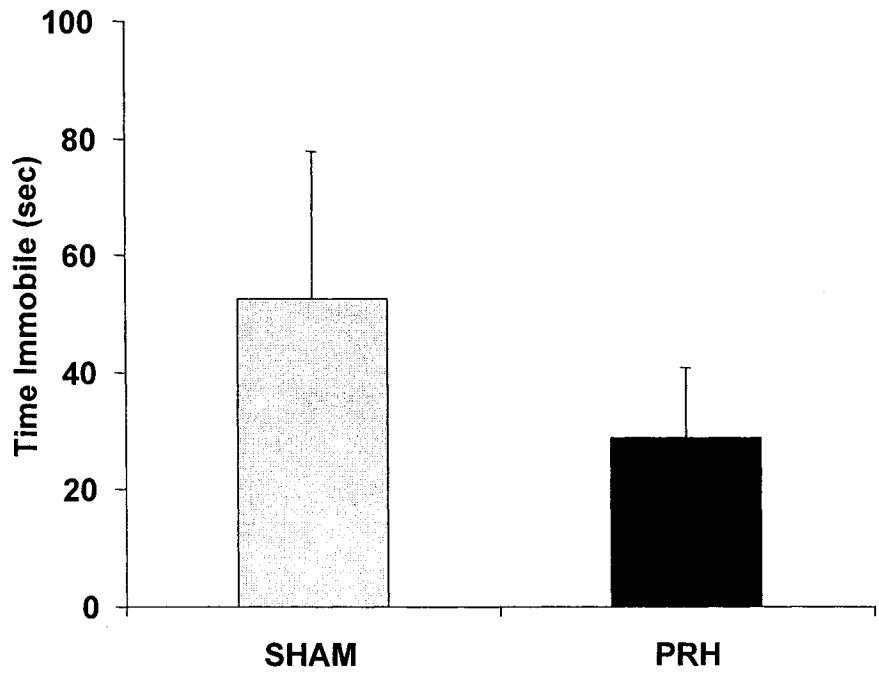

Figure A-4. Mean (± *SEM*) amount of time spent immobile during the retention tests by SHAM and PRH rats.

1.6 IMMOBILITY RESULTS FROM EXPERIMENT 5

Experiment 5 examined the effects of pre- and post-training neurotoxic lesions of the PRH in the shock-probe fear-conditioning test. Figure A-5 shows the amount of time the rats spent immobile during the retention test. No significant difference among the groups was found ($X^2_{(4)} = 0.641, p = .958$), suggesting that immobility did not influence the results in Experiment 5.

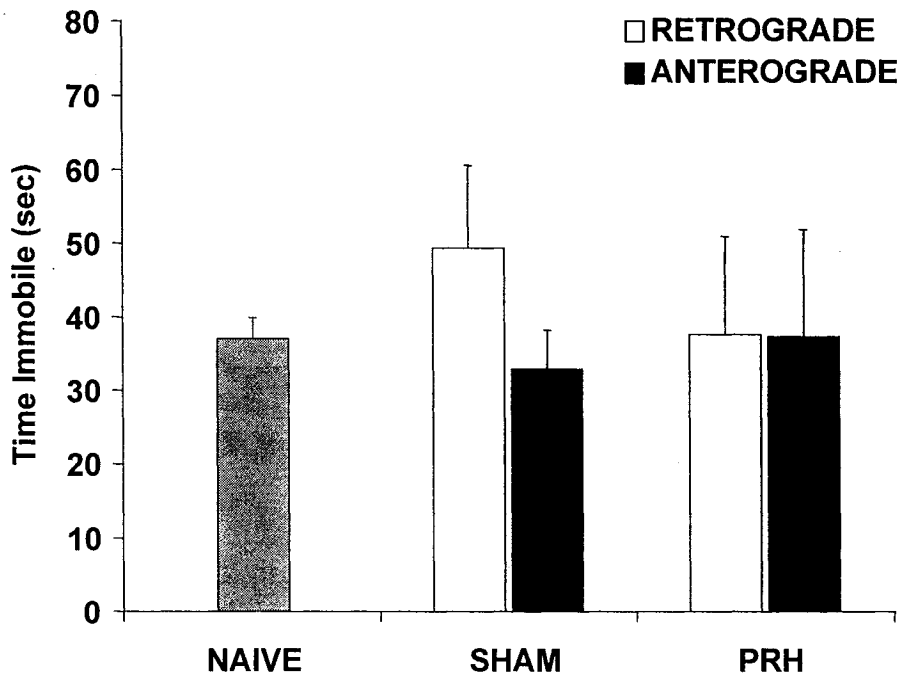

Figure A-5. Mean (± *SEM*) amount of time spent immobile during the retention tests by NAÏVE, SHAM, and PRH rats in the RETROGRADE and ANTEROGRADE conditions.

1.7 IMMOBILITY RESULTS FROM EXPERIMENT 6

Experiment 6 examined the effects of pretraining AMY lesions in the shock-probe fear-conditioning test. Figure A-6 shows the amount of time the rats spent immobile during the retention test. No significant difference among the groups was found ($X^2_{(2)} = 1.89, p = .389$), suggesting that immobility did not influence the results in Experiment 6.

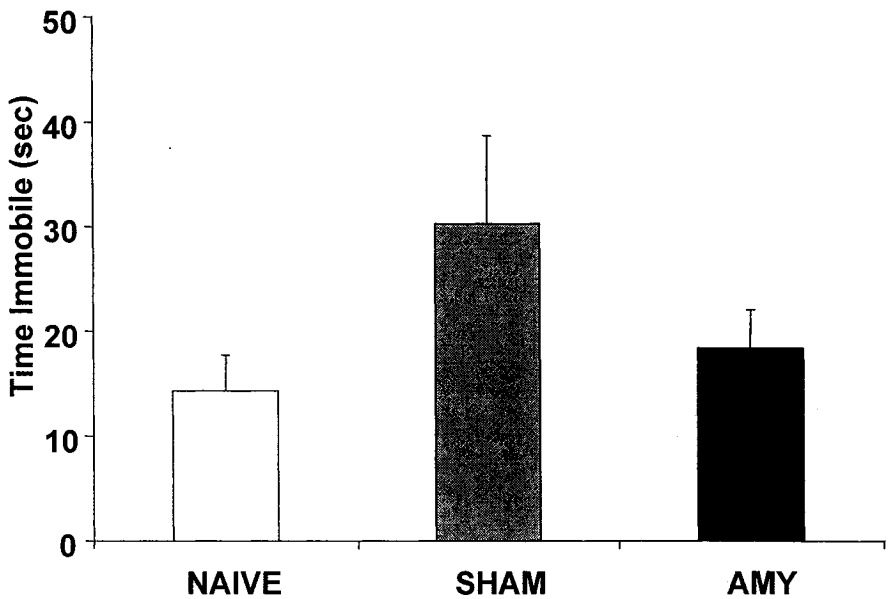

Figure A-6. Mean (± *SEM*) amount of time spent immobile during the retention tests by NAÏVE, SHAM, and AMY rats.

1.8 IMMOBILITY RESULTS FROM EXPERIMENT 7

Experiment 7 examined the effects of pretraining AMY lesions on ACTH levels in the shock-probe-fear-conditioning test. Figure A-7 shows the amount of time the rats spent immobile during the retention test. No significant difference among the groups was found ($X^2_{(2)} = 1.89, p = .389$), suggesting that immobility did not influence the results in Experiment 7.

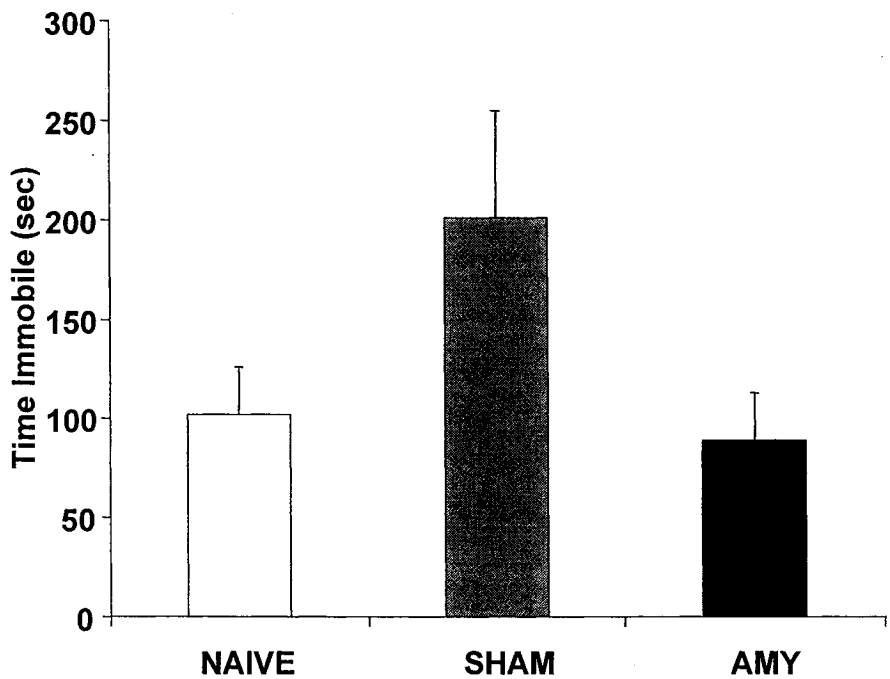

Figure A-7. Mean (± *SEM*) amount of time spent immobile during the retention tests by NAÏVE, SHAM, and AMY rats.

1.9 IMMOBILITY RESULTS FROM EXPERIMENT 8

Experiment 8 examined the effects of post-training AMY lesions in the shock-probe fear-conditioning test. Figure A-8 shows the amount of time the rats spent immobile during the retention test. A significant difference among the groups was found ($X^2_{(4)} = 25.934, p < .001$). Pair-wise comparisons indicated that the SHAM-REMOTE and -RECENT rats spent more time immobile than the NAÏVE rats (all $ps < 0.05$). More importantly, the comparisons revealed that the AMY-RECENT rats spent less time immobile than the NAÏVE, SHAM-RECENT, and AMY-REMOTE rats (all $ps < 0.05$). These latter results suggest that the AMY-RECENT rats were more active than the other three groups and possibly contributed to the avoidance and/or burying deficits on the retention test.

However, the AMY-RECENT rats spent less time in the probe area than the NAÏVE rats, which suggests that despite greater activity levels they were still able to direct their behaviour during the test. Thus, it is unlikely that decreased immobility greatly affected the other behaviours during the test. More notably, the AMY-REMOTE and -RECENT rats were similarly impaired on burying and avoidance during the retention test, even though the AMY-REMOTE rats did not have differences in immobility. Thus, it is highly improbable that the memory impairments in the AMY-RECENT rats were due to changes in activity levels.

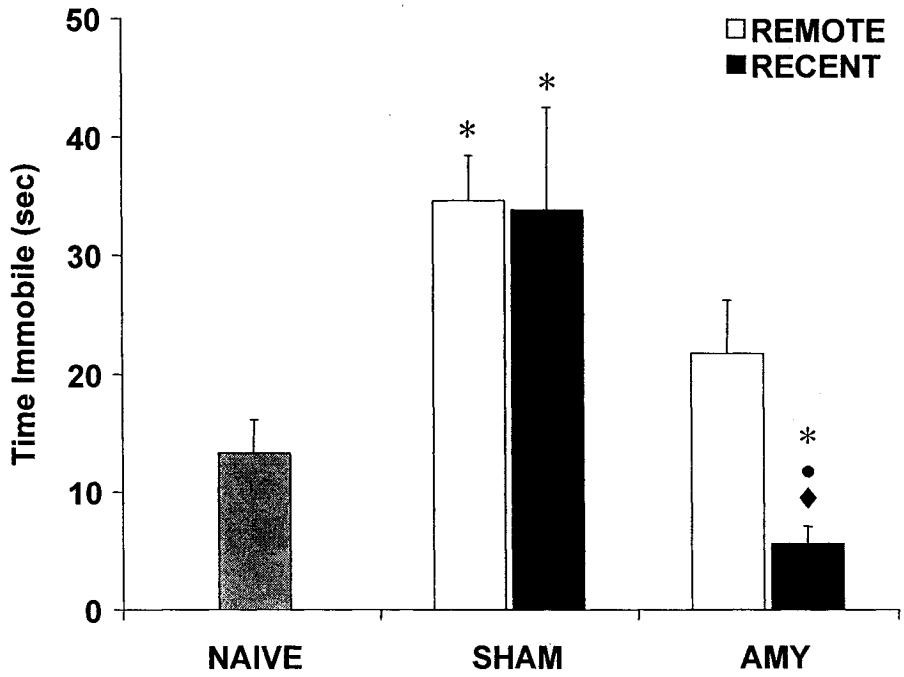

Figure A-8. Mean (± *SEM*) amount of time spent immobile during the retention tests by NAÏVE, SHAM, and AMY rats in the REMOTE and RECENT conditions (*$p < .05$ versus NAIVE; •$p < .05$ versus SHAM-RECENT; ♦$p < .05$ versus AMY-REMOTE).

APPENDIX B

Behavioural Data and Analyses for the NAIVE Rats

2.1 AIM OF APPENDIX B

The purpose of this appendix is to demonstrate that the pooling of the NAÏVE control groups in each experiment was warranted because the rats' scores on the acquisition session and retention test were not significantly different, with the exception of two instance that are discussed below. The following tables report the raw data for each rat in each NAÏVE group per experiment because in some experiments there were few rats in each NAÏVE group. The tables also describe the results of the statistical analyses conducted on the data. A Kruskal-Wallis test was conducted on the data for the experiments that include three or more NAÏVE groups, whereas Mann-Whitney U tests were conducted on the data for the experiments that include only two NAÏVE groups.

In both Experiments 4 and 6, a significant difference was found among the various NAÏVE groups for the latency to contact the probe during the retention test (see Table B-4 and B-6). Although, these significant differences undermine the pooling of the NAÏVE control groups, they did not affect the statistical outcome of the latency comparisons that were reported in these two experiments. Accordingly, the NAÏVE control groups in both Experiments 4 and 6 were still pooled.

TABLE B-1. Performance of the NAIVE rats in Experiment 1, which examined the effects of pretraining HPC lesions

		Behavioural Data				
		Acquisition	Retention			
NAÏVE Group	Rat	Burying (sec)	Burying (sec)	Latency (sec)	Time in Probe Area (sec)	Time Immobile (sec)
SHAM-CONGRUENT						
	1	0	0	2	301	4
	2	2	0	4	566	2
	3	0	0	5	299	41
	4	0	3	83	537	29
	5	1	0	4	413	3
	6	0	0	7	402	6
	7	0	0	4	415	32
	8	30	5	6	265	66
	9	0	0	6	278	48
	10	0	0	4	795	21
	11	0	1	10	188	70
	12	0	0	154	140	41
SHAM-INCONGRUENT						
	1	11	2	1	323	14
	2	0	4	6	312	19
	3	0	0	2	320	4
	4	1	2	15	435	11
	5	0	0	5	431	8
	6	0	0	8	535	25
	7	0	0	5	416	51
	8	0	1	15	431	15
	9	0	0	44	410	45
HPC-CONGRUENT						
	1	0	0	107	290	16
	2	0	0	13	278	22
	3	0	0	2	428	22
	4	0	0	56	492	168
	5	0	8	9	55	184
	6	8	1	13	39	9
	7	0	0	14	127	148
	8	0	0	13	365	98
	9	0	0	2	149	80
HPC-INCONGRUENT						
	1	0	44	58	268	0
	2	0	0	33	358	34
	3	0	0	5	461	2
	4	0	0	13	466	30
	5	0	0	7	396	28
	6	0	0	17	438	21
Kruskal-Wallis Test		2.075	1.167	3.758	5.807	5.654
Df		3	3	3	3	3
***p* value**		.557	.761	.289	.121	.130

TABLE B-2. Performance of the NAIVE rats in Experiment 2, which examined the effects of post-training HPC lesions

		Behavioural Data				
		Acquisition	Retention			
NAÏVE Group	Rat	Burying (sec)	Burying (sec)	Latency (sec)	Time in Probe Area (sec)	Time Immobile (sec)
SHAM-REMOTE						
	1	0	0	17	429	28
	2	2	0	19	343	18
SHAM-RECENT						
	1	0	0	8	368	21
	2	76	2	3	354	64
	3	1	0	3	369	9
	4	0	0	13	355	22
	5	0	0	4	191	14
HPC-REMOTE						
	1	0	0	4	197	124
HPC-RECENT						
	1	0	0	24	225	18
	2	28	0	9	171	87
	3	22	0	9	352	9
	4	0	0	3	253	15
Kruskal-Wallis Test		.818	1.400	4.278	4.327	2.880
Df		3	3	3	3	3
***p* value**		.845	.706	.233	.228	.411

TABLE B-3. Performance of the NAIVE rats in Experiment 3, which examined the effects of pretraining aspiration lesions of the PRH and PORH

		Behavioural Data				
		Acquisition	Retention			
NAÏVE Group	Rat	Burying (sec)	Burying (sec)	Latency (sec)	Time in Probe Area (sec)	Time Immobile (sec)
SHAM						
	1	7	13	14	426	11
	2	0	0	2	373	1
	3	0	0	6	376	61
	4	0	0	5	440	39
	5	0	0	2	657	37
PORH						
	1	0	2	2	438	8
PRH						
	1	0	0	5	669	34
	2	0	0	3	228	6
	3	0	0	4	446	7
	4	0	0	16	585	19
	5	0	0	6	442	14
Kruskal-Wallis Test		1.200	4.240	1.981	1.200	1.309
Df		2	2	2	2	2
***p* value**		.549	.120	.371	.549	.520

TABLE B-4. Performance of the NAIVE rats in Experiment 5, which examined the effects of pre- and post-training neurotoxic lesions of the PRH

		Behavioural Data				
		Acquisition	Retention			
NAÏVE Group	Rat	Burying (sec)	Burying (sec)	Latency (sec)	Time in Probe Area (sec)	Time Immobile (sec)
SHAM-RETROGRADE						
	1	0	0	7	303	15
	2	12	0	8	444	65
	3	10	5	4	475	48
	4	0	7	4	326	12
	5	0	0	5	425	28
SHAM-ANTEROGRADE						
	1	0	0	15	354	86
	2	0	0	47	282	11
PRH-RETROGRADE						
	1	0	0	8	522	36
	2	0	0	7	371	26
	3	1	38	13	417	25
	4	3	3	34	521	7
	5	4	0	19	393	19
PRH-ANTEROGRADE						
	1	0	0	5	239	103
Kruskal-Wallis Test		2.091	1.534	8.053	5.637	3.033
Df		3	3	3	3	3
***p* value**		.554	.674	.045*	.131	.387

* Significant difference

TABLE B-5. Performance of the NAIVE rats in Experiment 6, which examined the effects of pretraining AMY lesions

		Behavioural Data				
		Acquisition	Retention			
NAÏVE Group	Rat	Burying (sec)	Burying (sec)	Latency (sec)	Time in Probe Area (sec)	Time Immobile (sec)
SHAM						
	1	8	0	7	317	22
	2	0	0	10	347	26
	3	1	0	3	509	8
AMY						
	1	0	0	3	413	15
	2	3	0	77	339	5
	3	3	0	13	378	10
Mann-Whitney U Test		4.5	4.5	2.5	4.0	2.0
***p* value**		1.0	1.0	.400	1.0	.400

TABLE B-6. Performance of the NAIVE rats in Experiment 7, which examined the effects of pretraining AMY lesions on ACTH

		Behavioural Data						
		Acquisition	Retention					
NAÏVE Group	Rat	Burying (sec)	Burying (sec)	Latency (sec)	Time in Probe Area (sec)	Time Immobile (sec)	Δ ACTH MINUTE 5 (%)	Δ ACTH MINUTE 15 (%)
SHAM								
	1	1	1	237	347	203		-10.625
	2	0	1	16	188	146	37.723	16.552
	3	0	0	10	331	50	53.703	53.870
AMY								
	1	0	0	6	338	123	49.998	7.954
	2	0	0	3	313	67	21.599	-29.497
	3	1	0	6	304	109	-7.225	-12.314
	4	0	0	2	336	14	51.038	6.581
Mann-Whitney U Test		5.5	2.0	0.0	6.0	3.0	2.0	2.0
***p* value**		.823	.074	.032*	1.0	.289	.355	.157

* Significant difference

TABLE B-7. Performance of the NAIVE rats in Experiment 8, which examined the effects of post-training AMY lesions

		Behavioural Data				
		Acquisition	Retention			
NAÏVE Group	Rat	Burying (sec)	Burying (sec)	Latency (sec)	Time in Probe Area (sec)	Time Immobile (sec)
SHAM-REMOTE						
	1	0	0	5	398	16
SHAM-RECENT						
	1	15	0	3	327	10
	2	7	0	25	516	15
	3	0	0	54	422	39
	4	0	0	23	415	13
	5	9	0	16	411	12
AMY-REMOTE						
	1	0	0	24	372	2
AMY-RECENT						
	1	12	5	2	513	12
	2	0	0	14	602	10
	3	1	0	36	395	3
	4	3	0	7	421	14
Kruskal-Wallis Test		2.220	1.750	2.0	2.636	4.995
Df		3	3	3	3	3
***p* value**		.528	.626	.572	.451	.172