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**Perirhinal or Postrhinal Cortex Lesions:
Effects on Spatial versus Fear Learning, and on Kindling**

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**A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy**

**Department of Psychology
Carleton University
Ottawa, Ontario
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@February 2001

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Yves Bureau

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Abstract

This thesis investigates the influence of perirhinal and postrhinal cortex aspiration lesions on learning/memory, emotionality and amygdala/hippocampal kindled epileptogenesis. Learning and memory were explored using the Morris Water Maze (MWM) and inhibitory avoidance, while emotionality was assessed in the elevated plus maze (EPM) and startle in an open activity field. In the MWM, postrhinal cortex lesioned rats needed more time to find a submerged platform on later acquisition sessions than control rats. The nature of this effect suggested a working memory deficit only, and was not due to general visual or motor impairments as shown by their normal performance in two raised platform (Cued) sessions. Performance in the inhibitory avoidance and startle in an open field tasks were unremarkable between groups. In the EPM, postrhinal cortex lesioned rats spent more time in the enclosed arms compared to control rats, the implications of which are discussed.

In the epileptogenesis studies, kindling was mildly retarded in perirhinal lesion rats ($p=0.06$) during primary amygdala kindling but was significantly retarded during transfer hippocampal kindling in the same rats. Using the reverse kindling order in different perirhinal lesioned rats, this was not true. However, lesions in the reverse order group were slightly posterior compared to the former kindling group. There were no pronounced effects of postrhinal lesions on kindling of either the amygdala or hippocampus. In spite of the retarded kindling, the perirhinal lesioned rats exhibited convulsions that were quite normal. Further, perirhinal cortex lesioned rats sacrificed two hours after a triggered convulsion from the amygdala or the

hippocampus showed strong c-Fos immunohistochemistry throughout the parahippocampal cortices, indicating good, normal recruitment in association with a convulsive episode. Indeed, regardless of treatment group, convulsions triggered from either the amygdala or the hippocampus resulted in extensive c-Fos labeling in the parahippocampal and insular cortices, both of which project strongly to the frontal motor cortices and are suspected to drive the convulsive response. It is our position that the perirhinal cortex is one of the important neural conduits carrying information and seizures from the amygdala and hippocampus to the frontal motor networks but is not solely responsible for convulsive expression during kindling epileptogenesis.

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Table of Contents

Acceptance Form.....	i
Abstract.....	ii-iii
Acknowledgements.....	iv
Table of Contents.....	v-vii
List of Tables.....	viii
List of Figures.....	ix-xii
Introduction	
I. Prologue.....	1
II. Anatomy of the Perirhinal and Postrhinal Cortices.....	1
III. Intrinsic and Extrinsic Connections of the Perirhinal and Postrhinal Cortex.....	3
IV. Extrinsic Connections of the Perirhinal and Postrhinal Cortices.....	5
V. Learning and Memory.....	6
VI. Epilepsy.....	10
VII. Animal Models of Epilepsy.....	16
VIII. Neuroanatomical Substrates of Kindling.....	19
IX. C-Fos Protein and Neuronal Pathway Activation Mapping.....	22
General Methods	
I. Aspiration Lesions: Surgery.....	27
II. Behavioral Tasks.....	28
Experiment One: Morris Water-Maze.....	29
I. Introduction.....	29
II. Methods.....	29
III. Summary of Morris Water-Maze Protocol.....	31
IV. Hypotheses.....	31
V. Results	32
Perirhinal or Postrhinal Lesioned vs. Control Groups.....	45
Probe Trials.....	57
Flexibility Learning.....	57
VI. Discussion.....	65

Experiment Two: Inhibitory Avoidance.....	71
I. Introduction.....	71
II. Hypotheses.....	71
III. Methods.....	72
IV. Results.....	73
V. Discussion.....	84
VI. Conclusions.....	85
 Experiment Three: Elevated Plus Maze.....	 86
I. Introduction.....	86
II. Hypotheses.....	87
III. Methods.....	87
IV. Results.....	88
V. Discussion and Conclusions.....	94
 Experiment Four: Open-Field and Startle by Sound.....	 96
I. Introduction.....	96
II. Methods.....	96
III. Hypotheses.....	97
IV. Results.....	97
V. Discussion.....	107
 Experiment Five: Part One: Kindling.....	 108
I. Introduction.....	108
II. Method: Stimulating Electrode Implantation.....	109
III. Summary of Dependent Measures for Kindling.....	111
IV. Results.....	111
Electrode Placements.....	111
Analysis.....	118
Afterdischarge Thresholds (ADTs).....	118
Afterdischarge Durations (ADDs).....	118
Kindling Rate.....	121
Latency to Convulsion.....	125
Duration of Convulsion.....	130
V. Results Summary and Discussion.....	135
Afterdischarge Thresholds (ADTs).....	135
Afterdischarge Durations (ADDs).....	135
Kindling Rate.....	136
Latency to a Convulsion.....	137
Duration of a Convulsion.....	138
VI. Discussion.....	139

Experiment 5: Part II: C-Fos Labeling.....	143
I. Introduction.....	143
II. Hypotheses.....	143
III. Results.....	143
IV. Discussion.....	161
 Appendix I: Histology and Immunohistochemistry.....	 164
References.....	165

List of Tables

Table 1.	F ratios and probabilities for repeated measures analyses of variance on individual trials across place-learning sessions by perirhinal and postrhinal lesioned and control groups.....	54
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List of Figures

Figure 1.	A schematic of the rat brain. EC (entorhinal cortex), rs (rhinal sulcus), Prh (perirhinal cortex), Ins (insular cortex), POR (postrhinal cortex), Te2 (secondary temporal or auditory cortex), Te3 (tertiary temporal cortex), Oc2L (secondary visual cortex), 35 and 36 (areas 35 and 36 of the perirhinal cortex). The parasubiculum is not represented. Adapted from Burwell et al. (1995).....	3
Figure 1.1.	Minimal (A) and maximal (B) perirhinal lesions represented in lateral whole brain perspective, coloured in black.....	34
Figure 1.2.	Minimal (A) and maximal (B) postrhinal lesions represented in lateral whole brain perspective, coloured in black.....	36
Figure 1.3.	Photomicrographs of a coronal section through the perirhinal cortex of a control rat brain (A) and a rat brain with a typical perirhinal cortex lesion (B). The arrows show the dorsal and ventral borders of the perirhinal cortex.....	38
Figure 1.4.	Photomicrographs of a coronal section through the postrhinal cortex of a control rat brain (A) and a rat brain with a typical postrhinal cortex lesion (B). The arrows show the dorsal and ventral borders of the postrhinal cortex.....	40
Figure 1.5.	A coronal section through the perirhinal cortex of a control rat brain (A) stained with Cresyl Violet and using the Timm's method (B). Note the ventral border of the Timm's stained section (highlighted by the arrows), which defined the ventral edge of the perirhinal cortex.....	42
Figure 1.6.	A coronal section through the postrhinal cortex of a control rat brain stained with Cresyl Violet (A) and the Timm's method (B). Note the Timm's section and its darkened border at the ventral edge of the postrhinal cortex.....	44
Figure 1.7.	Latency swim times for all place sessions by perirhinal and postrhinal lesioned and control groups.....	47
Figure 1.8.	Latencies to reach a raised platform (cued learning) before place-learning and after the second probe session.....	50
Figure 1.9.	Morris Water-Maze: Session represented with individual trials. Reference vs. working memory deficits.....	56
Figure 1.10.	Latency times for the last place-learning session and the retention session by perirhinal and postrhinal lesioned and control groups.....	60

Figure 1.11.	Average latency times for all platform location change sessions by perirhinal and postrhinal lesioned and control groups.....	62
Figure 1.12.	Latency times for individual trials of the first session in the first platform location session by perirhinal/postrhinal area lesioned and control groups.....	64
Figure 2.1.	Step-down latencies for all inhibitory avoidance sessions by perirhinal, postrhinal lesioned and control groups.....	77
Figure 2.2.	Freezing by step-down latency ratios in the inhibitory avoidance task by perirhinal, postrhinal lesioned and control groups.....	79
Figure 2.3.	Rears by step-down latencies ratios during the inhibitory avoidance sessions involving perirhinal, postrhinal lesioned, and control groups.....	80
Figure 2.4.	Ratio of number of nose pokes by step-down latencies into the dark chamber during inhibitory avoidance sessions involving perirhinal and postrhinal lesioned, and control groups.....	83
Figure 3.1.	Time spent in the open or enclosed arm in the elevated-plus-maze by perirhinal, postrhinal lesioned and control groups. Note the arms main effect and the arms by groups interaction. The postrhinal group spends more time in the open arms compared to the other groups.....	90
Figure 3.2.	Time in seconds spent in either the open or enclosed arms on day 1 and day 2.....	93
Figure 4.1.	Number of Inside and Outside Grid Square Crossings: The data are presented by groups. Data averaged over epochs.....	100
Figure 4.2.	Number of grid squares crossed before and after the buzzer. The data are presented by groups.....	102
Figure 4.3.	Number of grid squares crossed. Data are presented for in and outside crossings by before and after the sounding of the buzzer.....	104
Figure 4.4.	Number of rears before and after the buzzer. Data presented by groups.....	106
Figure 5.1.	Hippocampal (A) and amygdala (B) electrode placements for the non-lesioned control group.....	113
Figure 5.2.	Hippocampal (A) and amygdala (B) electrode placements for the perirhinal cortex lesioned group.....	115
Figure 5.3.	Hippocampal (A) and amygdala (B) electrode placements for the postrhinal cortex lesioned group.....	117

Figure 5.4.	The current needed to produce an afterdischarge (ADT) in the hippocampus and amygdala at the time of threshold determination.....	120
Figure 5.5.	Amygdala and hippocampal kindling rates when kindled first and at transfer by Groups (Control(Ctrl), perirhinal (PER) and postrhinal (POR)).....	124
Figure 5.6.	Latency to convulsion from stimulating the amygdala on the first and sixth convulsion when stimulated first and at transfer by groups (Control (Ctrl), perirhinal (PER), and postrhinal (POR)).....	127
Figure 5.7.	Latency to convulsion from stimulating the hippocampus on the first and sixth convulsion when stimulated first and at transfer by groups (Control (Ctrl), perirhinal (PER), and postrhinal (POR)).....	129
Figure 5.8.	Duration of first and sixth amygdala stimulated convulsions when kindled first and at transfer by groups (Control (Ctrl), perirhinal (PER), and postrhinal (POR)).....	132
Figure 5.9.	Duration of first and sixth amygdala stimulated convulsions when kindled first and at transfer by groups (Control (Ctrl), perirhinal (PER), and postrhinal (POR)).....	134
Figure 5.10.	Left hippocampus (A) and insular cortex (B) in a non-stimulated rat. Note the absence of c-Fos labeling.....	146
Figure 5.11.	Right frontal (A) and perirhinal (B) cortex of a control rat experiencing a stage-5 convulsion triggered by stimulation from the amygdala. Note the c-Fos labeling in both cortices.....	148
Figure 5.12.	A second control rat with a stage-5 convulsion triggered from the amygdala, where the AD was shorter than 70 seconds. Note the absence of c-Fos labeling in the hippocampus (A), but positive labeling in the frontal (B) and the perirhinal (C) cortices.....	150
Figure 5.13.	A postrhinal lesioned rat stimulated from the left amygdala that had a severely protracted convulsion. Note the abundant bilateral c-Fos labeling of the left (A) and right (C) hippocampus, and the left (B) and right (D) insular cortex	152
Figure 5.14.	A perirhinal lesioned rat that experienced a right amygdala stimulated convulsion. Note the absence of c-Fos labeling in the hippocampus (A), some in the frontal (B), and heavy labeling in the insular (C) cortices. Because of leaking of the anti-fade, the left side was chosen for display. However, the right side displayed the same distribution and intensity of c-Fos.....	154

Figure 5.15.	A control rat stimulated from the right hippocampus, which did not have a convulsion. This rat has little or no labeling in the frontal cortex (A) and perirhinal cortex (C), but abundant labeling in the hippocampus (B).....	156
Figure 5.16.	A schematic of c-Fos labeling in a control rat that had a right amygdala stimulated convulsion. Each dot represented approximately 5 neurons that positively expressed c-Fos.....	158
Figure 5.17.	A schematic of c-Fos labeling in a control rat that had a right hippocampus stimulated convulsion. Each dot represented approximately 5 neurons that positively expressed c-Fos.....	160

Introduction

Prologue

This thesis is divided into several sections to increase reading ease and ensure clarity of conceptual constructs. There are five experiments, and the last experiment is subdivided into two sections. All experiments will be introduced to the reader in the same order that they were presented to the rats. Therefore, the learning experiments are introduced first, followed by the epilepsy experiment. The literature overview will begin with anatomical and connection considerations of the perirhinal and postrhinal cortices, followed by their role in learning/memory and epilepsy.

Anatomy of the Perirhinal and Postrhinal Cortices

The perirhinal and postrhinal cortices are central to this thesis, therefore, the anatomy of those cortices defined by their connections to other areas of the brain will be discussed first. It should be kept in mind that until recently the perirhinal and postrhinal cortices received little attention with respect to their anatomy and connectivity. In spite of this fact, there are some very good recent studies that have contributed to a better understanding of their role in learning and memory, and in epilepsy.

Historically, the perirhinal cortex was subdivided into two basic parts, including a rostral and caudal region. The rostral or anterior region has retained the parent name *perirhinal* cortex, whereas, the posterior region is now called the *postrhinal* cortex (Burwell & Amaral, 1998a; Burwell, Witter, & Amaral, 1995; Shi & Cassell, 1999) (see

Figure 1). The perirhinal cortex is bordered rostrally by the insular cortex, caudally by the postrhinal cortex (discussed below), ventrally by the entorhinal cortex and dorsally by the auditory association cortex. The postrhinal cortex, on the other hand, is bordered by the perirhinal cortex rostrally, retrosplenial cortex medially, visual association cortex dorsally, and by the entorhinal cortex ventrally for the anterior half but by the parasubiculum in the caudal half. The perirhinal cortex is invaginated throughout its length by the rhinal sulcus, whereas the postrhinal cortex is not (Burwell et al., 1995).

Burwell et al. (1995) determined that the areas 35 and 36 of the perirhinal cortex in the rat are the homologue of Brodmann's area 35 and 36 in the primate brain. They also reasoned that in the latter, the postrhinal cortex is the homologue to the parahippocampal area designated TF and TH in the monkey. They also suggested that the perirhinal and postrhinal cortices would subserve visual functions more in the monkey compared to the rat, whereas in the rat, these cortices would subserve more olfactory functions.

Figure 1.: Rhinal Cortices of the Rat

Figure 1. A schematic of the rat brain. EC (entorhinal cortex), rs (rhinal sulcus), Prh (perirhinal cortex), Ins (insular cortex), POR (postrhinal cortex), Te2 (secondary temporal or auditory cortex), Te3 (tertiary temporal cortex), Oc2L (secondary visual cortex), 35 and 36 (areas 35 and 36 of the perirhinal cortex). The parasubiculum is not represented. Adapted from Burwell et al. (1995).

Intrinsic and Extrinsic Connections of the Perirhinal and Postrhinal Cortex

To determine their connectivity, Burwell et al. (1998a) conducted an extensive investigation of the perirhinal and postrhinal cortices using both anterograde and retrograde tracers. The bulk of the following descriptions, therefore, will be taken from that paper, with some supplements from other sources.

Injections of retrograde and anterograde tracers result in the massive labeling of the areas closest to the injection site, as should be expected. It was found that cells in area 36 project heavily to area 35, which in turn reciprocate weakly. Since area 36 is dorsally positioned relative to area 35, the data suggest a dorsal to ventral projection system within the perirhinal cortex itself. The postrhinal cortex does not have such a clear-cut topography. Like the perirhinal cortex, it has an extensive intrinsic associational projection network but it abruptly ends at the border of area 36.

The perirhinal and postrhinal cortices do project to each other. The perirhinal cortex projections to the postrhinal cortex arise mainly from layers V and VI, and make contact primarily to the rostral-dorsal part of the postrhinal cortex. Projections from the postrhinal cortex arise mainly from layers II and V in the rostral postrhinal cortex, which terminate preferentially in the caudal half of area 36 of the perirhinal cortex.

Possibly the most important connections of the perirhinal and postrhinal cortices are those made with the entorhinal cortex, because the entorhinal cortex has been so heavily implicated in learning, memory and epilepsy. The perirhinal cortex projects preferentially to the lateral entorhinal cortex or area LEA. Area 35 is more strongly connected with LEA compared to area 36. The perirhinal cortex is less extensively connected to the medial entorhinal cortex or area MEA, when compared to its connections to the LEA (Burwell et al., 1998a; Naber, Caballero-Bleda, Jorritoma-Byham, & Witter, 1997). This is a similar projection organization to the perirhinal cortex, which has a dorsal to ventral projection organization.

The postrhinal cortex has reciprocal connections with the LEA and MEA, but unlike the perirhinal cortex, it has a more extensive reciprocal connectivity with the

MEA. In fact, this extensive MEA connectivity is the major differentiating characteristic that separates the postrhinal cortex from the perirhinal cortex.

Extrinsic Connections of the Perirhinal and Postrhinal Cortices

It has been found that the perirhinal cortex sends and receives many varicose terminals to cortical and subcortical sites that are quite distal to the rhinal cortices. In anterograde and retrograde tracing experiments in the rat, Shi and Cassell (1999) have found that the perirhinal cortex projects dorsally to the temporal cortices Te1 and Te3, but does not extend its projections to the somatosensory or visual cortices. Again, as described above, projections to the entorhinal cortex were found. It should be mentioned that Shi and Cassell (1999) found a sparse labeling of cells in Ammon's horn of the hippocampus and the subiculum, suggesting a small direct hippocampal influence from the perirhinal cortex. Supporting evidence for this connectivity has been found (Liu & Bilkey, 1997; 1996), but these findings have been challenged (Witter, Naber, & Lopes da Silva, 1999; Leung & Canning, 1999; Canning & Leung, 1999). The latter authors suggest that the perirhinal cortex's influence on the hippocampus is indirect, through the entorhinal perforant path system. Indeed, the majority of the labeling experiments show dense terminals in the entorhinal cortex when the perirhinal cortex is injected with anterograde tracers (Burwell et al., 1998). The hippocampus is rarely mentioned by other authors.

Shi and Cassell (1999) and McIntyre, Kelly, and Staines (1996) have also shown that the perirhinal cortex has dense projections terminating in the amygdala,

concentrating in the lateral, basomedial and posterolateral cortical nuclei. McIntyre et al. (1996), using both anterograde and retrograde tracers, found that the perirhinal cortex has strong reciprocal connections with the frontal cortex. Additionally shown by retrograde tracing was that the frontal cortex also receives dense projections from the amygdala, specifically, the basal lateral nucleus.

The postrhinal cortex has fewer known connections outside of the rhinal cortices compared to the perirhinal cortex. The postrhinal cortex receives strong input from visual association cortex, weak input from somatosensory association cortex, and little or no input from auditory and olfactory regions (Burwell et al., 1995). It also receives input from anterior cingulate cortex, the claustrum and the lateral posterior thalamic nucleus. Based on these and other data, it has been suggested that the postrhinal cortex may be important in visuo-spatial tasks (Naber et al., 1997), including the Morris water maze.

Learning and Memory

It is ironic that procedures initially developed to alleviate symptoms of epilepsy also would produce serious memory impairments as a consequence. One patient that was suffering from intractable seizures underwent a bilateral anterior temporal lobectomy that included the removal of the amygdala, hippocampus and the overlying cortex. This historic patient, referred to as H.M., subsequently experienced profound and permanent anterograde amnesia (Scoville & Milner, 1957). The authors concluded that the removal of the hippocampus was likely the critical structure responsible for the deficits in H.M.. Much supporting evidence for the role of the hippocampus in memory formation since

has come from animal lesion and human hippocampal activation studies (Zola-Morgan & Squire, 1990; Squire, Ojemann, Miezin, Petersen, Videen, et al., 1992).

Though it has been known for nearly a century that bilateral lesions to the medial temporal lobes or midline diencephalic structures could cause profound amnesia (e.g., Zola-Morgan, Squire, Amaral, & Suzuki, 1989), it was unknown at that time that bilateral surgical removal of the medial temporal lobes for therapeutic reasons would have such a devastating impact on learning/memory. Treatment for epilepsy using anterior temporal lobe removal is still practiced today. The resections, however, are now limited to one hemisphere or, if both hemispheres are involved, rigorous neuropsychological and neurological testing are conducted so that structures crucial to language and memory functions are left intact.

Cases with extreme damage to the medial temporal lobe structures, as discussed above, have been instrumental in showing that this general brain area is crucial for normal declarative information consolidation. However, other structures in the temporal lobes may subserve different aspects of memory consolidation. Indeed, isolation of either the hippocampus and/or the amygdala have demonstrated that selected lesions to these areas will have differential impact on learning and memory (McDonald et al., 1995).

The amygdala is classically associated with emotional based behaviors (e.g., LeDoux, 1992). In 1937, Kluver and Bucy (in LeDoux, 1992) observed that large temporal lobe lesions transformed normally wild and fierce monkeys into tame and indifferent animals. It was later discovered that restricted amygdala lesions alone produced this same effect (Weiskrantz, 1956; Downer, 1961; Horel, Keating, & Misantone, 1975; and Aggleton & Passingham, 1981). In rats, the amygdala has been

implicated in fear conditioning, stimulus-reward association learning, and anxiety just to name a few behavioral correlates (LeDoux, 1992). It is not surprising then that epileptics, with a verified EEG abnormality in the amygdala, often experience fear or some other type of unusual emotion during their focal seizure (Williams, 1956).

The hippocampus is not usually associated with emotional learning; generally it is associated with consolidation of information, particularly spatial learning and contextual learning (Scoville et al., 1957; Jarrard, 1993). Possibly, its special role, beyond those discussed above, is in ensuring that information remains accessible until it can be permanently stored elsewhere (Zola-Morgan et al., 1990). Presumably the information is ultimately archived in neocortical structures (Squire & Zola-Morgan, 1996).

There are numerous neocortical structures implicated in mnemonic processes. The entorhinal cortex may be very important within the memory consolidation pathway. Lesions of the entorhinal cortex before training, electrical stimulation shortly after training, or functional lesions from inactivation with TTX before training, disrupts retention of various tasks (Miwa & Ueki, 1996; Collier & Routtenberg, 1978; Baldi et al., 1998). More importantly, for our experiment, the entorhinal cortex is strongly implicated in spatial learning, as shown by bilateral or unilateral lesions in rats and mice, which impair their performance in a water maze task or in an eight arm radial maze (Oswald & Good, 2000; Glasier, Janis, Roof, & Stein, 1999; Glasier, Sutton, & Stein, 1995; Cho & Jaffard, 1995).

Also, the perirhinal cortex has recently been included as a possible important structure in the memory circuit. Its direct and indirect connections to the entorhinal cortex and hippocampus via the entorhinal cortex, respectively, make it reasonable to

suggest that the perirhinal cortex may contribute uniquely to learning and memory. For example, combined lesions of the perirhinal and entorhinal cortex result in spatial learning impairments in the radial arm maze and Morris Water-Maze (Otto, Wolf, & Walsh, 1997; Nagahara, Otto, & Gallagher, 1995). Similarly, lesions of the perirhinal cortex exacerbate the memory deficits observed following damage to the fimbria-fornix pathway as shown in a delayed non-matching to sample test (Wiig & Bilkey, 1995). However, these are dual lesions, making it somewhat difficult to understand the exact contribution of the perirhinal cortex versus the entorhinal cortex to mnemonic processes. Recently shown, selective lesions of the perirhinal cortex also resulted in impairments in learning and memory. In this regard, impairments in spatial learning have been found in the Morris Water-Maze, radial arm maze and delayed non-match to sample tasks (Liu & Bilkey, 1998a; Wiig et al., 1994a; Lui & Bilkey, 1998b; Liu & Bilkey, 1998c; Wiig et al., 1994b).

The postrhinal cortex on the other hand, has received even less attention compared to the perirhinal cortex with respect to learning and memory. There are, however, a few studies that suggest that a postrhinal cortex involvement in learning and memory. For example, Event Related Potentials (ERPs) measured from human epileptic patients showed that the parahippocampal cortex displayed event dependent voltage changes associated with correctly memorized words. Successfully memorized words elicited ERPs 300 milliseconds after the word event for the rhinal cortices and 500 milliseconds for the hippocampus, suggesting that the rhinal cortices processes information before the hippocampus (Fernandez, Effern, Grunwald, Rezer, Lehnertz, et al., 1999).

Combined lesions of the perirhinal and postrhinal cortex have resulted in a disruption of spontaneous object discrimination reversal learning in rats and impaired delayed non-matching to sample learning in monkeys (Bussey, Muir, & Aggleton, 1999; Zola-Morgan et al., 1989). Functional magnetic resonance imaging (fMRI) of human subjects shows that the parahippocampal cortex is preferentially involved in encoding new perceptual information about the appearance and layout of scenes (Epstein, Harris, Stanley, & Kanwisher, 1999). It is possible then that damage to the postrhinal cortex may disrupt the ability of rats to properly assess environmental cues that are novel.

In this thesis, the perirhinal or postrhinal cortex will be selectively aspirated to assess their potential involvement in various forms of learning and memory. Multiple behavioural tasks have been used in this assessment.

Epilepsy

As often occurs, many brain structures are associated with a variety of dynamic behavioral phenomena. Certainly this is the case for the CNS structures discussed above. Not surprisingly, therefore, structures associated with neuroplastic or mnemonic phenomena are also commonly associated with the development of epilepsy - the latter being the brain's most profound example of neuroplasticity. Indeed, the temporal lobes, as the seat of most experiential records (Gloor, 1992), are the most common and intractable site for epilepsy.

Almost two percent of the world's population is diagnosed with some sort of epileptic condition, (Prince, 1978) and close to nine percent will have one seizure during their lifetime (Hauser et al., 1975). Epilepsy is a condition of the brain characterized by *recurrent* sudden increases in the synchronization of large populations of neurons (Engel,

1998). On the other hand, a seizure is defined as a sudden and transitory abnormal phenomena of a motor, sensory, autonomic or psychic nature, resulting from transient dysfunction of part or all of the brain (Wallace, 1996). Therefore, epilepsy is diagnosed in part, when a seizure occurs more than once for an individual. Depending on the location and how much of the brain is involved during this synchronization of neurons, a person can experience as little as no response, to as much as a violent convulsion accompanied by unconsciousness.

Seizures can result from a multitude of events. They can be caused by a head injury, an irritating tumor, lesions from a stroke, from chemotoxic substances or alcohol withdrawal - to name a few. Many of these source/causes induce seizures that are brief in nature but occur periodically. Some conditions such as alcohol withdrawal can induce continuous seizures that if left uncontrolled can result in substantial brain damage and/or the death of the patient. Such a prolonged seizure is called status epilepticus (SE) and is defined as a condition in which a seizure lasts for thirty minutes or more without recovery (Barry & Hauser, 1993; Bleck, 1983). For this thesis, however, the typical seizures under discussion will be those that are brief in duration (<2 min).

During Hippocratic times, epilepsy was considered to be a sacred disease (Temkin, 1971). Those afflicted were said to have had the falling sickness, and during an attack, it was believed that they were 'seized' by evil spirits. Thus, the word *seized* was used for the first time, and continues to be the term used in describing seizures today.

In the early years, treatments for epilepsy comprised of special foods, environmental exposures such as extreme cold or the often-used blood letting by leeches. One of the first surgical approaches was trephening, which is the cutting away of a disk

shaped hole in the cranium to release the evil spirits. As one might surmise, this was not an effective treatment, and often life threatening infections would follow (Temkin, 1971).

Since that time, developments in techniques, equipment and philosophies have resulted in a greater scientific understanding of epilepsy. We now have the use of electroencephalographic recording machines that allow us to determine whether or not large populations of neurons are abnormally firing. Now at our disposal are imaging machines, such as Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI), which can help us in the determination of hyper -or hypometabolic areas, and localized lesion sites in the brain. In the event that abnormal *focal* firing is detected, we can now remove the local tissue that is believed to be the cause of epilepsy for individual patients. In spite of these advancements, epilepsy - of which at least 50 million people around the world suffer (Brodie, and Dichter, 1997) - remains the most common neurological disease (Goodridge & Shorvon, 1983).

Seizures associated with epilepsy are not uniform in manifestation, and involve focal brain activation or activation of large portions of the brain that may include both hemispheres. The seizures have been classified based on behavioral presentation and EEG recordings, which gives us an overall appreciation of the varied symptoms for the existing seizure types. Partial seizures occur in approximately 75% of patients with epilepsy and are characterized by EEG and behavioral changes that suggest focal or localized neuronal discharges (Long & McAuley, 1996). Partial seizures are sub-classed into simple and complex partial seizures (CPSs). Simple partial seizures, which do not induce a loss of consciousness, are sometimes called *auras*. The most common aura in temporal lobe epilepsy is abdominal visceral sensations (French, Williamson, Thadani,

Darcey, Mattson, et al., 1993), however, some patients hear strange noises or experience smells that are unrelated to the present environment. These auras are brief in duration, but may herald a more serious seizure episode.

CPSs, on the other hand, once begun, are associated with loss of consciousness. The person is not aware of what he or she is doing, which may involve behaviors such as staring and performing non-meaningful activities or automatisms in the form of lip-smacking, grabbing things, or walking without purpose. Secondary generalized partial seizures can be preceded by simple local partial seizures, or occur when abnormal synchronizations of neurons in one hemisphere generalize to the other hemisphere. These *secondarily* generalized seizures ultimately appear the same as *primary* generalized seizures. Yet early on, primary generalized seizures are different from those slowly evolving secondary generalized seizure as they immediately involve the entire cortex in seizure activity, where a localized onset cannot be identified. These seizures are always accompanied by a loss of consciousness and are the ones where a patient has the classic loud cry when the epiglottis closes and air is forced through the constricted opening.

The seizure conditions described above can involve several different brain areas. However, the most prevalent type of epilepsy is one that involves primarily the temporal lobes of the brain and is called temporal lobe epilepsy (TLE). In fact, TLE is so common among epileptic patients that one study found over 55% of all adult epileptic patients had temporal lobe symptoms associated with temporal lobe epilepsy (Gastaut, Goncalves e Silva, & Sanchez, 1975). The term TLE can easily be interchanged with the term CPS; however, seizures originating from temporal lobe structures do not necessarily result in

unconsciousness as is suggested by using the term CPS (Dreifuss, 1998; Williamson, Thadani, French, Darcey, Mattson, et al., 1998). Therefore, the term TLE will be used whenever possible with the understanding that many seizures originating in the temporal lobes can and often do result in unconsciousness and occasionally secondary generalized seizures.

Over 51% of individuals that have had a single initial seizure will have recurring seizures (Berg & Shinnar, 1991). It is generally accepted that most patients with recurrent epileptic seizures will require some sort of intervention. For partial seizures (i.e., TLE), anti-epileptic drugs such as carbamazepine, phenytoin, primidone and valproic acid are first recommended to reduce, and hopefully, eliminate seizure recurrence altogether (Brodie & Dichter, 1997). Unfortunately, some epileptic patients are medically intractable and may need resective brain surgery to control seizure development and expression. TLE is particularly resistant to pharmacological manipulation, and patients with TLE are often referred for surgery (Williamson et al., 1998).

There is an ongoing debate as to which temporal lobe structure or combination of structures is primarily involved in TLE. Two structures that have enjoyed a great deal of attention by neurologists and neuroscientists alike are the hippocampus and amygdala. By far the most popular structure to be blamed for causing TLE is the hippocampus. Bouchet and Cazauviel in 1825 were the first to identify abnormalities within the hippocampus of epileptic patients (Werner, Hatt, & Gottmann, 1998). The hippocampus was found to have some sort of lesion that was called *sclerosis* in 65% of temporal lobe epileptics that are refractory to medication (Hudson, Munoz, Miller, McLachlan, Girvin,

et al., 1993). It was not until the 1950's, when Penfield and Jasper demonstrated a reduction in seizures from anterior temporal lobe resection that included parts of the hippocampus, that this structure was intensely investigated for its role in TLE. Since that time, 'en bloc' resections of the anterior temporal lobe have become a popular treatment for patients with refractory TLE. The removal of the anterior temporal lobes has proven to be a worthwhile procedure. Approximately 55% of patients receiving this treatment become seizure free and remain so ten years later, while many more who are not cured, show a great reduction in the number of seizures and auras that they experience (Salanova, Marlan, & Worth, 1999).

It has been suggested that structures anterior to the hippocampus, like the amygdala, may be the cause of convulsions. Sclerosis of the amygdala has been implicated in producing seizures in the temporal lobe (Gloor, 1992; Hudson et al., 1993). In fact, abnormal synchronization of neurons in the temporal lobes begins in both the hippocampus and the amygdala (Gloor, 1992). Also, in patients with intractable TLE, 27% of the 'en bloc' resections show no pathology in the hippocampus suggesting that structures such as the amygdala may be important to TLE seizures (Hudson et al., 1993). In support of this suggestion, anterior temporal lobectomies that include the amygdala plus the hippocampus, or the amygdala alone have had roughly equal benefits for the patients (Feindel & Rasmussen, 1991). Regardless of these findings, anterior temporal lobe resections, including both the amygdala and the hippocampus continue to this time (Manno, Sperling, King, Jaggi, Alavi, et al., 1994; Sperling, Saykin, Glosser, Moran, French, et al., 1994; & Park, Bourgeois, Silbergeld, & Dodson, 1996).

An interesting study that suggests a reason for the equal success of amygdalectomy and amygdalectomy plus hippocampectomy was conducted by Parrent and Blume (1999). They performed radiofrequency lesions of the amygdala plus hippocampus in TLE patients while preserving the surrounding tissue. These patients were less likely to become seizure free, compared to those patients that received anterior temporal lobe resections. These authors suggested that neocortical resections such as the entorhinal cortex (or adjacent perirhinal/postrhinal cortices) might be sufficient for eliminating seizure recurrences. In fact, neocorticectomy of the lateral temporal cortex that includes the perirhinal cortex have been performed in TLE patients (Keogan, McMackin, Peng, Phillips, Burke, et al., 1992). In the latter case, the resections spared the amygdala and hippocampus, yet achieved therapeutic levels that were equal to that of the complete anterior temporal lobectomies. Thus, it is possible that the 'en bloc' anterior temporal lobe resections, which include the amygdala or amygdala plus hippocampus, achieve similar therapeutic success because a common crucial cortex for the expression of seizures is removed in both cases.

Animal Models of Epilepsy

As we have already seen, all epileptic conditions are not accompanied by unconsciousness or behavioral convulsions. However, most animal models of epilepsy involve behavioral convulsions that recruit much of the forebrain. This makes the study of partial epilepsy somewhat difficult. Fortunately for researchers, TLE often involves secondary convulsions, and therefore many animal models of epilepsy can be used in the investigation of TLE with secondary convulsions. There are several animal models that have been developed for the purpose of studying secondarily generalized convulsions,

supplying the researcher with excellent tools to study its development and expression in TLE.

Another problem with these animal models of epilepsy is that many use chemoconvulsants that, at high doses, induce status epilepticus (SE). Patients with TLE usually do not initially present themselves with SE, as the latter is often the result of some other cause, such as metabolic derangement, strokes or cardiac problems (Barry et al., 1993). There are two chemical models of epilepsy, however, that initially involve limbic structures (Turski, Cavalheiro, Schwarz, Czuczwar, Kleinrok, et al., 1983; Honchar, Olney & Sherman, 1983), and severely damage limbic structures, such as the hippocampus, amygdala, rhinal cortices and various thalamic nuclei (Fujikawa, 1996; Ben-Ari, 1985). These chemicals are pilocarpine and kainic acid. However, the damage in these models far exceeds that normally found in the brains of human epileptics. Yet these models have been useful for studying the effect of drugs on seizure thresholds and even on their effects in limiting SE-induced brain damage. However, it is difficult to study the *development* of convulsions with these models since the drugs are often systemically administered, work quickly (within one hour), and if the SE is not controlled using anti-epileptic drugs, can kill the animal.

Another model of epilepsy, called kindling, was developed following the accidental discovery in the 1960s. Kindling is defined as the progressive development of electrographic and behavioral seizures as a result of daily low intensity electrical stimulation to a particular forebrain site, such as the amygdala or the hippocampus (Goddard et al., 1969). Gradual behavioral progressions, which develop into full motor convulsions, can be produced by stimulating a brain site once or twice a day with an

electrical shock at low intensities. Because it can require several weeks to kindle a brain to the level of motor convulsions, a researcher has an opportunity to closely study brain-related changes during kindling development. Also, electrodes can be placed in selective forebrain areas, such as the amygdala and the hippocampus, for specific and controlled stimulations of those sites. Thus, the problem of systemic drug administration (of a convulsant) or spillover of the drug into adjacent brain areas following focal brain injections can be avoided. Because of these kinds of problems with drug administration, the kindling model has become the most commonly used technique for studying temporal lobe epilepsy.

For a model of epilepsy to be useful, it must satisfy certain criteria resembling human epilepsy. Wada, Osawa, Sato, Wake, Corcoran, et al. (1976), suggested that a model should allow for: (1) precise experimental control over the anatomy in terms of area as well as size of the epileptogenic lesion; (2) the ability to create an epileptogenic site without introducing identifiable destructive pathology; (3) accurate experimental control over the initiation and development of seizures; (4) ready induction of seizures by a discrete and identifiable experimental event; (5) eventual development of a spontaneous recurrent seizure state mimicking the previously established electroclinical pattern; and (6) evidence of persistence of the epileptic state for many months.

We know that humans can kindle, as shown in situations where stimulation electrodes are implanted into the brain, which then produce kindled epileptogenesis with repeated electrical stimulations. One criticism that has emerged with respect to the use of kindling as an animal model of human epilepsy is that there is little direct evidence that humans experience seizures due to kindling from events other than electrical stimulation.

Even though direct evidence suggesting a link between kindling and human epilepsy is speculative, there are some indirect reasons for suggesting that kindling does occur in man. Cases involving patients who have had a closed or open head injury that subsequently develop CPS provide intriguing data. Though some of the epilepsies are not considered to be progressive, it is believed that in other patients, a detached bone or damaged tissue may act as a repeated irritating stimulus, resulting in recruitment of neuronal pathways and eventually causing convulsions (Engel, Caldecott-Hazard, & Bandler, 1986; Goddard et al., 1969). These patients occasionally present themselves with CPS months to years after the initial injury, thus, suggesting that a kindling-like process may be responsible for their slowly acquired seizures.

Neuroanatomical substrates of kindling

The progressive process underlying kindling permanently changes recruitment of neuronal networks both in the stimulated focus (kindling site) and at distal brain sites (Goddard et al., 1969). These network changes are associated with behavioral changes as well. Stimulation of the amygdala can result in intermediate behaviors that eventually progress into full blown kindled convulsions with subsequent stimulations (as indicated above). These intermediate behaviors have been defined using amygdala kindling into 5 distinct stages (Racine, 1972). Stage one is defined by mastication; two, by head bobbing; three, by unilateral forelimb clonus; four, by rearing with bilateral forelimb clonus; and five, by rearing with forelimb clonus and loss of postural control. Stage-5 seizures are those that will be of primary interest in this thesis.

The number of stimulations needed to produce a kindled convulsion is called the *kindling rate*. It has been known for some time that different forebrain structures have different kindling rates (Goddard et al., 1969; Mohapel, Hannesson, Armitage, Gillespie, & Corcoran, 2000). It has been observed that excitable structures that are strongly connected to the motor cortices kindle quickest. For example, the amygdala, which is well-connected to the motor cortex via direct and indirect connections, kindles in approximately 15 stimulations, whereas the dorsal hippocampus, which has no direct connection to motor cortex, requires an average of 30 to 40 stimulations to produce stage-5 kindled convulsions (McIntyre & Kelly, 1993a; McIntyre, Kelly, & Armstrong, 1993b). In addition, recently, kindling rates in the temporal cortices also have been explored because of the growing interest in their involvement in TLE. It has been found that the piriform cortex kindles in approximately 5-10 stimulations while the perirhinal kindles even faster (McIntyre et al., 1993a; Mohapel et al., 1996). Further, during evocation of these stage-5 seizures, considering the latency from the stimulation onset to the beginning of the convulsive response, the hippocampus initially can take 30 seconds or more of recruitment for clonus to develop, compared to about 20 seconds from the amygdala and piriform cortex, while the perirhinal cortex may take as little as 1-2 seconds. These data suggest that the temporal cortices, particularly the perirhinal area, may be crucial players in the development and expression of convulsive seizures in TLE.

Instrumental to this thesis is the work of Mary Ellen Kelly (Ph.D. thesis, 1995). The goal of her work was to produce SE related temporal lobe brain damage in the hopes of discovering which anatomical networks were crucial to subsequent convulsive development during dorsal hippocampal kindling. Procedurally, her work involved first

producing forebrain commissurotomy to lateralize the experiment to a single hemisphere, followed by electrical SE of the amygdala, and then by hippocampal kindling. She found that when the SE-induced damage to the temporal lobe included both the piriform cortex and posterior perirhinal cortex (plus anterior postrhinal cortex), hippocampal kindling ipsilateral to this damage could not support clonus from that hemisphere. However, an incomplete forebrain commissurotomy allowed for the contralateral (non-kindled) hemisphere to be recruited, thus, accessing intact networks in that hemisphere and their associated clonus production. Of course, this unilateral clonus (supported by the contralateral hemisphere) was behaviorally ipsilateral to the kindled hemisphere from where the initial seizure discharge was triggered. Because behavioural contralateral clonus could not be induced (i.e., clonus supported by the kindled or stimulated hemisphere), she suggested that the posterior perirhinal cortex may be crucial for the behavioural or convulsive expression of hippocampal kindling in each associated hemisphere.

In support of Kelly (1995), Federico and MacVicar (1996) showed that the posterior perirhinal cortex (aka postrhinal cortex) was intimately involved in the temporal lobe seizure process. They used an *in vitro*, whole Guinea pig brain preparation, which was maintained by perfusion of artificial cerebral spinal fluid (ACSF). This brain was then implanted with stimulating and recording electrodes, from which electrical pulses could be delivered and field potentials recorded. They found that when the entorhinal cortex (the output structure from the hippocampus) was stimulated, the resulting seizure propagated strongly to the posterior perirhinal cortex, and then to other structures. Further, when perfused with bicuculline, a GABA_A receptor antagonist, they found that

the posterior perirhinal cortex was the most critical structure supporting spontaneous seizures, and that inhibitory mechanisms in the postrhinal cortex were likely important for controlling seizure activity.

The difficulty with the electrical SE model for producing brain damage is that brain damage cannot be confined to a single brain area. In Kelly's 1995 work, damage was found in the amygdala, hippocampus, piriform cortex and the perirhinal cortex. Even though it is reasonable to predict that the perirhinal cortex is crucially involved in the expression of hippocampal kindled convulsions, on the basis of her data, it cannot be confirmed that the perirhinal cortex alone is responsible for that effect; rather it may be that the perirhinal acts in conjunction with other temporal lobe structures to support the expression of hippocampal kindled convulsions.

C-Fos Protein and Neuronal Pathway Activation Mapping

As essential as lesion methods have been for understanding seizure propagation and the associated anatomy, other techniques involving metabolic increases in certain structures in *intact* brains during seizures have also been studied (Dragunow & Robertson, 1987; Baille-Le Crom, Collombet, Burckhart, Foquin, Pernot-Marino, et al., 1996). Metabolic mapping of the seizure process can be done by detecting the accumulation of radioactive glucose (C^{14} -2-deoxyglucose) in the brain that has been assessed at various characteristic stages of seizure development (White & Price, 1993).

Another metabolic marker for brain area activity is the staining of proteins formed from immediate early genes shortly after neuronal activation (Sato, Yamada, Morimoto, Uemura, & Kuroda, 1998; Ferland, Nierenberg, & Applegate, 1998; Teskey, Atkinson, &

Cain, 1991). Immediate early genes that are located in the nucleus of neurons quickly transcribe their code for the production of proteins (Dragunow et al., 1987; Dragunow & Robertson, 1988). Using immunohistochemical techniques these proteins can then be observed in brain areas that are the most active during a desired seizure event. In fact, for the purpose of mapping the pathways involved in seizures, c-Fos immunohistochemistry has already been demonstrated as reliable (Dragunow & Faull, 1989). C-Fos is turned on by the same neuronal activity that is required for expression of C¹⁴-2-deoxyglucose (White et al., 1993).

In recent years, investigators studying mechanisms of TLE have used c-Fos immunohistochemistry in conjunction with the kindling model to study the progression of convulsive seizure development. In one study, researchers kindled the amygdala, hippocampus and perirhinal cortex in separate rats followed by staining for c-Fos protein (Sato et al., 1998). They found that c-Fos expression was strongly in the perirhinal cortex when either the amygdala, hippocampus or perirhinal cortex was kindled. They also mentioned that some c-Fos was expressed in the contralateral perirhinal cortex when stage 1 or 2 (non-convulsive) seizures were observed, but that fully generalized seizures were usually needed to produce heavy c-Fos production in the perirhinal cortex, etc. depending on the structure being kindled.

Ferland et al. (1998) have shown that kindling the perirhinal cortex produces widespread c-Fos protein labeling in the hemisphere ipsilateral to the stimulation. What they also demonstrated was that in the contralateral hemisphere, perirhinal and frontal motor cortices preferentially expressed c-Fos along with the agranular insular cortex following a kindled convulsion from the perirhinal cortex. A curious observation was

that when the perirhinal cortex was kindled, the contralateral hippocampus was rarely labeled in rats that exhibited convulsions. As mentioned earlier, the hippocampus is hailed as being essential for the development of convulsions in TLE, however, these data suggest that the hippocampus may not be crucial for the development of convulsions during perirhinal kindling. Similar results were also observed by Sato et al. (1998).

In order to demonstrate that the perirhinal cortex may be crucial for behavioral convulsions, Ferland et al. (1998) injected KCl in the deep layers of the perirhinal cortex contralateral to the kindled amygdala stimulation site to produce a functional but reversible lesion called cortical spreading depression (CSD). They then attempted to trigger a convulsion from the amygdala. Only a unilateral forelimb convulsion contralateral to the stimulation site was expressed. The authors postulated that only the perirhinal cortex ipsilateral to the amygdala stimulation could be activated from the stimulation, which resulted in behavioral convulsions involving the contralateral forelimb. Because bilateral clonus could not be elicited in rats with the perirhinal cortex inactivated by unilateral KCl, it was concluded that the perirhinal cortex is crucial to the expression of motor convulsions in epilepsy. Unfortunately, they did not label for c-Fos in these rats to show that only the ipsilateral perirhinal cortex was activated.

It seems that in other animal seizure models, the perirhinal cortex may play an important role in seizure expression. Handforth and Treiman (1995), injected C¹⁴ 2-DG at various stages of SE in lithium-pilocarpine treated animals. They found that the perirhinal cortex was preferentially activated only when forelimb clonus became observable.

The perirhinal cortex, its frontal connections, and potential role in seizure expression are further supported by Scremin, Shih, and Li (1997). They showed that seizures produced by a cholinesterase inhibitor, Soman, increase the metabolic activity (as measured by C^{14} 2-DG) in the temporal and frontal cortices more than that of rats that were treated with vehicle or did not exhibit convulsions when Soman treated. Of interest was that the perirhinal, piriform and insular cortices were among the most activated structures followed by the frontal cortex. Further, the postrhinal cortex was also significantly activated above control levels. Importantly, the parietal cortex was never active above the levels found in control animals, indicating that cells in the temporal region are not equivalently activated during convulsive expression.

Further evidence of the possible importance of the perirhino-frontal cortex connection in the expression of kindled convulsive seizures from the amygdala, is implied by the results of Kelly, Battye, and McIntyre (1999). Because of the massive unilateral projection to the frontal motor cortices of the perirhinal cortex, these authors (McIntyre et al. 1996) examined the role of the frontal cortices in amygdala kindled rats using unilateral cortical spreading depression (CSD). These authors first kindled rats from the amygdala and then injected KCl unilaterally above the frontal cortex to produce CSD either contralateral or ipsilateral to the kindled amygdala. This was followed 3 min later by the stimulation of the kindled amygdala. The results were clear. When a KCl injection was followed by a stimulation of the ipsilateral amygdala, there was no convulsive activity. When a similar injection was followed by a stimulation of the contralateral amygdala, a convulsion occurred with a normal latency, however, all such convulsions were unilateral and, thus, supported only by the kindled hemisphere.

Clearly, amygdala kindled convulsions are primarily expressed by unilateral projections to the ipsilateral frontal cortices, where they become bilateral likely through the transcallosal, homotopic projections to the contralateral motor cortices.

Thus far, evidence suggests that the perirhinal cortex and the adjacent postrhinal cortex may be crucial to the development and expression of behavioral convulsions from either amygdala or dorsal hippocampal kindling. The intimate relationship between the perirhinal and the frontal motor cortex and the strong connections between the perirhinal cortex and limbic structures such as the amygdala, make the perirhinal cortex the best candidate to be a crucial interface between the limbic system and the frontal motor cortex. Thus, it is reasonable to hypothesize that the perirhinal cortex must be intact if behavioral convulsions are to be recruited during limbic kindling.

To test this hypothesis, and adequately understand the role of the perirhinal cortex in hippocampal and amygdala kindling, it is essential that selective lesions of the perirhinal cortex be created. In this thesis, selective perirhinal or postrhinal aspiration lesions are generated, followed by limbic kindling involving either amygdala or hippocampal, to determine the critical importance of those two temporal lobe cortical regions in the expression of limbic kindling.

Prior to limbic kindling studies, however, rats with perirhinal versus postrhinal lesions were examined in several behavioral tasks to be elaborated upon following the surgical descriptions below.

General Methods:

Aspiration Lesions:

Surgery:

Fifty-five Long-Evans-Hooded rats, 250-300g at the time of surgery, were used for this experiment. All rats were anesthetized using sodium pentobarbital (Somnotol; 65mg/kg, i.p.). Once the rats were no longer reactive to toe pinch, their heads were shaved and they were placed in a Krieg stereotaxic apparatus.

A longitudinal incision was made on the top of the head from the eye level to the base of the skull. The periosteum was then removed and retractors put in place to keep the skin from interfering with the experimenter's field of vision. Penicillin G, sodium 0.5 million units/cc (Novopharm) in sterile distilled water, was liberally applied to the surface of the skull to prevent subsequent infection. The masseter muscles were detached bilaterally from the top of the skull to the zygomatic arch. Once the skull was exposed, a 2.5 mm burr bit in a dentist drill was used to grind away bone to gain access horizontally to the perirhinal or the postrhinal cortex at the following coordinates (Pelligrino, Pelligrino, & Cushman, 1979):

	Perirhinal	Postrhinal
A-P	-0.6 to -3.6 mm	-3.6 to -7.6 mm
Depth	-7.0 $\pm$ 1.0 mm	-7.1 to -2.0 $\pm$ 1.0 mm*

*in a diagonal, guided by A-P coordinates

Excluding surgical controls, the aspirations were performed using a vacuum pump system with a waste reservoir. The nozzle used for the aspirations was a modified Pasteur pipette that had the tip heated and bent at approximately 15°. A hole was blown

out of the shaft of the pipette using a propane blow-torch to soften the glass, and air pressure to create the smooth edged makeshift valve. The valve was made so that the aspiration intensity could be adjusted. This pressure adjustment was accomplished by partially or completely covering the valve opening with the index finger. In conjunction with the use of a dissection microscope, the perirhinal or the postrhinal cortex was removed by aspiration. Subsequently, the lesion site was filled with dental foam packs (Gelfoam by Upjohn), and the wound closed with 9 mm surgical autoclip staples (MikRon Precision Inc). Fourteen days post-surgical recovery was given to all rats before behavioral testing in the learning experiments began. Of the original 55 rats, four died during surgery. In all, 51 rats were used in the analysis of Morris Water-Maze learning.

Behavioral Tasks

The following tasks are presented in the thesis in the same order as they were presented to the rats. The most important learning experiment was the Morris Water-Maze. As a result, it was conducted first, so that no other experiences would be confounding in its acquisition. All other learning experiments were conducted to extract additional information regarding the influence of the lesions on behaviour, but were considered secondary in importance compared to the Morris Water-Maze. The order in which the additional tasks were added beyond the Morris Water-Maze depended on the availability of the apparatus, which included: the inhibitory avoidance task; the elevated-plus-maze; and, the open-field test.

Experiment One

Morris Water-Maze

Introduction:

The Morris Water-Maze was developed to test the rats' ability to navigate in space. Rats swim in a pool until they find a submerged platform on which to rest. This test traditionally has been sensitive to hippocampal dysfunction or to dysfunction of hippocampally related networks, such as the fimbria/fornix and the entorhinal cortex (see general introduction).

Methods:

Our Morris Water-Maze is a pool 1.75m in diameter and 600cm in depth. The pool was filled with clear water at a temperature of 21°C. In order for the rats to habituate to the pool, they were given a single free swim with no platform session (first probe session) that lasted for 2 minutes. In the following 7 days, the rats had one session per day consisting of 4 trials per session. On those 4 swim trials each day, the rats were randomly released from one of four different start locations. The start quadrant was changed daily to minimize the memorization of release order. On each trial, there was a maximum time of 2 minutes for the rats to find the platform. When the rats failed to find the platform on a given trial, they were escorted by hand to the platform. A rest period of 60 seconds was given to each rat once they reached the platform.

Following the initial free swim session (probe session #1), the rats were allowed to find a *raised* platform. This was the *cued* session. During this session, the platform was covered with a black plastic bag and wrapped with a black headband to allow the rats

a better grip when climbing onto the platform, as well as to serve as a visual cue. The cued session was followed by 7 acquisition learning or *place-learning* sessions, in which the rats were forced to find a transparent platform *submerged* three centimeters below the surface. The platform remained fixed in place at a location different from the earlier raised cued session for all 7 learning days. Then, the rats had a second *probe* session to again test for location biases in the pool. This second probe session was followed the next day by second *cued* or raised platform session. For this session the platform was in a different location from that of the first cued session.

It is important to determine the specific strategies that the rats used while learning the Morris Water Maze. One of the strategies possibly used by the rats is orienting themselves using cues from the immediate environment. Some of these cues could be markings on the pool itself. In the next session, the water maze was rotated clockwise by 90° to see if the rats used *internal cues* such as imperfections of the pool's edge. The following day, the pool was again placed in its original position with the exception that all moveable external objects, such as visible furniture and posters, were rotated by 90° to see if the rats used these *external cues* to navigate in the maze. Again, the next day, the external cues were returned to the original acquisition session specifications and the rats were tested for one more regular learning session.

Subsequently, retention-over-time was tested by giving the rats 7 days without further maze exposure, which was followed by a *retention test* session with the hidden platform in the same location as that of the place-learning sessions. Lastly, to test behavioral flexibility and new learning, during the next three sessions, the rats had to find

a hidden platform in a location that differed from the original platform acquisition session. During those three sessions, the platform location was fixed.

Summary of Morris Water-Maze Protocol:

There is one probe session to ensure that there are no place preference biases (probe #1)→: one raised platform session (cued session) to show that the animals could see the platform + were equally motivated to escape→; 7 Acquisition (place-learning) sessions to investigate the rats' ability to learn the location of a *submerged* transparent platform over days →; a *second* probe session (probe #2) to show that the animals now had a preference for the location of the platform developed during the acquisition sessions→; a *second* raised platform session (cued session)→; one session with the maze rotated to determine if any internal cues are being used by the rats→; one session with external cues rotated to determine if distant objects are used for orientation in the pool→; one session with the original specifications of the place-learning sessions to serve as a reminder →; 7 days of rest →; One session to determine long-term retention of the hidden platform →; three sessions with a hidden platform in a new fixed location to assess behavioral flexibility.

Hypotheses:

It was hypothesized that lesions to either the perirhinal or postrhinal cortex would produce impairments in the acquisition of the MWM. Compared to control or sham operated rats, the performance for perirhinal or postrhinal cortex lesioned rats was predicted to be inferior as measured by (a) the latency to find a hidden platform during

MWM acquisition, (b) time spent in the quadrant with the hidden platform, and (c) quadrant preference during the second probe test. All data was reported using S.E.M when displaying statistical error.

Results:

Histology

Of all rats, 14 were sham operated, 18 received complete or partial perirhinal lesions and 18 received complete or partial postrhinal lesions. Minimal and maximal lesions for the perirhinal and postrhinal cortex lesioned groups are represented in lateral whole brain views (see Figure 1.1 and 1.2). For the perirhinal cortex lesions, 6 rats had a maximal, 5 had an intermediate and 7 rats had a minimal lesion. For the postrhinal cortex lesioned rats, 6 had a maximal, 5 an intermediate, and 7 had a minimal lesion. Analyses reported in this thesis was conducted for: control (sham), perirhinal and postrhinal lesioned groups. Further subdividing groups did not provide additional information to the three group analyses.

In order to determine electrode placements and the extent of perirhinal and postrhinal lesions, Cresyl Violet was used to stain the tissue (Figure 1.3 and 1.4). Staining tissue with the Timm's method was also done in order to assist in the determination of lesion location (Figure 1.5 and 1.6). According to Burwell et al. (1995), the Timm's method allows anatomists to fully determine the extent of the perirhinal and postrhinal cortices as these two areas are stained more darkly compared to the adjacent entorhinal cortex.

Figure 1.1: Minimal (A) and maximal (B) perirhinal lesions represented in lateral whole brain perspective, coloured in black.

A

B

Figure 1.2: Minimal (A) and maximal (B) postrhinal lesions represented in lateral whole brain perspective, coloured in black.

•

A

B

Figure 1.3: Photomicrographs of a Cresyl violet stained coronal section through the perirhinal cortex of a control rat brain (A) and a rat brain with a typical perirhinal cortex lesion. (B). The arrows show the dorsal and ventral borders of the perirhinal cortex.

Figure 1.4: Photomicrographs of a Cresyl violet stained coronal section through the postrhinal cortex of a control rat brain (A) and a rat brain with a typical postrhinal cortex lesion. (B). The arrows show the dorsal and ventral borders of the postrhinal cortex.

Figure 1.5: A coronal section through the perirhinal cortex of a control rat brain (A) stained with Cresyl Violet and counter stained using the Timm's method (B). Note the darkened ventral border of the Timm's stained section (highlighted by the arrows), that defined the ventral edge of the perirhinal cortex.

Figure 1.6: A coronal section through the postrhinal cortex of a control rat brain stained with Cresyl Violet (A) and the Timm's method (B). Note the Timm's section and its darkened border at the ventral edge of the postrhinal cortex.

Perirhinal or Postrhinal Lesioned vs Control Groups

In the following analyses, the word *session* will be equivalent to *day*. A session consisted of all four trials on any given day. Also, in these analyses, only those rats that have a verified lesion of the perirhinal or postrhinal cortices, whether minimal or maximal, are included.

A repeated measures analysis of variance was performed for all 7 place-learning (acquisition) sessions by groups to determine whether or not the groups of rats became differentially more proficient at finding the hidden platform. A significant main effect for sessions and for groups emerged but no significant sessions by groups interaction was observed [$F(6,282)=48.68$, $p<0.001$], [$F(2,47)=4.26$, $p<0.05$], and [$F(12,282)=0.85$, $p>0.05$], respectively. Generally, all groups showed progressive, daily decreases in latencies to reach the hidden platform, with the postrhinal group requiring more time than the control group (see Figure 1.7) (Note, there are two additional sessions in the figure, sessions 8 and 9, which refer to the internal + external cue sessions, respectively.). As described earlier, internal and external cues were rotated to determine their importance for finding a hidden platform. A repeated measures analysis of variance was performed that included all seven place-learning sessions and the intra and extra-maze rotation sessions by groups. No significant main effect for sessions or interaction for session by groups emerged [$F(2,94)=0.164$, $p>0.05$], and [$F(4,94)=0.677$, $p>0.05$], respectively. Therefore, in accordance with this analysis, the internal and external cue rotations sessions were considered as place-learning sessions 8 and 9.

Figure 1.7: Latency swim times for all place sessions by perirhinal and postrhinal lesioned and control groups.

Morris Water-Maze: Latency for all Place-Learning Sessions by Perirhinal and Postrhinal Lesioned and Control Rats

a=postrhinal lesioned group significantly different from the control group
($p < 0.05$).

A repeated measures analysis of variance was performed for all place-learning sessions, now including sessions 8 and 9, by groups. A significant main effect for sessions and groups was found [$F(8,376)=46.74$, $p<0.001$], and [$F(2,47)=6.12$, $p<0.01$], respectively, but no significant sessions by groups interaction was observed [$F(16,376)=0.75$, $p>0.05$] (see again Figure 1.7). Post-hoc multiple comparison Tukey tests ($p<0.05$) were performed on sessions in which significant one way ANOVAs were conducted, to determine how the groups differed. Groups were different in place-learning sessions 6, 8 and 9, with the control group performing better than the postrhinal group on all three sessions (see again Figure 1.7).

It is possible that postrhinal lesioned rats performed poorly compared to the other groups because of visual, motor, or motivational difficulties. To rule out these possibilities, a repeated measures analysis of variance was performed for the two *cued* sessions by groups. A significant main effect for sessions was found but no significant main effect for groups or significant sessions by groups interaction was observed [$F(1,47)=80.12$, $p<0.001$], [$F(2,47)=1.01$, $p>0.05$], and [$F(2,47)=1.88$, $p>0.05$], respectively (Figure 1.8). Thus, all groups performed similarly when escaping the pool to a raised platform, suggesting that visual, motor and motivational processes were similar regardless of lesion location or the time of testing, relative to the place learning experience.

Reference memory and working memory contributions to group differences were assessed next. A series of repeated measures analyses of variance were conducted selecting for individual trials, which included all place-learning sessions. These analyses were conducted in an identical fashion to the initial acquisition learning analysis. It was

Figure 1.8: Latencies to reach a raised platform (cued learning) before place-learning and after the second probe session.

Morris Water-Maze: Latency to Reach a Raised Platforms in two Seperate Sessions by Group

hypothesized that if group effects existed on trial one for any of the sessions, then, reference memory may be impaired. However, if the group differences existed on trials 2, 3, or 4, then, possibly short-term or working memory was impaired in the appropriate groups. Significant main effects for sessions but no sessions by groups interactions were found for all analyses. Significant main effects for groups were found for analyses selecting for trials two, three and four (Table 1 for statistics, Figure 1.9). Post-hoc multiple comparison Tukey tests ($p < 0.05$) showed that pair-wise group differences did not exist for analyses selecting trial two or three. It is possible that the differences existed only when comparing the combined lesion groups to the control group. For the analysis selecting for trial 4, differences were found on sessions 4, 6, and 9. On all of these sessions, the postrhinal group performed significantly poorer compared to the control group. Also, on session 9, the postrhinal group performed significantly poorer compared to the perirhinal group. This analysis suggested that groups differentiated from one another on later sessions and that the differentiation was relatively large on the later trials of those sessions. The data suggested that postrhinal lesioned rats are less likely to improve their performance across trials as do control rats or those with perirhinal lesions. It is reasonable to attribute this impairment to a possible working memory deficit, as cued learning ruled out obvious sensory and motor (performance) difficulties. This working memory impairment, however, does not seem to impact on long-term memory storage and retrieval as measured by the similar first trial latencies between groups.

Long-term retention of place-learning was tested using a repeated measures analysis of variance for the last place-learning and the 7 days after the reminder sessions by groups. A significant main effect for sessions and groups, but no significant sessions

by groups interaction was observed [$F(1,47)=7.13$, $p<0.05$], [$F(2,47)=6.36$, $p<0.01$], and [$F(2,47)=0.50$, $p>0.05$], respectively (Figure 1.10) . Post-hoc multiple comparison Tukey tests ($p<0.05$) for groups revealed that on both sessions, the control group performed better compared to the postrhinal lesioned group. No other groups significantly differed from one another. Thus, all three groups performed well on the retention session, but as found for the place learning analyses, the postrhinal lesioned group performed more poorly compared to the other two groups.

Table 1: F ratios and probabilities for repeated measures analyses of variance on individual trials across place-learning sessions by perirhinal and postrhinal lesioned and control groups.

Table 1.

Repeated Measures Analysis on Individual Trials for all Place-Learning Sessions by Perirhinal/Postrhinal Lesioned and Control Groups

Trial	Source	F
<i>Trial 1</i>	Group	$F(2,47)=0.50^{ns}$
	Session	$F(8,376)=29.35^c$
	Session by Group	$F(16, 376)=0.30^{ns}$
<i>Trial 2</i>	Group	$F(2, 47)=3.72^a$
	Session	$F(8, 376)=13.35^c$
	Session by Group	$F(16, 376)=0.69^{ns}$
<i>Trial 3</i>	Group	$F(2, 47)=3.91^a$
	Session	$F(8, 376)=5.62^c$
	Session by Group	$F(16, 376)=1.34^{ns}$
<i>Trial 4</i>	Group	$F(2, 47)=6.08^b$
	Session	$F(8, 376)=6.87^c$
	Session by Group	$F(16, 376)=1.31^{ns}$

^{ns}, non significant, $p>0.05$

^a, $p<0.05$

^b, $p<0.01$

^c, $p<0.001$

Figure 1.9: Morris Water-Maze: Session represented with individual trials. Reference vs working memory deficits.

Morris Water-Maze: Place-Learning with Trials by Perirhinal and Postrhinal Lesioned and Control Groups

a=postrhinal lesioned group is significantly different from the control group ($p<0.05$).

b=postrhinal lesioned group is significantly different from the perirhinal lesioned group ($p<0.05$).

Probe Trials:

As mentioned earlier, one probe session before the place-learning sessions and another immediately before the second cued session were performed by each rat. By measuring the amount of time spent in each quadrant, it is possible to determine a swim location bias. A 3-way repeated measures analysis of variance was conducted for time spent in each quadrant of the pool in both probe sessions by groups. A significant main effect for quadrants and a significant probe sessions by quadrants interaction was observed, $[F(3,129)=25.94, p<0.001]$, and $[F(3,129)=29.44, p<0.001]$, respectively. There were, however, no significant effects for groups. Thus, all groups spent an equal amount of time in each quadrant in the first probe session but more time in quadrant four during the second probe session. Quadrant four was the location of the hidden platform during the place-learning sessions. Additionally, it was found, using a oneway analysis of variance, that during the second probe session, all groups crossed the hidden platform location (the annulus) an equal number of times $[F(2,43)=2.16, p>0.05]$, indicating that all groups were equally accurate in their assessment of the previous platform location.

Flexibility Learning:

Flexibility learning was analyzed using a repeated measures analysis of variance. Platform location change sessions were used as the within subjects factor and groups was used as the between subjects factor. A significant main effect for sessions but no significant main effect for groups or significant sessions by groups interaction was found $[F(2,86)=11.72, p<0.001]$, $[F(1,43)=1.63, p>0.05]$, and $[F(4,86)=1.08, p>0.05]$, respectively (Figure 1.11).

Visual inspection of the first session of the first platform location change day suggested that it should be analyzed separately. A repeated measures analysis of variance was performed that included all four trials of this first session. A significant main effect for trials and significant trials by groups interaction but no significant main effect for groups was found, $[F(3,141)=19.43, p<0.001]$, $[F(6,141)=3.68, p<0.01]$, and $[F(2,47)=3.175, p=0.051]$, respectively. Post-hoc multiple comparison Tukey tests ($p<0.05$) revealed that group differences existed on trial one only, with the control group taking more time to escape to the hidden platform when compared to the postrhinal group. Differences between the control group and the perirhinal group only approached significance (Figure 1.12). Clearly the negative impact on performance with a change in platform location was only evident in the control group and only on the first of the 4 trials.

Figure 1.10: Latency times for the last place-learning session and the retention session by perirhinal and postrhinal lesioned and control groups.

Morris Water-Maze: Last Place-Learning Session and the Retention Session by Perirhinal and Postrhinal Lesioned and Control Groups

a=postrhinal lesioned group is significantly different from the control group ($p<0.05$).

Figure 1.11: Average latency times for all platform location change sessions by perirhinal and postrhinal lesioned and control groups.

Morris Water-Maze: Latency Averages for Platform Location Change Sessions by Perirhinal and Postrhinal Lesioned Rats and Control Groups

Figure 1.12: Latency times for individual trials of the first session in the first platform location session by perirhinal/postrhinal area lesioned and control groups.

Morris Water-Maze: Flexibility Learning by Trials During the First Platform Location Change Session; Perirhinal and Postrhinal Lesioned and Control Groups

a=postrhinal lesioned group is significantly different from the control group ($p < 0.05$).

Discussion:

Exposure to the MWM protocol resulted in a variety of interesting outcomes for the three groups. The initial exploration of the maze by the three groups during the first *probe* experience indicated that the groups were similar in their activity and none showed any particular quadrant biases. The next day the three groups again were similar when their escape latencies from the pool during the *cued* trials with the raised platform were assessed. These data are interpreted as showing a similarity between the groups in motivation and perception *prior* to testing for their spatial learning ability in subsequent testing.

Assessment of the spatial learning abilities of the rats showed a clear similarity between groups during the initial acquisition trials, on the first 5 days, but then significant differences appeared between groups when the postrhinal rats did not achieve the same rapid escape latencies as the controls. The escape scores of the perirhinal rats were positioned between these other two groups. Clearly, some disability in the postrhinal rats prevented them from performing at the same asymptotic level as the controls during the final stages of acquisition. This slight retardation in performance by the postrhinal rats was not evident on the subsequent *cued* session, where all groups again showed equally fast escape latencies, nor did it show on the next *probe* test, where the postrhinal rats spent as much time in the quadrant that previously contained the submerged platform as did the controls and perirhinal rats. Thus it appears that the motivation and sensory capacity of the postrhinal rats was equivalent to the other two groups, as well as the knowledge of where the platform should be located.

What is the nature of the postrhinal rats disability? Analysis of the four trials in each acquisition session suggested that the postrhinal rats did not benefit from the 4 trials each day by showing a progressive improvement across those trials like the other two groups. This suggested a slight working memory problem, but not a reference memory problem, since the first of the four trials in the postrhinal rats began as expeditiously as the other two groups.

The rotation of cue sessions did not add anything to answer this question, as those two sessions were identical to the previous and subsequent normal acquisition sessions. This fact, in the case of the external cue rotation session, was likely related to our inability to rotate all of the cues in that session. The door in one corner of the room and the sink in the other corner were clear cues to the rats. In addition, the rectangular fluorescent ceiling lights potentially acted as a directional beacon, reducing the confusion from rotating the cue that could be changed.

What addition information might be gleaned from the *permanence* (or retention) test? In that session, all groups performed much like their final session of acquisition, where the postrhinal rats were slight but significantly impaired; however, they were no worse off for having received 7 days without additional exposure to the task. Thus, like the relatively good daily reference memory (the first trial each day) showed by the postrhinal rats, their longer term reference memory was equivalently good.

Our last opportunity to challenge the learning/memory of the various groups was by moving the submerged platform to a new location and reassessing their behavioral flexibility in reacquisition and retention (over three days). The fascinating outcome of this experiment was the dramatic negative impact that this manipulation had on the

control group during the first session that was not shared by the two lesion groups. Indeed, inspection of Figure 1.12 shows that on the first trial of this session, the controls performed very poorly, but discovered 'their error' after ~45 seconds and corrected their behaviour over the next 3 trials. On the other hand, both of the lesion groups found the new location much more quickly than the controls, and further improved their approach speed on the next 3 trials. These results suggest that the strategy used by both lesion groups might be slightly different from the controls, resulting in their abandoning the old platform location for a new location more quickly than controls, and continuing to use that working information to improve their performance over the three remaining trials (although the postrhinal rats again were not as facile in their reacquisition as the perirhinal and control rats in this regard). Indeed, by the third session of this 'new learning', the three groups were performing at comparable levels to those that they showed during their final trials of initial acquisition -- trials where the control and perirhinal rats were superior to the postrhinal rats, which again reinforces the suggestion that the postrhinal rats could not use the information in a daily session to improve their performance across trials like the other two groups.

How does the fact that perirhinal lesions produced no spatial learning deficits in the MWM while postrhinal lesions did articulate with the accruing literature on these two areas? The evidence that supports the contention that the postrhinal cortex is important for spatial learning comes from Vann, Brown, Erichsen, and Aggleton (2000). They showed that rats that were trained on a standard, spatial eight-arm radial maze task, (as opposed to non-spatial running up and down a single runway) were more likely to show c-Fos staining in the postrhinal cortex, the dorsal hippocampus and the entorhinal cortex.

This was in contrast to the perirhinal cortex which did not show an increase in c-Fos labeling. These results suggest that the postrhinal cortex plays a role in spatial learning.

Although we did not show that perirhinal lesions produced impairments in the MWM, a number of recent papers have shown contradictory results concerning spatial learning and the involvement of the perirhinal cortex. Some show deficits in spatial learning (Lui et al., 1998a; Lui et al., 1998b; and Nagahara et al., 1995), and others show no spatial deficits (Bussey et al., 1999; Ennaceur, Neave, & Aggleton, 1996; and Wiig et al., 1994a).

Even though these several studies showed opposing results, certain subtle differences have been observed in their chosen behavioral protocols. The studies that did show deficits in spatial learning used delays between the learning trials, and between the information and choice runs in their behavioral tests. Nagahara et al. (1995) introduced delays in the MWM, where the rats were to find a randomly placed submerged platform after a single trial. They found that perirhinal/entorhinal lesioned rats performed poorly at delays that exceeded 5 minutes. With delays that were shorter like ours, the experimental lesioned groups performed equally to the control group.

Tests such as delayed non-matching to sample, which have been shown to be sensitive to hippocampal system lesions (Weiner et al., 1998; Aggleton, Keith, Rawlins, Hunt, & Sohgal, 1992) recently have been shown to be sensitive to perirhinal cortex lesions as well. Wiig et al. (1994b) showed that rats with perirhinal lesions began to show impairments at delays of 60 seconds or more in a delayed non-match to sample task. Wiig et al. (1995) then went on to show that perirhinal cortex lesions can exacerbate the effects of fimbria/fornix lesions in this same task. Mumby and Pinel

(1994) showed that rats with perirhinal cortex damage were particularly impaired in object recognition at delays of 15 seconds or longer. Mumby and Pinel (1992) using the delayed non-matching-to-sample tasks with objects found that if the hippocampus or the amygdala was lesioned but the entorhinal and the perirhinal cortices were spared, the observed impairments were mild. In fact, if either of these cortices were damaged, the delayed-non-matching-to-sample test showed severe impairments. Kornecook, Anzarut, and Pinel (1999) found similar results but used rats that had either mediodorsal thalamic or perirhinal cortex lesions. They found that only perirhinal cortex damage produced delay dependent impairments. It should be noted that in that study, lesions to the perirhinal cortex conducted two weeks or more after learning the task showed no impairments at testing. It can be suggested that the perirhinal cortex is important in the acquisition of the task and not crucial for information retrieval of the task after it has been learned. Thus, it is evident that the perirhinal cortex is involved in delay dependent information processing and that deficits in spatial learning may be secondary to delays when this cortex is lesioned.

There is, however, a little more specificity to the perirhinal cortex with respect to function than has been previously known. A review of neuronal responses and visual perception found that in general, perirhinal cortex neurons fire less intently to visual stimuli presented a second time (Brown, 1996). Similarly, in another study conducted by Wan, Aggleton, and Brown (1999), it was shown that novel and familiar pictures shown simultaneously, one to each eye, various brain structures were activated differentially. They found that the perirhinal cortex and the TE area of the temporal lobes were activated significantly more by pictures of *novel* rather than of familiar individual

objects. The hippocampus, however, was not differentially activated. They also investigated neuronal responses in the postrhinal cortex and found that it contrasted greatly from neuronal firing in the perirhinal cortex. Pictures of *novel arrangements* of familiar items produce significantly greater activation than familiar arrangements of these items in postrhinal cortex and subfield CA1 of the hippocampus. If this is true, then a rat with the postrhinal cortex lesions may not be as efficient in putting together familiar venues, which would be required in a daily session with the MWM.

In summary, the MWM is most sensitive to detecting spatial learning deficits, while the perirhinal cortex is most likely important in for tasks where delays are introduced. Thus, the MWM should be relatively insensitive to detecting perirhinal cortex damage when the task is purely spatial in nature without delays as in our study. On the other hand, the postrhinal cortex may be more important for successfully completing spatial tasks if integrating visual stimuli in a familiar setting is crucial. It was evident that the postrhinal cortex lesioned rats experienced impairments that were observed between trials within a session. These results are interpreted as working memory deficits since reference memory was relatively normal. Thus, we suggest that the postrhinal cortex is also important for storing or using information of a spatial nature for short durations, but does not prevent the encoding of long term memories for the future retrieval.

Experiment Two

Inhibitory Avoidance

Introduction:

Inhibitory avoidance testing was developed for the purpose of investigating the ability of rats to learn a conditioned fear response. In essence, a rat must learn that completely stepping off an insulated platform onto an electrified metal grid floor is an event to be avoided in the future. As the name of the task implies, resisting impulses to step off a safe platform would be considered inhibitory behavior and, therefore, a rat that can inhibit would avoid a shock.

Certain brain structures, when intact, have been linked to successful inhibitory avoidance learning. Specifically associated with fear learning is the amygdala (see general introduction). Because of the intimate connections between the amygdala and the perirhinal cortex, and the latter with the frontal motor cortex, it is reasonable to suggest that perirhinal lesions might result in impairments with respect to this behavior. As the postrhinal cortex is not directly connected to the amygdala, postrhinal lesioned animals should be relatively unimpaired compared to perirhinal lesioned animals.

Hypotheses:

It is hypothesized that the perirhinal lesioned rats will show poor retention of inhibitory avoidance when compared to postrhinal lesioned rats and that postrhinal lesioned rats will learn inhibitory fear less efficiently when compared to control rats. Perirhinal lesioned rats should freeze less compared to the other two groups when in the safe compartment of the test chamber. It is also hypothesized that perirhinal lesioned rats

will be more likely to test the safety of the environment when compared to control rats in the form of nose entries into the 'unsafe' environment.

Methods:

Approximately two days after completion of the Morris Water-Maze, all rats underwent inhibitory avoidance training. The apparatus used was a modified shuttle box ("Reflex-16", Columbus Instruments International Corporation). The box was constructed of stainless steel, measuring approximately 50cm long, 25cm wide, and 30cm high, and was divided half way by a stainless steel plate separating it into two distinct compartments. One compartment was darkened by black construction paper placed on the walls and ceiling. The floor of the shock compartment consisted of grid rods (3mm stainless steel spaced about 12 mm apart), which were connected to a constant current stimulator and scrambler programmed to deliver a 0.75mA (DC) shock. The other compartment (stainless steel) was lit by ambient light and had a wooden floor, which insulated the rat from the grid floor. The divider had a semicircular opening that was 9.5cm high and easily allowed a rat to pass. A guillotine door was used to close the opening. A clear piece of Plexiglas covered the box, which allowed for the rats to be filmed.

On the training day of inhibitory avoidance, each rat was placed onto the wooden platform with its head facing away from the dark half of the apparatus. *Step-down latency*, which is the time required for the rat to leave the wooden platform and place all four paws onto the grid floor in the dark chamber, was measured. Once the rat crossed into the dark chamber, the sliding door was lowered and a shock lasting two seconds with

a current of 0.75 mA was administered. While the rat was in the dark chamber, the top of the apparatus was removed and the rat was returned to its home cage. The following day, the rats were tested for fear learning. As in training, the rats were placed in the lit chamber and the latency to enter the darkened chamber was recorded. Any rat that did not cross to the dark side within the maximum time allotted (300 seconds) was removed from the lit area and returned to its home cage. If the rat made its way to the dark chamber, the door was slid into place and the rat was returned to its home cage without receiving a shock. In total, there were three testing days: 1) one, 2) two days, 3) seven days after training. Also measured during each session were the number of nose entries into the dark chamber, the number of rears in the safe chamber, and the amount of time the rats spent completely immobile while in the safe chamber.

Results:

Because of an accident involving a video-tape, on which some of the data were stored, a number of rats could not have freezing, nose entries, and rearing behaviours quantified. However, 33 rats could still be analyzed for these variables. Fortunately, step-down latencies were recorded as the rats were tested, making it possible to analyze those data for all of the rats.

Step-down latencies were analyzed using repeated measures analysis of variance for all inhibitory avoidance sessions. A significant main effect for sessions, but no significant groups main effect or sessions by groups interaction was observed [$F(3,141)=54.15, p<0.001$], [$F(1,47)=1.20, p>0.05$], and [$F(6,141)=1.51, p>0.05$], respectively (Figure 2.1). Multiple correlated t-tests with Bonferroni corrections were

conducted for all sessions. Step-down latencies were shorter for the training day when compared to all testing days [$t(32)$ ranged from -6.47 to -7.99 , $p < 0.001$]. The other days were not significantly different from one another.

Little to no freezing occurred on any session. That being stated, inferential statistics were still performed on the data. The data were adjusted for the step-down latencies by transforming the freezing times into freezing by step-down latency ratios and multiplying the transformed data by 100 to facilitate plotting. A repeated measures analysis of variance was performed on the transformed freezing data for the training and all three testing sessions. A significant main effect for sessions emerged but no significant main effect for groups or for the sessions by groups interaction was found [$F(3,90)=3.16$, $p < 0.05$], [$F(1,30)=0.10$, $p > 0.05$], and [$F(6,90)=0.53$, $p > 0.05$], respectively (Figure 2.2). Multiple correlated t-tests with Bonferroni corrections were conducted for all sessions. The amount of time the animals spent frozen per unit time was shorter for the training day when compared to sessions conducted one and two days after training but not for the session conducted seven days after training [$t(32)=-3.7$, $p < 0.01$], [$t(32)=-3.01$, $p < 0.01$], and [$t(32)=-1.55$, $p > 0.05$], respectively. No other days differed from one another.

Rearing behaviors were analyzed using repeated measures analysis of variance for all inhibitory avoidance sessions. However, before any analyses were performed, the data were transformed into rearing by step-down latency ratios to obtain rears per second. The transformed scores were multiplied by 100 so that they could be more easily plotted. Analyses revealed a significant main effect for sessions but no significant main effect for groups or sessions by groups interaction [$F(3,90)=9.34$, $p < 0.001$], [$F(1,30)=0.13$,

$p > 0.05$], and $[F(6,90)=1.08, p > 0.05]$ respectively (Figure 2.3). Multiple correlated t-tests with Bonferroni corrections were conducted for all sessions. There were fewer rears on the training session when compared to the sessions conducted two and seven days after training $[t(32)=-1.25, p > 0.05]$, $[t(32)=-2.98, p < 0.01]$, and $[t(32)=-3.53, p < 0.01]$, respectively. Also, the number of rears per unit time were fewer for the session one day after training when compared to sessions two and seven days after training $[t(32)=-2.89, p < 0.01]$, and $[t(32)=-3.55, p < 0.01]$, respectively.

Nose entry frequencies into the dark chamber were also evaluated using a repeated measures analysis of variance. Same as for the rearing and freezing data, a ratio of nose pokes by step-down latencies was calculated for statistical analysis. A significant sessions effect but no significant groups or sessions by groups interaction was found $[F(3,90)=18.20, p < 0.001]$, $[F(2,30)=1.74, p > 0.05]$, and $[F(6,90)=1.23, p > 0.05]$, respectively (Figure 2.4). Multiple correlated t-tests with Bonferroni corrections were conducted for all sessions. All rats reared less on the training session when compared to every other session after training $[t(32)=-4.80 \text{ to } -6.40, p < 0.001]$. All other groups were not significantly different from one another.

It should be mentioned that the training session in which the rats passed from the lit chamber to the darkened chamber was very short. Therefore, the rats did not appreciably freeze, rear, or engage in risk behaviors.

Figure 2.1: Step-down latencies for all inhibitory avoidance sessions by perirhinal, postrhinal lesioned and control groups

Latency for Dark Chamber Entry by Perirhinal and Postrhinal Lesioned and Control Groups

Figure 2.2: Freezing by step-down latency ratios in the inhibitory avoidance task by perirhinal, postrhinal lesioned and control groups.

Latency by Freezing Ratios in an Inhibitory Avoidance Task by Perirhinal and Postrhinal Lesioned and Control Groups

Figure 2.3: Rears by step-down latencies ratios during the inhibitory avoidance sessions involving perirhinal, postrhinal lesioned, and control groups.

Rears by Latency Ratios in an Inhibitory Avoidance Task by Perirhinal and Postrhinal Lesioned and Control Groups

Figure 2.4: Ratio of number of nose pokes by step-down latencies into the dark chamber during inhibitory avoidance sessions involving perirhinal and postrhinal lesioned, and control groups.

Nose Pokes by Latency Ratios in an Inhibitory Avoidance Task by Perirhinal and Postrhinal Lesioned and Control Groups

Discussion:

Of course, all rats entered the dark chamber in less time on the training session when compared to subsequent testing sessions. A similar pattern was observed for rearing, freezing, and nose pokes into the dark chamber per second. There were no group effects or interactions, which suggests that postrhinal or perirhinal cortex lesions do not create inhibitory avoidance impairments.

It cannot, however, be concluded with absolute certainty that the lesions do not have an effect on inhibitory avoidance learning. In this experiment, the rats were removed from the dark chamber once they had crossed from the ambiently lit one. This method may not have allowed for the maximum acquisition of the avoidance behavior making the task too difficult to acquire. Perhaps, it would have been preferable to allow the rats to cross back into the lit chamber, so as to show them that the lit chamber was safe. If this had been implemented, it is possible that the control rats would have demonstrated a greater avoidance response relative to the lesioned rats. As it is, the control rats did not perform at the ceiling score of 300 seconds.

It should be noted that there are other types of avoidance learning that were not tested in this experiment. Thus, it cannot be ruled out that lesions may have an effect on 'active avoidance', which is the moving away from an area or object that was previously paired with an aversive event. All that can be suggested in this experiment is that all groups were equally capable of remembering that the dark chamber was associated with foot shock based on remaining in the safe starting chamber.

Conclusions:

The most relevant other experiments to suggest perirhinal or postrhinal involvement in one trial inhibitory avoidance learning come from studies in which the entorhinal cortex is functionally lesioned, either immediately or minutes to hours after training. They show that bilateral injection of the glutamate NMDA antagonist AP5 (Izquierdo, Quillfeldt, Zanatta, Quevedo, Schaeffer, et al., 1997), or an AMPA receptor antagonist, DNQX, (Barros, Izquierdo, Souza, Arderghi, Pereira, et al., 2000) produce significant impairments in retrieval of avoidance learning. In our case, the chronic perirhinal and postrhinal cortex lesions were made well before training and, thus, ask a different question from the other acute reversible lesions studies indicated above.

Experiment Three

Elevated Plus Maze

Introduction:

Rats naturally move into dark enclosed areas. Presumably, those behaviours increase the rats' chances of eluding a predator in its natural environment. If this behaviour is dependent on the integrity of the limbic system, then perirhinal cortex lesions may impact on the rats' inclination to seek shelter. It is possible that an intact perirhinal/amygdala/hippocampal network allows for normal anxiety and its associated behaviours. Without a basal level of anxiety, a rat may not be apt to avoid open areas.

The elevated plus maze (EPM) consists of two intersecting runways of which two ends are enclosed by walls and the other two arms are open. The EPM has been shown to be a useful tool in determining anxiety levels in rats (Hogg, 1996), and traditionally, pharmaceutical companies have used this maze to screen their compounds for anxiolytic effects (Hogg, 1996; File, Zangrossi, Viana, & Graeff, 1993). In short, it is normal for rats to seek out the enclosed arms. However, drugs such as benzodiazepines may reduce this tendency, resulting in a rat that ventures onto the open arms (File et al., 1993). It has also been found that septal nuclei and mammillary body lesions have resulted in anti-anxiety effects on rats and mice, respectively, in the EPM (Pesold & Treit, 1992; Triet & Pesold, 1990; and Beracochea & Krazem, 1991). Also, bilateral injections of crystallin corticosterone into the amygdala resulted in increased anxiety-like behaviors in the EPM (Shepard, Barron, & Myers, 2000). Since the postrhinal and the perirhinal cortex are part of the rhinal system that can influence the limbic network (which includes the septal and

mammillary body nuclei) through the entorhinal cortex, it is a reasonable assumption to test their influence on emotionally related behaviors.

Hypotheses:

In this experiment, it was expected that the group with perirhinal lesions would display less anxiety than either the postrhinal lesioned or the control group because of the direct connections between the perirhinal cortex, the amygdala, and the entorhinal cortex. Thus, the perirhinal lesioned group was predicted to spend less time in the enclosed arms in favor of the open arms when compared to the postrhinal lesioned or the control group. It is also predicted that the postrhinal lesioned group would spend more time in the open arms when compared to the control group because of the strong connections between the perirhinal and entorhinal cortices. The same prediction was made for risk behaviors as measured by nose entries into open arms. The rats were tested in this task approximately two days after completion of the inhibitory avoidance task.

Method:

The maze consisted of two intersecting white plastic runways that were 105 cm in length, 14 cm in width and were elevated 49 cm off the ground. One of the runways was completely open without raised edges so that the rats could see over the edge, whereas the other runway had enclosed ends (with an open top) where relative shelter could be found. The enclosures at the ends of one of the runways measured 44 cm in length, 40 cm in height and were made of the same type of plastic as the rest of the maze. The only illumination was from the fluorescent ceiling lights.

The rats were placed randomly at the intersection of the two runways, facing either one of the enclosed arms or the open arms upon release. They were observed for a total of five minutes per session for *two* sessions (except for the first series of rats, in which there was only one session) run once a day. The time spent in open or enclosed arms as well as in the intersection of the runways was recorded. All rats were video-taped so that the playback could be use for counting arm entries, time spent in the arms, and the number of nose vacillations into the open arms. The nose poking (or vacillations) was counted as a risk-assessing behavior, which consisted of entries into the arm including up to half of the body. The runways were washed before each rat performed on the maze using Savlon for disinfectant and odor masking purposes.

Results:

A three-way repeated measures analysis of variance was performed for time spent in the open and enclosed arms. There were two within subjects factors: arms (open versus enclosed) and days (day 1 and day 2), and one between subjects factor: groups. A significant arms main effect, arms by groups, and days by arms interaction emerged [$F(1,28)=126.33$, $p<0.001$], [$F(2,28)=3.60$, $p<0.5$], and [$F(1,28)=5.27$, $p<0.05$], respectively (Figures 3.1, and 3.2). No other significant main effects or interactions were observed. Multiple comparison post hoc Tukey tests ($p<0.05$) showed that the control group remained in the open arms for longer periods compared to the perirhinal lesioned group and the perirhinal lesioned group remained in the open arms longer compared to the postrhinal lesioned group. Not surprising, a reverse pattern emerged for the amount of time that the groups remained in the enclosed arms. The arms by days interaction

Figure 3.1: Time spent in the open or enclosed arm in the elevated-plus-maze by perirhinal, postrhinal lesioned and control groups. Note the arms main effect and the arms by groups interaction. The postrhinal group spends more time in the open arms compared to the other groups. Data averaged over days.

**Time Spent in the Open or Enclosed Arm of the Elevated-Plus-Maze
by Perirhinal and Postrhinal Lesioned and Control Groups:
Averaged over days**

showed that all groups spent more time in the open arms on day one compared to day two (see again Figure 3.2).

Statistical analyses were conducted for risk behaviors (nose pokes) into the open arms. A repeated measures analysis of variance for nose pokes for both days by groups was performed. No significant main effects or interactions emerged. Thus, as measured by nose pokes, rats from all groups evaluated the risk of being in the open arms equally.

A general index of activity in the EPM is arm entries. The number of entries into the open or enclosed arms was analyzed for day one and day two by groups using a three-way repeated measures analysis of variance. One significant finding emerged. There was a significant main effect for the type of arm that was preferred [$F(1,28)=209.84$, $p<0.001$]. The average arm entries for all rats on both days was 1.93 (0.28 SEM) times for open arms and 6.95 (0.27 SEM) times for closed arms. These results are in agreement with the time data in this experiment, in which all rats preferred the relative 'security' of the enclosed arms. There were, however, no differences between groups, suggesting that general activity was the same and could not explain any of the group related time differences.

Figure 3.2: Time in seconds spent in either the open or enclosed arms on day 1 and day 2.

Time Spent in Open or Closed Arms on Day 1 and Day 2 of the EPM

Discussion and Conclusions:

As expected, all rats remained in the enclosed arms for longer periods of time compared to time spent in the open arms. Rats also tended to reenter the enclosed arms more often than the open arms when they ventured off from the intersection of the maze. Similar to other literature (File et al., 1993), these data indicate that the open arms are more anxiety provoking than the enclosed arms.

All rats tended to stay longer in the open arms on day one compared to day two. It is possible that, from their previous experience in the maze on day one, rats learned that the enclosed arms resulted in less anxiety, thus motivating them to spend a greater proportion of their time in the enclosed arms on day two. This finding is not unique. Treit, Menard, and Royan (1993) showed that open-arm avoidance increases in the second trial (day), but that no habituation occurred in subsequent exposures to the EPM up to 18 days. This suggests that there is something unique about the first trial.

Bertoglio and Carobrez (2000) devised an experiment in which rats were confined to open or enclosed arms in various configurations of the maze. They found that when the rats did not have the opportunity to explore open versus enclosed arms on the first trial, on the second trial, in a normal EPM, they behaved as though they were exploring the maze for the first time. They spent a greater amount of time in the open arms when compared to rats that experienced the normal EPM on the first trial. These authors suggest that rats need to experience contrast in the EPM in order for them to express normal anxiety over the open arms. This fear then 'overrides' the initial tendency to explore their environment. Thus, similar to controls, rats with perirhinal or postrhinal lesions displayed similar fear sensitization behaviors in the EPM.

It was found, however, that the control rats were more likely to remain in the open arms compared to the postrhinal or the perirhinal cortex lesioned rats for both days. Thus, the hypothesis that perirhinal cortex lesioned rats would stay in the open arms longer compared to the other groups was not supported. Perhaps, the perirhinal and postrhinal cortex have an inhibitory influence on neuronal networks associated with fear, so that when absent, an exaggerated fear response results. It is equally plausible that the postrhinal cortex is very important for environmental perceptions. With the postrhinal cortex absent, the rats may have more difficulty making a judgment on their environment, thus remaining in the enclosed arms, which resembles fearful behavior. This may explain why the postrhinal cortex lesioned group showed a greater exaggeration of open arm avoidance overall.

Therefore, rats, regardless of treatment group, preferred to spend more time in the enclosed arms in general but more so on the second day of maze exposure. Also, the lesions seem to exaggerate a fear response in the maze. Thirdly, based on findings in the literature other, it is reasonable to suggest that previous experiments (MWM + inhibitory avoidance) conducted on these rats may not have adversely compromised the interpretation of the result for this experiment. Thus, this experiment likely produced results that would be generalizable to a study in which the EPM alone is experienced by rats with perirhinal or postrhinal cortex lesions.

Experiment Four

Open-Field and Startle by Sound

Introduction:

In the colony room, a chance observation was made. Rats with postrhinal lesions did not startle when food was placed in the food bin situated on the cage cover. Control rats on the other hand tended to react more strongly. It was decided to incorporate startle in an open-field paradigm to see if a loud buzzer would disrupt the rats' activity in the open field. All rats were exposed to this paradigm two days after experiencing the elevated-plus-maze.

Method:

The open-field apparatus was a box constructed of white Plexiglas with side walls 50 cm high and a 100cm X 100cm floor with 25 equal (10cm X 10cm) squares marked off with thin black lines. A camera was mounted above the apparatus, which allowed the observer to record the rats' activities. A buzzer was suspended approximately 60cm above the center of the box by a broom handle measuring approximately 60cm in length.

All rats were placed in the center of the box and observed for three minutes. The buzzer was then activated and the rats observed for a further three minutes for a total observation time of six minutes. The rats were scored in two three-minute bins for the number of squares they crossed and the number of times they reared. Also scored, was the amount of time necessary for them to move away from the starting center-square, and the amount of time they froze to the buzzer.

Hypotheses:

As mentioned in the general introduction, the perirhinal cortex has intimate connections with the frontal cortex. It is conceivable that lesions to the perirhinal cortex may have some adverse effects on the rats' tendency to explore a novel environment due to motivational or subtle motor influences. It was hypothesized that if the perirhinal cortex has a significant positive influence on exploratory behaviour, the perirhinal lesion group will show the smallest number of grid crossing.

Results:

A 3-way repeated measures analysis of variance was performed for the total number of grid squares crossed immediately bordering the walls as opposed to the squares in the center of the open-field box (in/out), before and after the buzzer (epoch), and by groups. A significant main effect for in/out, epochs, as well as significant in/out by epochs interaction was observed [$F(1,32)=218.53$, $p<0.001$], [$F(1,32)=83.78$, $p<0.001$], and [$F(1,32)=29.56$, $p<0.001$], respectively (see Figure 4.1, 4.2 and 4.3, respectively). All rats showed thigmotaxic behaviors, choosing to spend most of their time near the walls of the enclosure. Also, all groups were equally more active before the buzzer was activated as opposed to after. Relative to the number of outside squares crossed, the rats were more likely to cross inside squares before as opposed to after the buzzer.

A repeated measures analysis of variance was performed for the number of rears before and after the buzzer (epochs) by groups. A significant main effect for epochs was observed (Figure 4.4) but no significant main effect for groups or significant epochs by

groups interaction emerged [$F(1,32)=5.79$, $p<0.05$], and [$F(2,32)=1.51$, $p>0.05$], and [$F(2,32)=0.50$, $p>0.05$], respectively.

A oneway analysis of variance was performed using the time needed for the rats to leave the starting center square by groups. The analysis revealed that all groups required an equal amount of time to leave the center starting square [$F(2,32)=0.66$, $p>0.05$]. Thus, neither the control nor the lesioned groups displayed different levels of urgency to seek out the edge of the open-field box.

Figure 4.1: Number of Inside and Outside Grid Square Crossings: The data are presented by groups. Data averaged over epochs.

**Number of Inside and Outside Grid Squares Crossed:
Average of Before and After Buzzer**

Figure 4.2: Number of grid squares crossed before and after the buzzer. The data are presented by groups.

Number of Grid Squares Crossed Before and After The Buzzer

Figure 4.3: Number of grid squares crossed. Data are presented for inside and outside crossings by before and after the sounding of the buzzer.

Number of Inside and Outside Grid Squares Crossed Before and After the Buzzer

Figure 4.4: Number of rears before and after the buzzer. Data presented by groups.

Number of Rears Before and After the Buzzer

Discussion:

There were no differences between groups on any measure. All groups were equally motivated to leave the starting center square. Also, all groups equally preferred to be active (cross grid squares) near the walls of the maze compared the center (out/in), but did so more before the buzzer compared to after the buzzer. The buzzer sounding is likely not the reason for this reduction in activity since no groups froze at the sound of the buzzer. Most likely, this reduced activity represents a simple habituation effect. The more likely reason for the change in location for the rats from inside to outside activity, is experience in the maze. Once the rats had explored the maze, they simply stayed at the periphery of the which was more sheltered. As time went on, this effect became more pronounced. Just as in the elevated plus maze, the more experience the rats had in the open field apparatus, the less likely they were to risk being in the open, as shown by their tendency to move along the wall.

All groups reared an equal number of times regardless of time spent in the maze. Traditionally, rearing behaviours are an anxiety indicator. In this experiment, as measured by rearing, all groups demonstrated the same amount of stress related behaviours, which did not increase as testing progressed. This suggests that the lesions were not effective in influencing the rat's anxiogenic behaviours. Thus, the hypothesis that perirhinal cortex lesions may influence activity in the open-field was not supported. All groups displayed equal activity levels and motivational levels to move away from the center grid squares. This further supports the suggestion that the data from the other three experiments are not confounded by motor or motivational differences between the treatment groups.

Experiment 5: Part I

Kindling

Introduction:

In the general introduction it was mentioned that kindling is the progressive development of electrographic and behavioural seizures as a result of daily low intensity electrical stimulations to a particular forebrain site. As kindling progresses, electrographic and behavioural changes can be observed. Specifically, the length of the AD, convulsions, stimulation to convulsion latency, and the quality of the behaviors can be measured. Experimental treatments can be closely evaluated as a result of the progressive development of kindling. The most important question asked in this thesis is whether or not the perirhinal or postrhinal cortex is crucial in the development of kindling and expression of stimulated convulsions in kindled epilepsy. This question was answered using perirhinal and postrhinal aspiration lesioned rats.

Not mentioned in the general introduction is the phenomenon of transfer kindling. Transfer kindling is an accelerated kindling of a brain structure following the kindling of some other brain structure (Burnham, 1975). It has been observed that when a brain structure, usually limbic, is kindled after another limbic structure has already been kindled, fewer stimulations are necessary to produce a generalized behavioral convulsion (Burnham, 1975; Racine, 1972; Goddard et al., 1969; and McIntyre & Goddard, 1973).

During transfer kindling, when the hippocampus is first kindled, the ipsilateral amygdala, consequently, can usually be kindled in 2 to 3 trials versus 10 to 15 trials if the amygdala is kindled first. This phenomenon is measured as the number of trials necessary to kindle the second structure. This is called the 'transfer rate', which is an indication of how much epileptogenesis during hippocampal kindling transferred to the

amygdala. A reduction in the number of stimulations necessary to kindle the amygdala after hippocampal kindling has already been shown to occur in rats where all brain structures were intact.

The same rats that were used in the behavioral experiments were used for the kindling experiments. Following the behavioral experiments, all rats were implanted bilaterally with stimulating/recording electrodes in both the amygdalae and the hippocampi.

With respect to primary site kindling, it was hypothesized that the perirhinal lesion would either block convulsive development or at the very least, that more stimulation sessions would be necessary to produce convulsive seizures during amygdala kindling. Because of the anatomical distance of the dorsal hippocampus from the perirhinal cortex and the multiple unaffected pathways, we predicted that dorsal hippocampal kindling should be less affected. Conversely, we predicted that postrhinal cortex lesions might block convulsive development or require a greater number of stimulation sessions from hippocampal kindling, but not for the amygdala, which we expected to kindle normally. In transfer kindling, the same hypotheses were applied.

Method:

Stimulating Electrode Implantation

All rats were anaesthetized with sodium pentobarbital (Somnotol; 65 mg/kg i.p.) and supplemented with the inhalation anaesthetic Halothane as necessary. The rats were prepared for surgery as described in the initial surgery for the aspiration lesions, and implanted bilaterally with four electrodes. The electrodes were constructed of 2 twisted

strands of 0.127 mm diameter Nichrome wire, Diamel-insulated, and were attached to male Amphenol pins. The coordinates relative to bregma for the amygdala were 0.0 mm posterior, 4.5 mm lateral to the midline, and 8.0 mm below the skull surface. The coordinates for the hippocampus were 3.5 mm posterior to bregma, 3.5 mm lateral to midline and 3.0 mm below skull surface (Pelligrino, Pelligrino, & Cushman, 1979). The electrodes were secured with dental acrylic and anchored to the skull by six jeweler's screws. Penicillin G was injected subcutaneously as a prophylactic against infection.

Seven to ten days following electrode implantation, the afterdischarge threshold (ADT) was determined for the amygdala for all rats. One day later, the ADT was determined for the hippocampus ipsilateral to the previously stimulated amygdala for all rats. A two second, 60 Hz sine wave stimulus of progressively increasing intensities (15, 25, 35, 50, 75, 100, 150, 200, and by 100 μ A increments peak-to-peak from then on) were used to trigger the afterdischarge (AD). The interval between stimulations was one minute until an ADT was determined.

Kindling began 24 hours after the determination of ADTs, and was conducted twice a day with at least six hours time between stimulations. The rats were stimulated until six stage 5 (Racine, 1972) generalized convulsions were recorded. Half the rats in each group were kindled first from the amygdala and half from the hippocampus. The kindling rate for these structures was then determined. Twenty-four hours following primary kindling, the kindling rate was determined for transfer structure (amygdala to hippocampus or vice versa).

Summary of Dependent Measures for Kindling

After discharge thresholds (ADTs) and after discharge durations (ADDs) were determined for both the first (primary) brain site kindled (amygdala or hippocampus) and the second (transfer) ipsilateral brain transfer site kindled (amygdala or hippocampus, depending on which structure was initially kindled). Kindling rates were scored for both the first brain site and the transfer site.

Results:

Electrode Placements

Figures 5.1, 5.2, and 5.3, show electrode placements for the control group, the perirhinal and postrhinal lesioned groups, respectively. Only those rats that had proper placements for both the amygdala and hippocampus are presented. Some rats had an electrode in the dorsal part of the fimbria/fornix, but kindled normally. These placements were considered to be hippocampal in nature.

Figure 5.1: Hippocampal (A) and amygdala (B) electrode placements for the non-lesioned control group.

A

B

Figure 5.2: Hippocampal (A) and amygdala (B) electrode placements for the perirhinal cortex lesioned group.

A

B

Figure 5.3: Hippocampal (A) and amygdala (B) electrode placements for the postrhinal cortex lesioned group.

A

B

Analysis

Afterdischarge Thresholds (ADTs)

A two way repeated measures analysis of variance was performed for ADTs that were determined before kindling was conducted, taking into consideration the stimulated structure and to which treatment group the rats belonged. A significant main effect for structure was observed, which showed that the amygdala had higher ADTs compared to the hippocampus [$F(1,25)=4.47$, $p<0.05$] (Figure 5.4). Also, as shown in the same figure, a near significant structure by treatment group interaction was observed with the amygdala showing a tendency for a higher threshold in the perirhinal group when compared to any other treatment-structure combination [$F(2,25)=3.09$, $p=0.063$]. Because the error was large in the perirhinal group for the amygdala thresholds, a non-parametric Wilcoxon Signed Ranks test was performed to confirm that thresholds for the amygdala were truly different from those for the hippocampus. As with the parametric ANOVA, a significant structure difference was observed [$Z=-2.32$, $p<0.05$]. Thus, the amygdala ADTs were significantly larger compared to those found for the dorsal hippocampus.

Afterdischarge Durations (ADDs)

A two-way repeated measures analysis of variance for the dependent measure ADD before kindling was conducted, taking into consideration the structure kindled, and in which treatment group the rats belonged. No significant differences were observed. ADDs for all groups and structures were the same (data not shown).

Figure 5.4: The current needed to produce an afterdischarge (ADT) in the hippocampus and amygdala at the time of threshold determination.

Afterdischarge Thresholds for the Hippocampus and Amygdala

Kindling Rate

Because of the nature of the database, complex multi-factorial analysis would not have been valid. Instead, multiple oneway analyses of variance were performed on kindling rate data for the amygdala and the hippocampus when kindled first and at transfer by treatment groups.

There were no differences for kindling rate by treatment groups for first structure kindling for either the amygdala or the hippocampus [$F(2,14)=3.19$, $p>0.05$] and [$F(2,14)=1.31$, $p>0.05$], respectively. Even though there were no statistical differences in kindling rate for the amygdala, there was a tendency for the perirhinal lesioned group to require a greater number of stimulation to produce an initial convulsion (Figure 5.5). At transfer kindling, a significant group difference was observed for the hippocampus [$F(2,13)=9.20$, $p<0.01$], but not for the amygdala [$F(2,16)=0.26$, $p>0.05$] (Figure 5.5). A post hoc multiple comparison Tukey test ($p<0.05$) showed that the perirhinal lesioned group needed a greater number of hippocampal stimulations to have its first transfer convulsion, suggesting a retardation of kindling when the perirhinal cortex is lesioned.

Correlated t-tests were conducted for amygdala and hippocampal kindling rates selecting for the amygdala or the hippocampus when kindled first, to verify that first structure kindling had an effect on the kindling rate of the transfer structure. When selecting for the amygdala, the number of stimulations necessary to produce a kindled convulsion was the same for the two structures [$t(15)=-1.27$, $p>0.05$]. However, when the hippocampus was kindled first, it took significantly more stimulations to produce a convulsion compared to the amygdala [$t(16)=-9.14$, $p<0.001$].

A correlated t-test selected for all groups was conducted to verify that the hippocampus requires a greater number of stimulations compared to the amygdala to produce its first convulsion. The hippocampus required a significantly greater number of stimulations [$t(32)=-5.42$, $p<0.001$] to produce a stimulated convulsion compared to the amygdala (Figure 5.5).

Figure 5.5: Amygdala and hippocampal kindling rates when kindled first and at transfer by Groups (Control (Ctrl), perirhinal (PER) and postrhinal (POR)).

Amygdala and Hippocampal Kindling Rates when Kindled First and at Transfer by Groups

Latency to Convulsion

As for the kindling rate section, the latency data from stimulation to convulsion were analyzed similarly for identical reasons. For first structure kindling, latencies did not differ between treatment groups for either the amygdala or the hippocampus on the first stimulated convulsion [$F(2,14)=0.18$, $p>0.05$], and [$F(2,16)=0.16$, $p>0.05$], respectively, or on the sixth stimulated convulsion, [$F(2,14)=0.11$, $p>0.05$], and [$F(2,16)=0.21$, $p>0.05$], respectively (Figure 5.6 and 5.7). When transfer kindled, only the amygdala on the sixth stimulated convulsion differed with respect to treatment [$F(2,14)=5.13$, $p<0.05$]. A post hoc multiple comparison Tukey test ($p<0.05$) was conducted to show that the postrhinal lesioned group required significantly more time to exhibit a convulsion after stimulation.

In order to determine that latencies changed with the number of elicited stimulated convulsions, correlated t-tests were conducted for latencies for the first and sixth stimulated convulsions. When kindled first, latencies were significantly longer to produce the first stimulated convulsion from either the amygdala or the hippocampus, [$t(16)=4.45$, $p<0.001$], and [$t(18)=3.38$, $p<0.01$], respectively, compared to latencies on the sixth convulsion. When transfer kindled, latencies were also significantly longer on the first convulsion for the amygdala and the hippocampus, [$t(16)=3.32$, $p<0.01$], and [$t(18)=2.61$, $p<0.05$], respectively. Thus, latencies shorten with the increased number of stimulated convulsions regardless of kindling order.

Figure 5.6: Latency to convulsion from stimulating the amygdala on the first and sixth convulsion when stimulated first and at transfer by groups (Control (Ctrl), perirhinal (PER) and postrhinal (POR)).

Latency to Convulsion from Stimulating the **Amygdala** on the First and Sixth Convulsion when Stimulated First and at Transfer by Groups

Figure 5.7: Latency to convulsion from stimulating the hippocampus on the first and sixth convulsion when stimulated first and at transfer by groups (Control (Ctrl), perirhinal (PER) and postrhinal (POR)).

Latency to Convulsion from Stimulating the Hippocampus on the First and Sixth Convulsion when Stimulated First and at Transfer by Groups

Duration of Convulsion

A similar analysis to latencies was performed for duration of convulsion. For structures kindled first, there were no group differences for either the first or the sixth convulsions [$F=0.13$ to 1.06 , $p>0.05$]. During transfer kindling, the amygdala on the *sixth* convulsion and the hippocampus on the *first* convulsion showed group differences [$F(2,14)=5.38$, $p<0.05$], and [$F(2,13)=6.32$, $p<0.05$], respectively (Figure 5.8 and 5.9). Multiple comparison post-hoc Tukey tests ($p<0.05$) showed that for the amygdala at transfer, the convulsion duration was shorter for the postrhinal lesioned group compared to the other groups. The hippocampus, at transfer, showed shorter convulsion durations when the perirhinal cortex was lesioned.

In order to show that convulsion duration changes with increasing numbers of stimulated convulsions, multiple correlated t-tests were conducted for duration on the first and sixth convulsions selecting for first and transfer kindled groups. For first structure kindling, both the amygdala and the hippocampus had longer convulsion durations on the sixth stimulated convulsion compared to the first [$t(14)=-3.28$, $p<0.01$] and [$t(18)=-4.06$, $p<0.01$], respectively. At transfer, there were no differences between first and sixth stimulated convulsions with respect to convulsion duration for either the amygdala or the hippocampus.

Figure 5.8: Duration of first and sixth amygdala stimulated convulsions when kindled first and at transfer by groups (Control (Ctrl), perirhinal (PER) and postrhinal (POR)).

Duration of First and Sixth Amygdala Stimulated Convulsions when Kindled First and at Transfer by Groups

Figure 5.9: Duration of first and sixth hippocampal stimulated convulsions when kindled first and at transfer by groups (Control (Ctrl), perirhinal (PER) and postrhinal (POR)).

Duration of First and Sixth Hippocampal Stimulated Convulsions when Kindled First and at Transfer by Groups

Results Summary and Discussion:

Afterdischarge Thresholds (ADTs)

The amygdala ADTs were approximately 80 μ A in this experiment, which match fairly closely those found by Mohapel et al. (1996). The hippocampal ADTs of approximately 50 μ A were also in accordance with the literature (Kelly & McIntyre, 1994). The amygdala in this experiment showed a higher threshold when compared to the hippocampus. This is perhaps due to the tendency for higher amygdala ADTs in the perirhinal group. It is possible that without access to the frontal motor networks through the perirhinal cortex, the amygdala must find other pathways that require more electrical current to produce a synchronization of neurons. We should, however, be careful in making the assumption that the perirhinal cortex lesion was responsible for the higher amygdala ADTs, since no analyses have shown significant effects with respect to lesion when considering ADTs.

Afterdischarge Duration (ADD)

The ADDs were unremarkable in this experiment at ADT determination. It would seem that regardless of the structure kindled, the ADDs had approximately the same length, which is in agreement with the literature. Also, lesions had no significant effect on ADDs. Common networks of structures, in which neurons are synchronized during amygdala and hippocampal ADT determination, may also be stimulated in both control and lesioned brains. Thus, these common structures may be largely independent from the structures lesioned, and remain synchronized for periods of equal duration regardless of the stimulated site or the location of the lesion in this experiment.

Kindling Rate

In this experiment, kindled convulsions developed from amygdala and hippocampal electrode sites were normal in appearance regardless of location of the lesion. It can only be assumed that lesions in the perirhinal cortex or the postrhinal cortex do not sever connections to motor areas that are influential in the “quality” of motor seizures. We suggest that once the frontal motor network is involved in the seizure, thalamic nuclei and structures downstream that are independent of the perirhinal and postrhinal cortex, are likely recruited. Also, as expected from the literature, fewer stimulation sessions were necessary to produce a kindled convulsion from the amygdala as compared to the hippocampus. Thus, regardless of treatment, the amygdala kindled more quickly.

Even though convulsions in the lesioned rats were expressed qualitatively the same when compared to non-lesioned control rats, there was a tendency (non-significant, $p=0.06$) for the perirhinal lesioned rats to take longer to kindle from the amygdala and for the postrhinal lesioned rats to kindle more quickly from the hippocampus. Of significance, however, was the finding that the hippocampus at transfer in the perirhinal group required significantly more stimulations compared to the postrhinal lesioned and control groups. It is possible that in spite of a fully kindled amygdala, the hippocampus when stimulated could not access the already facilitated pathways. This pathway block being a likely scenario, the hippocampus would have to gain access to collateral pathways to recruit the frontal motor cortex convulsion mechanism.

Latency to a Convulsion

Even though a greater number of stimulations were necessary to produce a convulsion from the amygdala during primary site kindling (tendency) and the hippocampus at transfer in the perirhinal lesioned group, kindled convulsion latencies for first structures were the same across these groups. Only at transfer did the amygdala kindled groups show longer latencies. Perhaps, in postrhinal lesioned rats, the hippocampus when kindled first, selects pathways that do not correspond to those used by the amygdala. This is not unreasonable since the postrhinal lesioned group have a slight tendency to kindle more quickly from the hippocampus. Even though the facilitation of kindling is not significant in the postrhinal group, it may be significant enough to influence pathway selection during amygdala kindling, and thus, forcing the amygdala to recruit networks primarily developed and recruited by the hippocampus. Therefore, the latencies were only influenced at transfer for amygdala kindling in postrhinal lesioned rats.

As expected, latencies decrease with increasing number of stimulations. This observation was made for structures both kindled first and at transfer. Thus, even though a structure is transfer kindled, the greater the number of provoked convulsions, the less time was necessary to recruit a convulsion. This suggests that in all groups, every stimulation recruits more networks so that the next stimulation triggers a more immediate convulsive effect.

Duration of Convulsion

As was found for the latencies, lesions only produced effects on convulsion durations at transfer. The amygdala at transfer had a shorter convulsions for the sixth convulsion in the postrhinal lesioned group compared to all other groups. This was possibly for the same reasons discussed in the latency section, where fewer common networks were facilitated by previous hippocampal kindling. Therefore, when the amygdala was kindled at transfer, new novel networks had to be recruited. The convulsion durations were also shorter for the hippocampus at transfer for the first convulsion for the perirhinal lesioned group. This may have been the result of unshared network recruitment as well. The hippocampus may have had difficulty accessing networks facilitated by the amygdala, not because the amygdala was stimulated fewer times, which would have resulted in fewer recruited networks, but because the hippocampus would normally have used the now non-existent perirhinal cortex to access shared frontal motor pathways.

Convulsion duration was found to increase with an increasing number of stimulations. This phenomenon, however, was observed for first structure kindling only, whereas, transfer kindled structures exhibited roughly the same convulsion durations on the first and sixth convulsions. Therefore, the initially convulsion durations increase, but ultimately reach a plateau value, independent of whether they are triggered from the amygdala or the dorsal hippocampus.

Discussion:

These specific findings, integrated more broadly with other evidence from the kindling literature, support the suggestion that the perirhinal cortex is functionally related to the amygdala and can influence the amygdala's excitability. Lesions of the perirhinal cortex resulted in an elevation of the amygdala ADT and slightly retarded its rate of kindling, although the statistical evidence for these effects was not strong. These results are very similar to those described recently by Schwabe et al. (2000), where they placed several small chemical lesions (using cannulated ibotenic acid) in the perirhinal cortex before kindling the amygdala of their rats. The influence of those small lesions on amygdala kindling was even less than in our study, but a tendency towards retardation in the appearance of the later stages (3-5) of seizure genesis was evident.

In our study, the perirhinal lesioned rats that showed nearly statistically significant retardation of amygdala kindling, did show significantly retarded hippocampal transfer kindling. This finding is new, and suggests that amygdala kindling and hippocampal kindling might make use of the same neural networks to express a convulsive seizure, as originally proposed by Kelly and McIntyre (1996). By contrast, the group of rats with perirhinal lesions that were kindled first from the hippocampus and then transferred to the amygdala showed fairly normal hippocampal kindling and normal positive transfer to amygdala kindling. What could explain the result profiles of those two different perirhinal cortex lesioned groups? Closer inspection of the histological data from the two perirhinal lesioned groups suggests an answer. In the rats that showed the retarded primary amygdala and transfer hippocampal kindling, the bilateral perirhinal lesions included nearly all of the anterior portion of that structure, near the borders where

it engages the insular cortex. On the other hand, in the other group, this anterior portion of the perirhinal cortex was left intact, and no negative effect on kindling rates from either structure (amygdala or hippocampus) was seen. This observation is consistent with evidence that the anterior perirhinal area and insular cortex project much more heavily to the frontal motor cortex than the more posterior areas (McIntyre et al., 1996), and should be more importantly involved in the recruitment of convulsive seizures during limbic kindling. Indeed, one of the two studies that showed a reversible blockade of convulsive amygdala kindled seizures involved *acute* injections of the NMDA receptor antagonist, APV, into the junction between the insular and perirhinal cortices shortly before triggering a seizure in amygdala kindled rats; an AD was triggered but a convulsion did not ensue (Holmes, Bilkey, & Lavery, 1992). In a similar vein, use of the reversible neuronal blocker, spreading depression, via KCl injection into the perirhinal cortex, also blocked convulsive seizure expression from that hemisphere in kindled rats (Ferland et al., 1998). Thus these two *acute* activity blockers of the perirhinal area were effective in disrupting convulsive kindled seizures. Yet, no chronic assessment of those agents has been made during the course of kindling.

We suspect that chronic treatments like aspiration and ibotenic lesions are reacted to by a neural network differently than are acute, reversible treatments, such as temporary transmitter blockade or spreading depression. Possibly, because of the plasticity of the nervous system, particularly in limbic areas, we suspect other parallel pathways and networks become available to chronically lesioned rats that are not normally available to uninjured brains or brains experiencing only acute disruptions. In the chronically lesioned rats, where these 'new' or alternative pathways are recruited, chronic interference (a

lesion) with the principle pathways might simply delay the genesis of a particular behaviour, e.g., the development of a kindled convulsion, but cannot completely block it, unless most of the alternative pathways are also compromised, as has been reported by Kelly and McIntyre (1996). Thus we expect that acute insults could create a greater interference effect with behaviour than chronic insults.

The postrhinal cortex lesions before kindling provided a very different scenario than the perirhinal lesions. We predicted that they might compromise hippocampal kindling, but they did not. Indeed there was a very slight tendency for them to facilitate hippocampal kindling. We predicted involvement of this area in hippocampal kindling based on anatomical grounds and evidence of heavy c-Fos activation therein during stage 4-5 seizure expression (Clark, Post, Weiss, Cain, & Nakajima, 1991). On the other hand, such connectivity and neuronal activation does not define the cell phenotypes (principle cells versus interneurons) that are involved in the activity or the nature of the network behaviour, since the latter might normally oppose rather than promote convulsive seizure expression. Indeed, compromising such antagonistic networks should more easily promote convulsive seizure expression.

Related to these possibilities is the fact that the postrhinal cortex normally has a very high threshold for seizure recruitment, as its local ADT to a kindling stimulus is often in excess of 1000 μ A. As recruitment during kindling increases progressively over days, either by directly kindling the postrhinal area or transynaptically by kindling the hippocampus or amygdala, the ADTs in the postrhinal area decrease dramatically, often by an order of magnitude (Felstead, 1997; and unpublished findings). Thus, frank lesions of the postrhinal area, or alterations of its activity through kindling, might make other

connected areas, like perirhinal cortex, much more seizure prone, which should facilitate kindling from other limbic structures, like the amygdala or hippocampus.

Experiment 5: Part II

C-Fos Labeling

Introduction:

As was shown previously in the kindling section, kindling from either the amygdala or the hippocampus developed eventually (normally) in spite of lesions to either the perirhinal or postrhinal cortices. The possible neuronal pathways associated with activation of these kindled convulsions are suggested by the c-Fos protein labeling in the rats that received a single convulsion 2 hours before being perfused for histological assessment. It was expected that the c-Fos would be most evident in the neurons that were highly activated (depolarized) during the seizure event. Thus, the pattern of neurons activated by c-Fos should help defined the neuroanatomy of the convulsion in both controls and lesioned rats following provocation from either the amygdala or dorsal hippocampus.

Hypotheses:

If the perirhinal cortex is important for seizure to be expressed convulsively, c-Fos should be observed in this cortex in rats sacrificed 2 hours after a final stimulated convulsion from either the amygdala or the hippocampus (see Appendix I for Methods).

Results:

In non-stimulated control rats, little or no c-Fos could be detected in the histological material, indicating that the immunolabeling of c-Fos was specific to seizure activation and not a consequence of normal bodily functions (Figure 5.10). In two kindled control rats, a convulsion triggered from the amygdala showed prominent c-Fos

labeling in the amygdala, perirhinal, postrhinal, piriform, insular and frontal cortices. In both of these cases, there was an absence of labeling in the hippocampus (Figures 5.11 and 5.12), suggesting the latter is not highly activated during an amygdala triggered seizure. Only when the seizure induced from the amygdala lasted longer than 40 seconds did the hippocampus show c-Fos labeling (as represented in this postrhinal lesioned rat, Figure 5.13).

Similar patterns of c-Fos labeling were evident in rats with either perirhinal or postrhinal lesions, independent of the trigger site. The non-lesioned cortical sites of greatest activation were always the insular, piriform and frontal cortices (Figures 5.13 and 5.14).

On the other hand, when the hippocampus was the trigger site, it always showed bilateral activation, along with the perirhinal and postrhinal cortices, if they were intact. When the hippocampus was activated, triggering an AD but *without* a convulsive response, the c-Fos labeling was restricted to the hippocampus bilaterally (Figure 5.15); in this case, few or no cells were labeled in the perirhinal, insular or frontal cortices. Clearly a generalized convulsion was necessary to activate these other cortical areas. These data are further depicted in schematic drawings, which present a more complete representation of the c-Fos labeling following both amygdala (Figure 5.16) and hippocampal (Figure 5.17) triggered convulsions. One will note that in addition to the cortical, amygdala and hippocampal labeling, several subcortical structures expressed c-Fos. They included the hypothalamus, midline thalamic nuclei and the nucleus accumbens.

Figure 5.10: Left hippocampus (A) and insular cortex (B) in a non-stimulated rat. Note the absence of c-Fos labeling.

Figure 5.11: Right frontal (A) and perirhinal (B) cortex of a control rat experiencing a stage-5 convulsion triggered by stimulation from the amygdala. Note the c-Fos labeling in both cortices.

Figure 5.12: A second control rat with a stage-5 convulsion triggered from the amygdala, where the AD was shorter than 70 seconds. Note the absence of c-Fos labeling in the hippocampus (A), but positive labeling in the ipsilateral frontal (B) and the perirhinal (C) cortex.

Figure 5.13: A postrhinal lesioned rat stimulated from the left amygdala that had a severely protracted convulsion. Note the abundant bilateral c-Fos labeling of the left (A) and right (C) hippocampus, and the left (B) and right (D) insular cortex.

Figure 5.14: A perirhinal lesioned rat that experienced a right amygdala stimulated convulsion. Note the absence of c-Fos labeling in the hippocampus (A), some in the frontal (B), and heavy labeling in the insular (C) cortices. Because of leaking of the anti-fade, the left side was chosen for display. However, the right side displayed the same distribution and intensity of c-Fos.

Figure 5.15: A control rat stimulated from the right hippocampus, which did not have a convulsion. This rat has little or no labeling in the frontal cortex (A) and perirhinal cortex (C), but abundant labeling in the hippocampus (B).

Figure 5.16: A schematic of c-Fos labeling in a control rat that had a right amygdala stimulated convulsion. Each dot represented approximately 5 neurons that positively expressed c-Fos.

Figure 5.17: A schematic of c-Fos labeling in a control rat that had a right hippocampus stimulated convulsion. Each dot represented approximately 5 neurons that positively expressed c-Fos.

Discussion:

These results show that stimulations, which produce a generalized convulsion from either the kindled amygdala or the hippocampus in non-lesioned rats, result in strong perirhinal and postrhinal cortex activation as shown by c-Fos immunoreactivity. By the same token, convulsions that were triggered from the kindled amygdala and hippocampal sites in rats with either perirhinal or postrhinal lesions showed the same pattern of c-Fos immunoreactivity in the temporal and frontal cortices, except for the areas that was surgically removed.

Thus, it can be suggested that the perirhinal and postrhinal cortices are not crucial for kindled convulsions to be elicited or to the network that supports the convulsive response. Even with the perirhinal or postrhinal cortices removed, large portions of the temporal lobes were activated by amygdala or hippocampal stimulated convulsions. Strongly labeled were the insular, piriform, entorhinal, frontal, cingulate, and to a lesser extent, the parietal cortices. Some subcortical structures were also activated from stimulated convulsions. It is likely that the cumulative activation of these several cortical structures represent parallel networks that support the development of convulsions in temporal lobe kindling and can operate independently from of perirhinal and postrhinal cortices.

Also, independent of the amygdala stimulated convulsions in this study, was the hippocampus. Our results show that the hippocampus does not need to be highly activated to a level that gives a c-Fos response to elicit a convulsion from the amygdala. Clark et al. (1991) showed similar results in that amygdala kindling at stages 4 and 5 resulting in *severe* convulsions were likely to activate the hippocampus bilaterally,

whereas, less severe convulsions stimulated from the amygdala show little or no c-Fos hippocampal labeling. Sitkoske, O'Shea, Rosen, Post, & Weiss (2000) found that hippocampal c-fos mRNA expression could be observed only after a stimulated amygdala convulsion lasted for 80 seconds or more. Our data showed that a convulsion of 40 seconds or longer with an ADD in the 70 second range was necessary.

An interesting discovery in this c-Fos study was the potential role of the insular cortex in kindled convulsions. In rats that experienced stimulated convulsions elicited from either amygdala or hippocampal stimulations, c-Fos labeling was always seen in the insular cortex. For those rats that did not experience a convulsion from stimulation, c-Fos labeling in the insular cortex was sparse or non-existent. Thus, it is reasonable to suggest that the insular cortex is important in recruiting convulsive seizures.

Perhaps, the insular cortex, because of dense enervation of the frontal motor network, contains the crucial projections that can elicit convulsive seizures from both amygdala and hippocampal stimulated convulsions. In fact, McIntyre et al. (1996) showed in rats injected into the frontal cortex with a retrograde tracer (Fluorogold) that many cells were labeled in the insular cortex. This may be significant since the amygdala communicates extensively with the insular cortex (Sun, Yi, & Cassell, 1994; Yasui, Breder, Saper, & Cechetto, 1991; and Veinante & Freund-Mercier, 1998). Thus, the amygdala should be able to activate the insular cortex and provoke convulsions in the absence of the perirhinal cortex. Work by Sitcoske et al. (2000) lends support for this hypothesis. They found that in amygdala kindled rats sacrificed 30 minutes after their first unilateral forelimb (stage 3) convulsion, the perirhinal and insular cortices bilaterally expressed c-fos mRNA. Similarly, Clark et al. (1991) found that in their rats, c-fos

mRNA was always expressed bilaterally in the insular, entorhinal, perirhinal and parietal cortices during amygdala stimulated convulsions.

The hippocampus, on the other hand, may activate the frontal cortex through the insular cortex by first recruiting other temporal cortices surrounding the perirhinal cortex or by activating the amygdala. Although it is likely that hippocampal seizures activate the cortices surrounding the perirhinal cortex in the absence of the perirhinal cortex, it also activated the amygdala, even though the amygdala disproportionately sends efferents to the hippocampus compared to the reverse order (Amaral et al, 1992). Thus, because of the lack of reciprocal amygdala/hippocampal connections, we predict that the stimulated hippocampal convulsions could be produced by directly recruiting the surrounding parahippocampal cortex, which then activated the insular and the frontal cortex in the absence of the perirhinal and postrhinal cortices.

Appendix I

Histology and Immunohistochemistry:

Two hours after their final seizure, all rats were anesthetized with sodium pentobarbital (Somnotol) before perfusion. They were then transcardically gravity perfused with a 120cc saline solution, followed immediately by 160cc of a 0.37% buffered sulphide solution (pH=7.2), followed again by 120cc of a saline solution, and finally, followed by 300cc of a buffered 4% paraformaldehyde solution (pH=7.2). The whole animal remained under the fumehood over-night. The brains were then removed and postfixed for two hours in 4% paraformaldehyde solution at 5°C. In preparation for tissue sectioning, the brains were exposed to 15% and then 30% sucrose in 100mM phosphate buffer solution (pH=7.2). The brains were cut only when they sunk to the bottom of the container. All brains were then sectioned into 40µm slices using a freezing microtome.

In addition to the slide preparation, there was one tube per brain filled with cryoprotectant (Lu et al., 1992), in which the slices destined for c-fos immunohistochemistry were stored. These sections were mounted onto slides in 10mM phosphate buffered solution in saline (PBS). This was followed by a 5 minute incubation in PBS containing 0.3% Triton-X followed by a wash in PBS. All sections were then incubated in C-fos primary antibody (Santa Cruz, sc-52-rabbit polyclonal IgG.) at 1/500 in PBS-Triton overnight at 4°C in a humid box. They were rinsed with PBS the next day. The sections were covered with biotinylated anti-rabbit IgG (H+L) secondary antibody (Vector Laboratories) made at 1/50 in PBS and Triton-X for one hour at 37°C to then be rinsed with PBS. This was followed by a Streptavidin Cy3 (Sigma) solution at 1/100 in PBS and Triton-X for 45 minutes at 37°C. The sections were then rinsed in PBS and coverslipped with an anti-fade medium.

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