Characterization of Kindling’s Effects on Spatial Cognition: Implications for the
Mechanisms of Kindling-Induced Mnemonic Dysfunction

Darren Keith Hannesson

College of Graduate Studies and Research
PhD
Department of Psychology
University of Saskatchewan
Fall, 2001
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0-612-63874-X
Abstract

Kindling refers to a procedure in which repeated electrical stimulation of a discrete brain region is used to evoke seizures that progress in their severity with successive stimulations. This process results in enduring changes in brain function that are manifest in an enhanced susceptibility to seizure-evoking stimuli and alterations in at least some types of behavior. As a result, kindling is widely used as a means for studying both epileptogenesis and neural plasticity in general. In my dissertation, I investigated the relation between brain changes associated with epileptogenesis induced by kindling and spatial cognition in rats. This topic is of interest for both its clinical relevance to memory dysfunction observed in patients with epilepsy and the insights it may provide into the mechanisms underlying learning and memory function. My approach was twofold. First, I examined the effects of kindling on a wide variety of behavioral tasks in order to characterize the nature of the effects of kindling on spatial cognition. Second, because kindling produces a collage of brain changes, some of which, at least, vary in relation to the site of the electrode used for kindling, the severity of the seizures elicited, and the interval since the last kindling stimulation, I investigated the relation between these kindling-related variables and behavioral task performance. To the extent that any effects observed were found to vary in relation to kindling-related variables, this approach was expected to help identify possible underlying mechanisms for the behavioral effects of kindling. In a series of studies, I investigated the effects of: i) kindling in the dorsal hippocampus (dHPC), perirhinal cortex (PRH), or amygdala (AM), ii) kindling until partially or fully generalized seizures were elicited, and iii) testing at intervals from one to 28 days following kindling on tasks that assessed sensorimotor
functions, motivation, anxiety, object-related cognition, and spatial cognition in rats. I found that kindling was capable of producing highly selective and enduring behavioral effects, which varied depending upon the site being kindled and the severity of the seizures elicited. These included: i) a selective disruption of spatial learning, which was produced by kindling in the dHPC but not in the PRH or AM and by kindling of fully generalized but not partially generalized seizures, ii) a partially selective impairment of object-related mnemonic functions, which was produced by kindling in the PRH but not the AM or dHPC, and iii) an anxiogenic effect, which was produced by kindling in the PRH or AM but not the dHPC. Based on these findings, I generated a specific hypothesis regarding the underlying mechanisms of the effects of kindling on spatial mnemonic function as follows. Epileptogenesis disrupts spatial learning through metaplastic effects that alter hippocampal mechanisms regulating induction of experience-dependent plasticity. Consistent with this hypothesis, kindling has been shown to produce enduring changes in factors that might be expected to alter plasticity including NMDA receptor function, PKC activity, and CA\(^{\text{++}}\) channel density. Importantly, available evidence suggests that these effects show a relation to the site and extent of kindling that is consistent with the conditions under which the deficit is observed. This hypothesis makes a variety of novel, testable predictions and, if verified, would provide evidence in support of the proposed role of synaptic plasticity in experience-dependent behavioral change. It would also offer a potential target for therapeutic interventions aimed at treating epilepsy-associated mnemonic dysfunction.
Acknowledgements

I’d like to thank my rats for behaving in a manner that helped provide me with interesting insights into the neural functions involved in epilepsy, cognition, and behavior. To my control rats ... thanx, so many of you were great. To those control rats that acted as if they were in a hemispherectomy group, may you wind up in such a condition in a future life! To the rats in my various treatment groups ... in so many different ways you guys really sucked. Thanx, I appreciated it.

I’d like to thank a number of fellow students that helped me with various parts of the projects in this dissertation including Paul Mohapel, Amy Wallace, Mike Pollock, John Howland, Ward Plunet, and Shannon Corley.

I’d like to thank my committee members, Deb Saucier, Mark Evered, and late-addition Lorin Elias. You all helped the process go smoothly and your contributions to the defense were both enjoyable and informative.

I’d like to thank my supervisor, Michael Corcoran. He made this work all possible. His large reservoir of kindling stories was a great source of both inspiration and amusement throughout my program and his relaxed but supportive supervisory style created an ideal environment for me to thrive in.

Lastly, I’d like to thank my wife, Teresa. No work of this magnitude is ever accomplished without it making significant and frequent intrusions into one’s family life and visa versa. Teresa agreeably accepted the former and helped minimize the latter. Her patience was greater than my own.
Dedication

I'd like to dedicate this dissertation to my father, Richard Stephen Marino Hannesson, who passed away on Feb. 7, 1999. He got me interested in science at an early age by nurturing my nearly unquenchable curiosity about dinosaurs and then by encouraging me to investigate whatever piqued my interests next. The hippocampusaurus has proven to be pretty cool, dad.
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1. INTRODUCTION

Thirty years ago, a particularly exciting type of research in behavioral neuroscience utilized discrete brain stimulation to investigate the role of different brain regions in behavior. A confound sometimes encountered in these studies was that some subjects developed seizures in response to the stimulation. Initially regarded as an annoying nuisance, this phenomenon was eventually recognized as inherently interesting by Graham Goddard (Goddard, McIntyre, & Leech, 1969), who coined the term kindling to describe it. In one of science’s many examples of serendipitous progress, kindling has subsequently proven to be a powerful and well-used preparation for studying neural function.

The present dissertation comprises a collection of studies investigating the kindling phenomenon. These studies examined the impact of kindling on a variety of subsequent behaviors with the goal of furthering our understanding of the relation between neural changes associated with kindling and functions involved in learning and memory (i.e., mnemonic functions).

1.1. Plasticity, Metaplasticity, Learning and Memory

One of the most fundamentally important properties of the nervous system is plasticity, or the capacity to undergo orderly and enduring changes in function. Among its many roles, plasticity is generally assumed to be essential for learning and memory
(Baudry, 1998; Eichenbaum, 1996; Elgersma & Silva, 1999; Jeffery, 1997; Martin, 
Grimwood, & Morris, 2000; Morris, 1989; Paulsen & Moser, 1998; Wang, Ko, & 
Kelly, 1997). According to this line of thinking, learning requires that experience causes 
changes in the brain and memory then requires that these changes persist over time. 

Recently, neuroscientists have become aware that plasticity itself is a dynamic 
property of the brain and thus is inherently modifiable by a variety of factors. An 
obvious example, that has not always been recognized as such, is development. It has 
long been known that the plastic capacity of the brain varies with the developmental 
stage of an organism (e.g., De Toledo-Morrell, Geinisman, & Morrell, 1988). This 
potential for regulating plasticity has been labeled metaplasticity and has recently been 
demonstrated by observations that capacity for long-term potentiation (LTP), an 
important form of synaptic plasticity, can be altered by the recent activational history of 
the synapses involved (Abraham & Tate, 1997). One interesting potential consequence 
of metaplasticity is that functions dependent upon plasticity have the potential to be 
subtly disrupted or regulated by metaplastic effects. For example, metaplasticity might 
result in changes in the efficacy of learning and memory functions by regulating the ease 
or strength of induction of plasticity in mediating circuitry.

1.2. Definition and Basic Characteristics of Kindling

Kindling can be defined most generally as an enduring enhancement of 
responsiveness to a stimulus resulting from repeated exposure to the same or a similar 
stimulus. Corcoran (unpublished) has argued cogently that the usefulness of the term 
may be diminished by such a broad definition and has suggested that kindling be
reserved to describe enhanced responsiveness to seizure-evoking stimuli resulting from repeated exposure to subconvulsant levels of the seizure-evoking stimulus (usually electrical or chemical stimulation). In the present dissertation, I will primarily concern myself with kindling using electrical stimulation and unless otherwise indicated will use the term to refer to this kind of kindling exclusively.

Given the above definition, it is apparent that kindling simultaneously refers to both a procedure and a process. As a procedure, then, kindling refers to the repeated application of focal electrical stimulation via a chronic indwelling electrode to a discrete brain region. This procedure includes a number of important parameters that are worth considering including the intensity, pattern, and duration of electrical stimulation, the interval between stimulations, and the brain region stimulated.

The intensity of stimulation required for kindling is quite low, with as little as 5 μA sufficient in some brain regions (personal observations). However, the stimulation must be of sufficient intensity to evoke afterdischarge (AD) in the region being stimulated (Racine, 1972). This intensity, termed the AD threshold (ADT), varies significantly between structures and can be as great as 10000 μA in some cortical regions (personal observations). Kindling is effective with a variety of patterns and durations of stimulation. Typically, a 1 sec train of balanced biphasic square wave pulses at 60 Hz is used. However, kindling can be achieved with trains as long as 60 secs, sine wave stimulation, and frequencies of 25 to 150 Hz (Goddard et al., 1969). Kindling with very low frequencies (e.g., 3 Hz) has also been shown to be effective if sufficiently high stimulation intensities are used (Corcoran & Cain, 1980).

The interval between kindling stimulations is also a critical factor (Goddard et
al., 1969; Racine, 1978). A minimum interval of 20 minutes is required for kindling to occur suggesting the presence of some kind of post-ictal inhibitory process that dissipates fairly rapidly. Kindling at relatively short intervals leads to slower kindling rates, at least as measured by the total number of stimulations required to elicit seizures of a given severity, compared to kindling at intervals of 8 hours or more. Kindling in rats has been shown with stimulation at intervals of as long as 7 days.

The stimulation site used for kindling has a significant impact on the kindling process. Two general patterns have been identified – limbic and cortical kindling (Burnham, 1975; Seidel & Corcoran, 1986). The limbic kindling pattern is observed with stimulation of subcortical sites such as the amygdala or basal forebrain and cortical sites such as the HPCal formation, pyriform cortex, or olfactory bulbs. It is characterized by low ADTs, the absence of clinical signs with initial ADs, progressive increases in AD duration, and the appearance of clinical signs that progress across stimulations through a predictable sequence of stages and culminate in clonic- tonic generalized seizures. In contrast, the cortical kindling pattern is observed with stimulation of sites in the anterior neocortex including the motor cortex, parietal cortex, dorsal prefrontal cortex, and claustrum. It is characterized by high ADTs, short AD durations that remain relatively constant, the appearance of clinical signs early into kindling and often during the stimulation train, and refractoriness such that stimulation of a previously effective intensity sometimes fails to elicit AD on a subsequent stimulation trial. It is noteworthy that if kindling is continued, fully generalized cortical-type seizures will progress to a state that more closely resembles limbic-type seizures (e.g., AD durations will grow, limbic type clonic-tonic convulsions are observed).
It is also important to note that, although stimulation in most sites can be generally classified according to these patterns, some variation exists. For example, some sites, such as the PRH, exhibit a pattern that is intermediate between these two classifications (personal observations). Also, even for sites that exhibit the same general pattern, considerable variability between sites exists in terms of kindling rates, ADTs, AD durations, and both quantitative and qualitative aspects of the accompanying clinical signs.

As a process, kindling can be broken down into two classes of phenomenon. First, kindling produces a gradual decline in ADT. This is an exclusively local phenomenon since ADT is altered only in the site being stimulated (Racine, 1972a). Secondly, kindling produces progressive increases in the complexity, amplitude, duration, and propagation of AD. Propagation of AD to other areas is associated with the development of increasingly severe seizures, which have been classified by Racine (1972b) according to a 6 point scale. Stage 0 is indicated by little or no convulsive signs but may include an increase in exploratory behavior; Stage 1 is indicated by immobility, blinking, and rhythmic mastication; Stage 2 is indicated by rhythmic head nodding; Stage 3 is indicated by unilateral forelimb clonus; Stage 4 is indicated by bilateral forelimb clonus; and Stage 5 is indicated by hindlimb clonus, rearing, and postural instability. Each stage also includes the clinical signs of the previous stages. Stage 5 is often considered an endpoint for kindling since seizure severity remains stable at this stage for a considerable period of time. However, more severe seizures eventually occur and have been classified by Pinel up to a 9 point scale (Pinel & Rovner, 1978). Changes in AD characteristics are at least in part a transynaptic phenomenon.
This is evident in the phenomenon of transfer where kindling in one site usually leads to an increase in the subsequent kindling rate from a second site. This is observed even if the primary site is lesioned providing strong evidence that transfer is due to transynaptic changes outside the initial kindling focus (Racine, 1972b). While the substrates of kindling transfer have not been definitively established, it is generally believed that the circuitry that specifically drives motor aspects of kindled seizures is similar regardless of the site of stimulation and it is changes in this circuitry that underlies transfer. Moreover, this data, as well as that from a variety of other types of studies suggests that a common or preferred motor substrate is activated regardless of the site being kindled and this, in turn, underlies the similarities in the clinical seizures observed following kindling in different regions (Applegate, 1998; Burchfiel, Applegate, Samoriski, & Nierenberg, 1998; McIntyre & Kelly, 1998).

In summary, kindling refers to a procedure in which weak levels of electrical stimulation, sufficient to evoke AD, are applied repeatedly to a focal brain region via a chronic indwelling electrode at intervals ranging from hours to days. Kindling also refers to a process involving progressive and enduring changes in neuronal responsivity that are manifest in a decline in local ADT, increases in the duration, complexity, and propagation of AD from the stimulation site, and a predictable progression in the severity of the accompanying convulsive response.

1.3. Reasons for Studying Kindling

The most common rationale for investigating kindling phenomena is their potential relevance to epilepsy. This is not surprising since kindling as both a procedure
and a process is inherently defined in terms of epileptic criteria. As a procedure, kindling uses stimuli that must meet the criterion of being sufficient to evoke AD and as a process, kindling is monitored in terms of changes in AD and the accompanying convulsive response. Thus, kindling is widely viewed as one of the best preparations available for studying epilepsy and the processes of epileptogenesis in particular (Engel, 1998; Sato, Racine, & McIntyre, 1990).

However, since its discovery, kindling was also recognized as being of more general interest as a preparation for studying neural plasticity in general (Goddard et al., 1969). Indeed, much of the initial excitement surrounding kindling derived from analogies drawn between kindling and mnemonic processes.

1.4. Theoretical Consideration of the Consequences of Kindling

Kindling involves the repeated application of focal stimulation to a discrete brain region. This stimulation induces epileptiform patterns of electrical activity and, across stimulations, is eventually accompanied by convulsive behavior. Based on these observations it can be inferred that the nervous system changes as a result of kindling since the stimulation parameters remain invariant yet the evoked response changes. Thus kindling utilizes nervous system plasticity. Theoretical considerations illustrate that at least three types of consequences might be expected to accompany kindling-induced changes in the brain (see Figure 1).

First, kindling-induced changes in the nervous system might be expected to have epileptogenic effects. Thus, the input of an invariant epileptogenic stimulus to a nervous system altered by kindling would be predicted to evoked enhanced epileptiform
Figure 1. Schematic figure describing the procedure of kindling (A) and three possible consequences (B) of this procedure.
A. The procedure of kindling:

1) Input = epileptogenic stimulus $\rightarrow$ Nervous System $\rightarrow$ Output = epileptiform activity (electrographic and behavioral)

2) Repeat 1)

B. The consequences of kindling:

i) *Epileptogenic*

Input = epileptogenic stimuli $\rightarrow$ A kindled nervous system $\rightarrow$ Output = enhanced epileptiform activity (electrographic and/or behavioral)

ii) *Behavioral*

Input = non-epileptogenic environmental stimuli $\rightarrow$ A kindled nervous system $\rightarrow$

Output = altered behavior

iii) *Metaplastic*

Input = non-epileptogenic environmental plasticity-inducing stimuli $\rightarrow$ Altered plasticity in a kindled nervous system $\rightarrow$ Output = altered behavioral change (e.g., differences in learning and memory, development etc.)
output relative to expected or pre-kindling levels as might be evident in increases in
electrographic and/or behavioral seizure activity. Indeed, since this is in fact the
defining characteristic of kindling (i.e., an enhanced output to epileptogenic stimuli), the
theoretical prediction that kindling has epileptogenic effects is consistently borne out in
experimental findings.

Second, kindling-induced nervous system changes might be expected to have
non-epileptic behavioral effects. Thus, the input of novel environmental stimuli to a
nervous system altered by kindling would be predicted to evoke altered behavioral
output relative to expected or pre-kindling levels. Note that this model does not restrict
the direction or nature of behavioral change that might be predicted to result from
kindling. As will be discussed later in more detail, this prediction is supported by some
experimental findings indicating that sensorimotor responses and anxiety-related
behaviors can be altered by kindling.

Third, kindling-induced nervous system changes might be expected to have
metaplastic effects. Thus, the input of novel plasticity-evoking environmental stimuli to
a nervous system altered by kindling would be predicted to evoke altered degrees of
plasticity relative to expected or pre-kindling levels and resultant changes in function
dependent upon that plasticity. Note that this model does not restrict the direction or
nature of behavioral change that might be predicted to result from kindling. Metaplastic
effects might come in at least two obvious forms. First, mnemonic-type metaplastic
effects might be observed. In this case, the repeated input of environmental stimuli to a
nervous system altered by kindling would be predicted to evoke differences in the nature
or extent of altered behavioral output relative to expected or pre-kindling levels.
Second, developmental-type metaplastic effects might be observed. In this case, the input of environmental stimuli across developmentally relevant time intervals to a nervous system altered by kindling would be predicted to evoke differences in the nature or extent of altered behavioral output relative to expected or pre-kindling levels. As will be discussed later in more detail, the prediction that kindling has mnemonic-type metaplastic effects is supported by some experimental findings, which indicate that several classes and stages of memory function can be affected by kindling. The prediction that kindling has developmental-type metaplastic effects is both theoretically and clinically interesting but has received limited investigation.

The present dissertation is primarily concerned with the behavioral and metaplastic effects of kindling. Therefore, I would like to consider in greater detail the theoretical circumstances under which kindling, which clearly produces epileptogenic consequences, would also be predicted to have behavioral and/or metaplastic effects. The consequences of kindling might take three general forms. First, neural changes associated with the kindled state might be restricted in their functional impact to epileptogenicity (i.e., only epileptogenic consequences are produced). For example, kindling-induced neural changes might remain in a latent state until the nervous system is activated in an epileptogenic fashion and then, and only then, do they become manifest in the sole activity they impact — the generation of a seizure. Second, neural changes associated with the kindled state might have a minimal impact on functional capacity of altered neurons or circuits. That is, kindling-induced neural changes might produce minor changes in neural or circuit function that are amplified in their impact by epileptogenic stimuli but are relatively unimportant under the physiological conditions
associated with normal behavior. Thus, under normal physiological conditions, these changes might be evident in a way that is measurable with sophisticated physiological techniques but not by behavioral analyses, perhaps because minor alterations in normal neuronal or circuit activity may be rendered behaviorally meaningless by redundancy or other forms of compensation within the systems or mechanisms mediating behavior.

Third, neural changes associated with the kindled state might have a meaningful impact on the normal functional capacities of altered neurons or circuits. That is, kindling-induced neural changes might affect the activity and/or plasticity of neurons and/or circuits under normal physiological conditions in a way that impacts the normal functions of these neurons and/or circuits. Thus, they would be evident in inter-ictal changes in the behavioral functions mediated by affected neurons or circuits.

The studies in the present dissertation investigated the impact of kindling on a variety of subsequent behaviors in order to assess changes in mnemonic functions. Although such effects might be mediated by a variety of mechanisms, one distinct possibility is that kindling does induce metaplastic effects, as described above, which result in alterations in plastic processes involved in information storage (and thus changes in learning or memory dependent behavior). Before considering the background literature for previous evidence of such effects and then outlining the details of my experiments, I will briefly consider two important reasons for studying the mnemonic effects of kindling.

1.5. Reasons for Studying the Mnemonic Effects of Kindling

The most obvious reason for investigating the effects of kindling on mnemonic
function is their relevance to the mnemonic dysfunction frequently observed in patients with epilepsy. It is well documented that impairments in a variety of mnemonic functions are observed more frequently in patients with epilepsy compared to other comparable populations (Pedersen & Dam, 1986), and that mnemonic disturbances are most common in a subset of patients with complex partial seizures (Hermann, Seidenberg, Schoenfeld, & Davies, 1997; Pedersen & Dam, 1986; Perrine & Kiolbasa, 1999). Disturbances in verbal memory are most frequently observed (Breier et al., 1996b; Hermann et al., 1997; Kilpatrick et al., 1997; Perrine et al., 1995; Perrine & Kiolbasa, 1999; Sass et al., 1995); however, deficits in non-verbal memory such as visuospatial memory are also observed (Breier et al., 1996a; Breier et al., 1996b; Kilpatrick et al., 1997). In addition, working memory (WM) deficits that are accompanied by abnormalities in event-related potentials have been noted (Grippo et al., 1994; Grippo, Pelosi, Mehta, & Blumhardt, 1996). Mnemonic impairments are observed early on in epileptic disorders, as evidenced by verbal memory impairments that are seen in patients with newly diagnosed cryptogenic partial seizures (Aikia, Kalviainen, & Riekkinen, 1995) and are a source of frequent complaints for patients (Giovagnoli, Mascheroni, & Avanzini, 1997; Thompson & Corcoran, 1992). Moreover, patient’s reports of their own memory dysfunction, if anything, underestimate the severity and extent of impairments apparent on laboratory memory tests (Giovagnoli et al., 1997; Thompson & Corcoran, 1992). Finally, the clinical significance of memory dysfunction in epilepsy is underscored by the fact that memory problems correlate inversely with measures of quality of life in this population (Perrine et al., 1995).

The mechanisms underlying mnemonic dysfunction in epilepsy are unknown.
Some contribution is likely made by damage to structures involved in memory.

However, not all patients with memory problems have detectable lesions (Aikia et al., 1995; Grippo et al., 1996), and measures of pathology are highly variable in the extent to which they correlate with aspects of memory performance (Hermann et al., 1997; Paradiso, Hermann, & Robinson, 1995). Thus other factors are also likely to make a significant contribution to the mnemonic dysfunction seen in patients with epilepsy.

Unfortunately, attempts to determine the full causes of mnemonic dysfunction in epilepsy are made difficult by the presence of a variety of potentially significant variables that are difficult to control in the clinical setting. These include seizure focus, seizure severity, seizure frequency, type of seizure, duration of the disease, the presence of underlying or associated pathology, age of onset, the nature and presence of an obvious precipitating event, the interval between the last seizure and behavioral testing, premorbid or baseline levels of functioning, drug history and current status, and a variety of psychosocial factors related to having an unpredictable and potentially traumatic disorder. Therefore, a suitable animal preparation is needed to disentangle the many factors that may be of significance to changes in mnemonic function in epilepsy. Kindling is an especially suitable model because one has precise control over the focus, severity, history, and frequency of seizures as well as the interval between the last seizure and behavioral testing. This latter fact is important because it enables one to assess the effects of kindling-induced brain changes independent of the acute after-effects of a seizure.

If kindling is to be used to investigate the mechanisms underlying epilepsy-associated mnemonic dysfunction, it is important to consider what aspects of epilepsy
are best modeled by kindling. Due to its focal induction, propensity to widespread propagation, ease of induction in limbic structures, and characteristic convulsive symptoms, kindling is most relevant to one type of epilepsy frequently labeled temporal lobe epilepsy or, more appropriately, complex partial seizure disorder. Like their kindled counterparts, complex partial seizures are focal in origin but subsequently show widespread generalization. They are accompanied by a loss of consciousness and frequently, although not invariably, have motor components. These typically involve orofacial automatisms but sometimes increase in severity to the point of being manifest in fully generalized tonic-clonic convulsions.

Kindling can also be considered most relevant to select aspects of epileptic disorders. Specifically, kindling best reflects processes related to epileptogenesis and progression in the severity of an existing epileptic condition (see Engel, 1998). For example, processes by which seizure threshold decreases (a presumed necessity for the onset of spontaneous seizures), a subclinical irritative focus progresses to a clinical seizure, and clinical seizures become more severe are mirrored in kindling by progressive decreases in the threshold stimulus required to elicit AD, progressive increases in the duration and propagation of initially focal AD, and the well recognized characteristic progression in the severity of convulsions that accompany kindling.

Notable aspects of epilepsy not reflected in kindling as conventionally used in rodents include the appearance of spontaneous seizures and HPCal sclerosis, a neuropathological feature often observed in patients with complex partial seizure disorders (Paradiso et al., 1995).

In summary, the effects of kindling on mnemonic function can be considered
most relevant to the roles of epileptogenesis and progression in seizure severity in mnemonic dysfunction accompanying complex partial seizure disorders, in the absence of the confounding influences of spontaneous seizures and HPCal sclerosis. It should be noted, however, that when continued beyond periods typically employed (often 200 or more stimulations), kindling in rats can produce both spontaneous seizures (Pinel & Rovner, 1978; Wada, Sato, & Corcoran, 1974) and neuropathology resembling HPCal sclerosis (Cavazos, Das, & Sutula, 1994; Sutula, Lauersdorf, Lynch, Jurgella, & Woodard, 1995), and thus may be useful for investigating the role of these factors in changes in mnemonic function in epilepsy as well.

A second and equally compelling rationale for investigating the effects of kindling on learning and memory lies in a basic interest in brain-behavior relations. Kindling is one of a few manipulations of the brain that lead to a variety of enduring changes in function in an adult mammal in the absence of gross damage. These include changes in gene activity, receptor structure and function, synaptic connectivity and structure, neurotrophic factor activity and a variety of other intracellular and extracellular functions, which, in turn, are manifest in changes in the excitability of individual neurons and the transmission of signals within multi-synaptic circuits (Corcoran & Moshe, 1998). Investigating the effects of kindling on mnemonic function addresses the curiosity driven question “what are the behavioral consequences of inducing such a collage of enduring changes in the brain?” In addition, this approach may be useful in the search for the mechanisms involved in mnemonic function as a convergent method that is distinct from yet complementary to more traditional approaches involving lesions, stimulation, recording, or pharmacological interventions.
(e.g., Rosen, Hamerman, Sitcoske, & Glowa, 1996). The use of kindling in this
capacity can capitalize on an existing body of knowledge about the molecular,
anatomical, and electrophysiological consequences of kindling that will continue to
compound since kindling remains a heavily investigated preparation for the study of
experimental epilepsy.

1.6. General Approach to Brain-Behavior Investigations

The design of the studies in this dissertation was guided by a general approach
to brain-behavior investigations. The investigation of most brain-behavior relations can
be divided into three general steps. In the first step, the question – “Does brain
manipulation A produce behavioral effect B?” is investigated. At this stage, research
primarily involves characterizing the nature of a given effect and exploring the
conditions under which it is observed. In the second step, the question – “Why or how
does brain manipulation A produce behavioral effect B?” is investigated. At this stage,
research involves testing hypotheses regarding potential mechanisms underlying the link
between A and B. Hypothetical mechanisms are usually generated based on
characterization of the phenomenon in the first step and relations made to other areas of
research. In the third step, the question – “How can the effects of brain manipulation A
on behavior B be modulated or reversed?” is addressed. At this stage, research often
involves further exploration of mechanistic links and, when the relation between A and
B has clinical relevance, interventions with therapeutic potential are frequently
investigated.

In the case of my research on the mnemonic effects of kindling these questions
take on the form – "Does kindling affect mnemonic function?", "What are the mechanisms mediating the effects of kindling on mnemonic function?", and "How can normal mnemonic function be restored after kindling?".

At the outset of my research, then, it was necessary to determine the current stage of investigations into the mnemonic effects of kindling to identify the types of research questions I should be addressing.

1.7. Review of the Effects of Kindling on Mnemonic Function – Studies Prior to 1996

In the following section, I will review the literature on the effects of kindling on learning and memory that was available when I began my dissertation research. The majority of these studies support two general findings: 1) kindling of the amygdala (AM) can disrupt aversive conditioning and, 2) kindling of the hippocampus (HPC) can disrupt spatial cognition.

A number of studies have shown that kindling can disrupt performance on aversively motivated conditioning tasks. These tasks share the common feature that performance requires the formation of an association between sensory stimuli and their affective significance, and hence they can be classified as assessing emotional cognition. It should be noted, however, that although all the tasks rely generally upon emotional cognition, they do differ notably in the types of stimulus processing they require and in the types of responses they demand. Hence these tasks might be expected to show differences in their relative sensitivity to qualitatively and quantitatively different kinds of kindling-induced impairments.
The earliest studies on the effects of kindling on mnemonic function showed that aversive conditioning was sensitive to disruption by prior kindling of the AM. In the first such study, McIntyre and Molino (1972) found that full AM kindling coupled with a contralateral AM lesion impaired acquisition of a conditioned emotional response. Subsequently, Boast and McIntyre (1977) showed that bilateral AM kindling was also effective in disrupting emotional memory using a passive avoidance (PA) task. This result was later replicated by Peele and Gilbert (1992), and then extended by Stone and Gold (1988) who showed that unilateral AM kindling could also disrupt PA. However, negative results were obtained by Bawden and Racine (1979) who found that full bilateral kindling of either the AM or lateral septum failed to disrupt PA performance. The reason for this discrepancy is not immediately clear but could relate to procedural variables that may have affected baseline levels of performance in the Bawden and Racine study (see Hannesson & Corcoran [2000] for a discussion). Finally, full AM kindling has been found to disrupt retention of a shock-motivated brightness discrimination in a Y-maze (Becker & Grecksch, 1992; Becker et al., 1992) further strengthening the conclusion that AM kindling can impair aversive conditioning. However, a general defect in emotional memory is likely not produced by AM kindling since acquisition of a shock-motivated active avoidance task is spared (Becker et al., 1992) as is acquisition of a conditioned taste aversion (Peele & Gilbert, 1992). These data suggest that the nature of the kindling induced impairment on aversive conditioning tasks may involve an interaction between emotional memory and other behavioral or cognitive demands that may relate quite specifically to subtle variations in demands between tasks.
The second grouping of studies investigated the effects of kindling on spatial cognition, defined as the ability to acquire, maintain, and use allocentric (i.e., viewer independent) spatial information (O'Keefe & Nadel, 1978). Because much of the remainder of this dissertation will be concerned with spatial cognition and only two spatial tasks have been used in all of the studies I will be discussing, it is worth describing these two tasks and some of their procedural variations in some detail.

The two tasks that have been employed to assess the effects of kindling on spatial cognition are the radial-arm maze (RAM) (Olton & Samuelson, 1976) and the Morris water maze (MWM) (Morris, 1984). In a typical RAM, 8 arms radiate from a central platform, each containing an appetitive reward located in a well near the end of the arm. Optimal performance requires that the subject visit each arm once, retrieve the bait, and then move on to unvisited arms until all the bait has been retrieved and no arms have been revisited. Because the arms are identical, they must be differentiated based on their position relative to extra-maze cues, and the task thereby demands the use of allocentric spatial information. It also requires the use of a short-term memory (STM) store, termed WM, which represents information about which arms have been visited within a trial. This trial specific memory is distinguished from memory for information that is held constant within and between trials, termed reference memory (RM). A common variation of the RAM task, which I will call the WM/RM version, involves baiting only a fraction of the arms, such that baited arms assess WM as in the standard RAM and unbaited arms assess RM since they require a constant response (i.e., avoidance) within and between trials.

In a typical MWM, a circular pool with undifferentiated inner walls is filled with
cool opaque water and an escape platform is positioned just below the surface of the water in a constant location. Because no local cues mark the position of the platform, the subject must locate it by its position relative to the configuration of extra-maze cues, and, like the RAM, the task thereby demands the use of allocentric spatial information. Using the terminology evolved for the RAM, the standard MWM task requires RM since the platform position remains constant both within and between trials. The MWM can also be modified to assess WM-like performance by moving the platform between sessions of trials, thereby requiring subjects to change responses (i.e., approach a new location) across trial sessions.

In the first study of kindling's effects on spatial cognition, Lopes da Silva and colleagues (Lopes Da Silva, Gorter, & Wadman, 1986) investigated the effects of full kindling in the dorsal hippocampus (dHPC) on performance in the combined WM/RM version of the RAM. They found that kindling impaired both WM and RM during testing concurrent with kindling but only RM when testing was continued after the completion of kindling. These results were subsequently supported and extended by Leung and colleagues who showed that WM performance in the standard RAM was disrupted by either full or partial dHPC kindling (Leung & Shen, 1991; Leung, Zhao, & Shen, 1994; Leung, Boon, Kaibara, & Innis, 1990; Leung, Brzozowski, & Shen, 1996). The above studies used electrode placements concentrated in the CA1 region. Feasey-Truger and ten Bruggencate (1993) then showed that full kindling in the dentate gyrus could also disrupt spatial cognition as evidenced by impaired RM performance in the WM/RM version of the RAM. Another notable design feature of all of the above studies was that subjects were trained before being kindled. Thus, the impairments
observed could be attributed to either retrograde or anterograde effects. Since, seizures are well known to produce a retrograde disruption of memory-related performance (McGaugh, 1966), the anterograde and or retrograde nature of kindling’s effects in these studies cannot be ascertained with certainty. However, Sutula and colleagues (Sutula et al., 1995) subsequently showed that in at least some cases kindling can produce an anterograde disruption of spatial cognition. They found that extended kindling of the olfactory bulb impaired RM in the combined WM/RM version of the RAM in subjects that received all testing after the completion of kindling. While these studies indicate that spatial cognition in the RAM is sensitive to the disruptive effects of kindling, there are also two studies showing that RAM performance may remain intact following kindling. Robinson and colleagues (Robinson, McNeill, & Reed, 1993) found that neither unilateral nor bilateral kindling of the perforant path disrupts acquisition in the RAM and Letty and colleagues (Letty, Lerner-Natoli, & Rondouin, 1995) found that full AM kindling also does not disrupt RAM acquisition.

Findings in the MWM also raise questions regarding the generality of kindling’s disruptive effects on spatial cognition. Full kindling of the lateral septum or basolateral AM does not disrupt acquisition in the MWM (McNamara, Kirkby, dePape, & Corcoran, 1992; McNamara, Kirkby, dePape, Skelton, & Corcoran, 1993) nor does full kindling of the ventral HPC in juvenile rats (Holmes et al., 1993). Similarly, Nieminen and colleagues (Nieminen et al., 1992) found that full kindling of the AM fails to disrupt MWM acquisition. Full kindling of the perforant path produces a mild impairment of acquisition in the MWM restricted to the first day of testing (McNamara et al., 1992). However, the authors in this study suggest that the impairment was non-spatial in nature
and may relate to a non-specific disruption of sensorimotor processes by the last kindled seizure induced 24 hours prior to testing.

Reasons for the discrepancy between the effects of kindling in the RAM, which predominantly showed impairments, and results in the MWM, which exclusively showed sparing, were illuminated by a study in our laboratory in which I acquired my first experience investigating the mnemonic effects of kindling. We found that full but not partial kindling of the dHPCal CA1 region disrupted acquisition in the MWM, whereas both full and partial kindling disrupted retention in rats trained prior to kindling (Gilbert, McNamara, & Corcoran, 1996). These findings suggested several features of kindling’s effects on spatial cognition. First, the site being kindled appears to be important, with the dHPC being an especially sensitive region. Second, the extent of kindling appears to be important with full but not partial kindling capable of producing a strictly anterograde impairment. Last, anterograde and retrograde effects appear to differ in their sensitivity to the disruptive effects of kindling with retrograde effects being more susceptible to less extensive degrees of kindling.

Collectively, the above data indicate that knowledge about the effects of kindling on learning and memory was still in its relative infancy when I began my research. Using the terminology introduced earlier, then, investigation into this area was still in the first stage where the relation between manipulation A (kindling) and behavior B (learning and memory related behaviors) was still being characterized. Thus, numerous questions regarding the nature of kindling’s effects on mnemonic function and the conditions under which these effects were observed still remained. Thus, as the purpose of my dissertation I chose to further characterize the effects of kindling on mnemonic
function. Moreover, I chose to focus on the effects of dHPCal kindling on spatial
cognition for several reasons. First, our laboratory was equipped to study spatial
cognition in the Morris water maze. Second, spatial cognition has been extensively
studied and its critical dependence on the HPCal system is well established. Third,
spatial cognition may represent one example of a more general form of memory,
declarative memory (Eichenbaum, 1999; Eichenbaum & Otto, 1992; Eichenbaum, Otto,
& Cohen, 1994), which is highly developed and broadly used by humans (Squire, 1992).
Thus, studying spatial cognition may be directly relevant to an important class of human
memory function. Fourth, although it is currently unclear whether this involvement is
causative or consequential, many researchers believe the HPC is particularly involved in
complex partial epilepsy (Engel, 1998; Sutula, 1990) and thus the effects of dHPC
kindling on mnemonic function may have particular clinical relevance.

1.8. Unanswered Questions Concerning the Effects of Hippocampal Kindling on
Spatial Cognition

Our current understanding of the effects of kindling on mnemonic function
leaves several fundamental questions that still require adequate characterization. These
can be broken down into two general categories: 1) Questions about the nature of
kindling's effects on mnemonic function, and 2) Questions about the impact of kindling-
related variables on mnemonic outcome.

Of primary importance in studying the effects of kindling on mnemonic function,
is understanding the precise nature of any such effects. This involves several steps.
First, behavioral tasks that are sensitive to changes induced by kindling must be
identified. Second, the role of non-mnemonic effects in these tasks must be considered in order to determine whether performance changes are indeed related to effects on mnemonic function. Last, it has become increasingly apparent that memory does not represent a unitary global function but instead comprises a set of abilities that exhibit stimuli and/or processing specificity and unique operating characteristics. Thus, the nature of the mnemonic disruption needs to be determined including differentiating both the class (e.g., declarative vs procedural memory; Squire, 1992) and stage (e.g., learning vs STM; Eichenbaum, 1996; Rolls, 2000) of mnemonic function affected.

Of secondary importance, then, is elucidating the relation between kindling-related parameters and mnemonic outcome. The list of potentially relevant parameters could be rather lengthy. Hence, I have chosen to consider only a few parameters that are most likely to have an impact on mnemonic outcome. These are: 1) the site of kindling, 2) the extent of kindling, and 3) the interval between kindling and testing. Variations in each of these parameters are associated with differences in the molecular, morphological, and electrophysiological consequences of kindling and hence might be expected to produce variations in associated mnemonic outcome.

Above, I have considered, in general, the types of questions that should be addressed to expand our understanding of the effects of kindling on mnemonic function. The answers to these questions should serve to direct subsequent investigation into the mechanisms underlying such effects.

The experiments in this dissertation will, to varying degrees, speak to the general questions as outlined above. However, the number and scope of these questions is greater than could be sufficiently addressed in any detail in one series of projects. Thus,
the focus of my studies can be further narrowed to several questions within the context of primarily one behavioral paradigm. Specifically, I have investigated the following questions regarding the effects of dHPCal kindling on spatial cognition in the Morris water maze: 1) Does dHPC kindling specifically disrupt spatial cognition?; 2) If so, what stage or stages of mnemonic processing is/are affected?; 3) Is this effect specific to kindling of the dHPC?; and 4) How does this effect relate to the extent of kindling in the dHPC? Answers to these questions should have significant theoretical implications regarding the mechanisms of dHPCal kindling-induced effects on spatial cognition and will be considered in some detail in my discussion sections.

This dissertation consisted of three groups of experiments, which were specifically designed to address the above questions regarding the effects of dHPCal kindling on spatial cognition but which also have implications within the broader context of the general effects of kindling on mnemonic function. In the next section, I will briefly describe the design of each of the experiments and both the specific and general questions each was designed to address.

1.9. The Design and Goals of the Studies in the Present Dissertation

1.9.1. Experiment 1a

In Experiment 1a, I investigated the effects of full kindling of the dHPC on subsequent: 1) acquisition and retention of spatial and object information in two water mazes, and 2) performance on two control tasks. This study addressed whether dHPCal kindling indeed impairs spatial task performance, whether non-mnemonic factors underlie any such impairment, whether an observed mnemonic impairment is specific to
spatial cognition, and whether learning/STM and/or long-term memory (LTM) processes are affected.

1.9.2. Experiment 1b

In Experiment 1b, I investigated the effects of full kindling of the perirhinal cortex (PRH) on the same tasks as in Experiment 1a. The primary purpose of this study was to examine the specificity of the effects observed in Experiment 1a to kindling of the dHPC. This study also addressed whether PRH kindling impairs spatial and/or object-related task performance, whether non-mnemonic factors underlie any such impairment, whether any mnemonic impairment is specific to spatial or object-related cognition, and whether learning/STM and/or LTM processes are affected. Since the role of the PRH in the tasks investigated in this study had not been thoroughly studied, the effects of bilateral partial lesions of the PRH were also investigated. This enabled the effects of PRH kindling to be considered relative to those of comparably placed lesions.

1.9.3. Experiment 2

In Experiment 2, I investigated the role of extent of kindling in the effects of dHPC kindling on spatial cognition in a delayed match-to-place (DMTP) task in the Morris water maze. Rats were kindled until 1, 6, 11, and 16 ADs, and 1 stage 1, and 1 stage 5 seizure were evoked. After each extent of kindling, rats were tested on the DMTP task. This study addressed the effects of dHPC kindling on spatial cognition when a WM component is required and has implications regarding anterograde and retrograde effects. The study also systematically explored the relation between extent of kindling and the disruptive effects of dHPC kindling on spatial cognition.
1.9.4. Experiment 3a

In Experiment 3a, I investigated the effects of full dHPC kindling on subsequent performance in: 1) the elevated plus maze, 2) an open field task, 3) an object recognition task in an open field, and 4) delay-dependent DMTP performance in the MWM. This study addressed the following issues: 1) the role of non-mnemonic effects, including changes in anxiety-related and exploratory behaviors, in spatial deficits, 2) the mnemonic specificity of any deficits, by comparing effects on spatial and object recognition memory, 3) the stage of mnemonic processing affected (learning versus STM), by testing DMTP performance at delays of 0.25, 1, and 4 minutes, and 5) the role of the interval between kindling and testing since both spatial and object recognition memory were tested at longer intervals (2 weeks or greater) than in any previous studies in this dissertation.

1.9.5. Experiment 3b

In Experiment 3b, I investigated the effects of full kindling of the PRH on the same tasks as in Experiment 3a. The primary goal of this study was to determine the site specificity of effects observed in Experiment 3a to kindling of the DHPC. However, this study also addressed the role of the following in any of the observed mnemonic effects of PRH kindling: 1) non-mnemonic effects, including changes in anxiety-related and exploratory behaviors, 2) the class of mnemonic function being tested, including spatial and object recognition memory, and 3) the interval between kindling and testing.

1.9.6. Experiment 3c

In Experiment 3c, I investigated the effects of full kindling of the AM on the same tasks as in Experiment 3a. The primary goal of this study was to determine the
specificity of effects observed in Experiments 3a and 3b to kindling of the DHPC and PRH respectively. However, this study also addressed the role of the following in any of the observed mnemonic effects of AM kindling: 1) non-mnemonic effects, including changes in anxiety-related and exploratory behaviors, 2) the class of mnemonic function being tested, including spatial and object recognition memory, and 3) the interval between kindling and testing.

1.10. Overview

Before proceeding to my studies, I will briefly review and elaborate the main line of questioning developed in this dissertation. The first question was: Does kindling produce a specific disruption of spatial cognition? This is made up of two sub-questions: a) Does kindling disrupt performance on tasks that require allocentric spatial processing? and b) Can spatial tasks deficits be accounted for by non-mnemonic effects including changes in sensorimotor function, motivational/emotional state, and/or general aspects of cognitive function (e.g. attention)? To address question a), performance was tested on three different versions of the MWM task, a 1-day learning version (Experiments 1a and 1b), a DMTP version (Experiment 2), and a variable-delay DMTP version (Experiments 3a, 3b, and 3c). To address question b), performance was tested on a variety of control tasks including visible platform (VP) tasks in the standard water maze and a modified water maze (Experiments 1a and 1b), the elevated plus maze (Experiments 3a, 3b, and 3c), an open field task (Experiments 3a, 3b, and 3c), and two object memory tasks (Experiments 1a, 1b, 3a, 3b, and 3c).

The second question was: What stage of spatial memory processing is affected
(learning, STM, and/or LTM)? To address this question, performance was tested on a 1 day acquisition procedure in the MWM which required learning/STM (Experiments 1a and 1b), a 7 and 28 day retention procedure in the MWM which required LTM, and a variable-delay DMTP procedure which enabled differentiation between effects on learning and STM (Experiments 3a, 3b, and 3c).

The third question was: Do the effects of kindling on spatial cognition relate to the site being kindled? To address this question the effects of kindling of the dHPC and PRH cortex on acquisition and retention performance in MWM were compared (Experiments 1a and 1b). Also, the effects of kindling of the dHPC, PRH, and AM on performance on a variable-delay DMTP version of the MWM were compared (Experiments 3a, 3b, and 3c).
2. EXPERIMENT 1A: DHPC KINDLING'S EFFECTS ON SPATIAL AND OBJECT MEMORY

2.1. Introduction

Kindling of the dHPC has been shown to disrupt performance in the RAM and MWM (Gilbert et al., 1996; Leung & Shen, 1991; Leung et al., 1994; Leung et al., 1990; Leung et al., 1996; Lopes Da Silva et al., 1986). Although these studies provide evidence that kindling of the dHPC can disrupt performance of spatial tasks, a number of questions regarding the exact nature of the underlying impairment remain. Kindling inherently affects activity in widespread brain regions and thus might be expected to alter a variety of different functions capable of compromising spatial task performance. Indeed, kindling has been shown to alter sensory evoked potentials, motor function, emotional behavior, and performance in some nonspatial tasks (Adamec, 1998; Becker & Grecksch, 1992; Boast & McIntyre, 1977; Ehlers & Koob, 1985; Pinel, Kalynchuk, & Treit, 1998; Robinson, Port, & Berger, 1989; Tsuru & Shimada, 1984). Thus, the contribution of non-mnemonic or nonspatial mnemonic impairments to deficits in performance on spatial tasks needs to be seriously investigated. Also, if the kindling-induced deficit is mnemonic, it is not clear from previous studies what stage of memory processing is affected. Thus, kindling's impact on different memory stages also needs to be determined.
The present study was designed to extend our understanding of the effects of dHPC kindling on spatial cognition by addressing some of these questions. First, I wanted to further investigate whether dHPC kindling produces non-mnemonic deficits that are likely to affect performance in spatial tasks. Previous work in the Corcoran lab (Gilbert et al., 1996) has tested rats on the VP variant of the MWM task, in which the rat can ignore spatial cues and swim directly to a platform that protrudes above the surface of the water. This task utilizes sensorimotor functions (e.g., goal-directed swimming, mounting the platform) and motivation (escape from water) similar to those in the conventional spatial MWM task. This study found that dHPC kindled rats were unimpaired, thus providing evidence against the presence of a non-mnemonic impairment. However, rats were tested on the VP task after spatial testing was concluded, at which time short-lasting sensorimotor or motivational deficits may have disappeared due to either the passage of time or the extensive practice rats had received with the water maze task by that point. In the present study, therefore, I tested rats prior to the start of spatial testing and also used a slightly more difficult VP task, in which the position of the VP was changed from trial to trial.

Second, I wanted to investigate the specificity of the mnemonic deficit induced by dHPC kindling to spatial cognition. In addition to testing in the MWM, therefore, I also tested rats on the acquisition and retention of a novel object discrimination (OD) task in a modified water maze. This task shares many of the sensorimotor and motivational features of the spatial MWM task, but differs in its cognitive demands. Namely, it requires object associative memory.

Finally, I wanted to investigate whether dHPC kindling alters learning/STM,
LTM, or both. Although no consensus on the exact time periods constituting these stages of memory processing is available, I will divide the temporal nature of any deficits for convenience according to approximate intervals, with STM representing memory for less than 1 hour and LTM representing memory for greater than 24 hours. Learning/STM has been investigated following dHPC kindling, but, to my knowledge, the long-term retention of spatial information acquired following kindling had not been directly investigated previously. In the Corcoran lab’s previous MWM study (Gilbert et al., 1996), learning/STM (memory between trials within an hour) and LTM (memory between trials across days) were confounded, since acquisition was distributed over 7 days of training. Therefore, in the present study I dissociated the effects of dHPC kindling on learning/STM and LTM by using a testing schedule wherein rats acquired the platform’s location in one day and were tested for retention 7 and 28 days later.

To summarize the procedure, seizures were kindled using stimulation of field CA1 of the dHPC until 3 consecutive fully generalized, or Stage 5, seizures were evoked and, 48 hr later, the rats were tested on a nonspatial VP variant of the MWM task. On the next day, rats were tested for acquisition of a constant HP location in the MWM and were subsequently tested for retention 7 and 28 days later. Forty-eight hr following the last MWM retention test, kindling stimulation resumed until 1 Stage 5 seizure was evoked (rekindling). After another 48 hr, rats were tested for two days on a VP variant of the OD task in a modified water maze. On the next several days, rats were tested for acquisition of an OD problem in the modified water maze, followed by retention testing 7 and 28 days later.
2.2. Methods and Materials

Twenty-two male Long-Evans hooded rats (Charles River) weighing 325-400 g at the beginning of the study were used as subjects. Food and water were available ad libitum throughout the experiment. Rats were maintained in pairs in shoe box cages prior to surgery and were housed individually for the remainder of the experiment. All experimental procedures were carried out during the light portion of the 12:12 hour light/dark cycle. All rats were handled each day throughout the experiment except during the first four days following surgery.

Subjects were randomly assigned to either the kindled group (n=11) or the control group (n=11). The 11 control rats were each yoked to one of the kindled rats.

2.2.1. Surgery

In preparation for surgery, animals were anaesthetized with Somnotol™ (sodium pentobarbital, 60 mg/kg) and given methyl scopolamine (1 mg/kg) to reduce respiratory congestion. Rats were placed in a stereotaxic apparatus, the skull was leveled, and bipolar enamel-insulated nichrome wire electrodes (127 μm dia.) were implanted bilaterally at the following coordinates relative to bregma: -3.5 mm (AP); 2.6 mm (ML); - 3.1 mm (DV). The electrode tips were separated by 0.4 to 0.5 mm, with the lower tip used as the stimulating electrode. Five jeweler’s screws were used to secure the electrode assembly to the skull, with one screw over the anterior cortex serving as the reference electrode. The electrode assembly was affixed to the skull with dental acrylic, and a topical antibiotic/steroid, Topagen™, was applied to the wound. Finally, a subcutaneous injection of Anafen (0.5 cc/kg) was given for postsurgical analgesia.
2.2.2. Kindling

One to two weeks following surgery, the intensity of stimulation required to induce an afterdischarge (AD) in all rats to be kindled and half of the control rats was determined as follows. A Grass S8800 stimulator was used to deliver a 1 sec train of balanced biphasic square wave pulses at 60 pps at an initial intensity of 20μA (base-to-peak). If AD greater than 5 sec in duration was not evoked, intensity was increased step-wise in 20μA increments every 2 minutes until AD was elicited. The minimal intensity triggering at least 5 sec of AD was arbitrarily defined as that rat’s ADT. On the following day, the ADT was determined in the contralateral hemisphere. Note that half of the control rats were also stimulated to determine whether assessing ADT alone might have effects on any of the measures taken in this study.

One day following the completion of ADT determination, kindling was started. Rats in the kindled group received daily stimulation in the right (n=5) or left hemisphere (n=6) at an intensity of 50 μA above ADT until 3 consecutive stage 5 seizures (Racine, 1972b) were evoked. Control rats were handled and then placed in the kindling box, but were not stimulated. Behavioral testing in the MWM commenced approximately 48 hr following the last evoked seizure.

Forty-eight hr after the completion of spatial testing in the MWM, rats were rekindled with daily stimulation at 50 μA above ADT until a single stage 5 seizure was evoked. Behavioral testing in the modified water maze commenced approximately 48 hr following the last evoked seizure.
2.2.3. Behavioral Assessment

2.2.3.1. Apparatus

Behavioral assessment was conducted in a circular fibreglass pool that was 150 cm in diameter, had 45 cm high featureless white walls, and was filled to a height of 26 cm with $22 \pm 1^\circ\text{C}$ water rendered opaque with 1500-2000 ml of skim milk powder. A vertically telescoping platform (11 x 13 cm upper face) that could be adjusted remotely from 8 to 28 cm in height was used. The maze was located in a windowless room with white walls and two doors. Various items placed on the walls (e.g., posters) provided visual cues. Two overhead ventilation fans and a radio placed on the floor facing away from the pool produced background noise. The movement of rats in the pool was recorded and analysed with a video camera coupled to a microcomputer by an image analyser (Chromotrack, San Diego Instruments). A remote switch was used to start and stop recording.

The pool was used for Morris water maze testing as described above, but was modified for OD testing in the following manner. The pool was sectioned into 6 wedges by 3 dividers made of corrugated plastic (see Fig. 2). Each divider formed two walls that were each 45 cm high and 35 cm long. Two objects were hung from the pool wall by wire mesh hooks such that their bottom edge was positioned 2-3 cm above the water’s surface in the corner of two adjacent wedges. Serving as stimuli were a blue plastic circular object with a diameter of 11 cm and a white 10 cm by 10 cm square plastic object with a black cross painted on it.

2.2.3.2. Procedure

The following procedures were common to all types of trials run in the water
Fig. 2. Schematic of the modified water maze as set up for an OD trial. An error was counted on any trial in which the rat's head crossed a line half way into any wedge except the one containing the platform. O' = object over the platform's location, O' = incorrect object, and S = start location.
maze: i) the rat was placed in the pool with its head facing the wall of the pool; ii) trials were terminated after the rat found the platform or a specified time expired; iii) if the rat did not find the platform after a duration specific to each trial type, it was gently guided to the platform; iv) the rat was left on the platform for approximately 10 sec at the end of each trial, after which it was removed to a holding pen 2.5 m from the pool with a 250 W heat lamp located 45 cm above; and v) an inter-trial interval of 2 to 4 min was employed.

2.2.3.2.1. Morris Water Maze

Nonspatial pretraining consisted of VP trials in which a black-sided attachment was added to the platform such that it protruded 3 cm above the water's surface. The platform was placed in a different location, none of which corresponded to platform locations in spatial testing, on each of six trials. The maximum trial duration was 60 sec. Distance and latency to escape were recorded. This trial type enabled rats to habituate to general testing procedures and was used to assess possible sensorimotor, motivational, or general mnemonic deficits.

Spatial testing consisted of hidden-platform (HP) and probe (P) trials. On HP trials, the platform was submerged 3 cm and held in a constant location. Three points equally spaced along the pool wall were used for start locations and were randomly selected without replacement every 3 trials. The use of 3 rather than 4 starting points enabled starting locations to be conveniently chosen in 6 blocks of 3 over the course of the 18 trials that pilot work suggested was ideal for MWM acquisition within our paradigm. The maximum trial duration was 60 sec. Escape distance, escape latency, and direct swims, determined in the following manner, were recorded. A direct swim
was scored each time the rat swam directly to the platform while remaining within a 20 cm wide alley from the start position to the platform's location. On P trials, the platform was lowered to its completely submerged position (18 cm below the water's surface) and remained in the same position as on HP trials. The start position was randomly chosen between one of two possible locations found 45° to either side of the point on the wall most distal to the platform. After 30 sec the platform was raised to 3 cm below the water's surface and the rat was gently guided to the platform. Time spent in each quadrant of the pool and crossings of the platform's location and the corresponding location in each of the other three quadrants were recorded. Quadrant preference was calculated as: time spent in the platform's quadrant / 30 * 100 %.

Crossing preference was calculated as: platform crossings - [other crossings / 3].

2.2.3.2.2. Modified Water Maze

Pretraining consisted of VP trials in which the black-sided platform protruded 3 cm above the water's surface and was placed in a corner position of an open wedge in the modified water maze (see Fig. 2). Rats were gently placed in the pool facing the center of the wall of the wedge farthest from the platform and were permitted to swim for 120 sec or until the platform was found. The wedge that had previously contained the platform served as the start location for the next trial. The platform was moved to one of the two most distant wedge corners with right and left locations chosen pseudo-randomly under the restriction that they were balanced within each block of ten trials. This obviated the successful use of a simple alternating strategy. Latency to escape was recorded, as were errors, counted as any trial in which, after leaving the starting wedge, the rat's head crossed more than half way into either of the two wedges that did not
contain the platform.

Object memory testing consisted of object OD trials in which the platform was submerged 3 cm below the water's surface and placed beneath the correct object (O+). The remainder of the procedure was identical to that for VP trials, except that the corner that was most distal from the start location and did not contain the platform was marked with the incorrect object (O'). Errors, defined as on VP trials, and latency to escape were recorded.

2.2.3.3. Behavioral Testing Schedule

2.2.3.3.1. Morris Water Maze

The schedule for testing in the MWM is shown in Table 1. Two days following the last kindling stimulation, nonspatial pretraining began with one session of testing consisting of 6 VP trials. The following day, spatial training began with acquisition of a constant HP location using the following sequence of trials: 15 HP, 1 P, and then 3 HP trials. To ensure satisfactory acquisition of the platform location prior to retention testing, an additional day of training using a sequence of 3 HP, 1 P, and then 3 HP trials, was administered to any rat that failed to achieve criterion performance, chosen a priori based on pilot data as 5 of the last 6 HP trials with escape distances < 200 cm or 4 of the last 6 HP trials < 200 cm and a correct quadrant dwell % > 40 on the P trial. At both 7 and 28 days after acquisition, retention of the hidden platform location was tested with 3 HP trials and 1 P trial.

2.2.3.3.2. Rekindling

Forty-eight hr after the completion of spatial testing in the MWM, daily stimulation resumed and continued until a single stage 5 seizure was evoked.
Table 1. Schedule for testing in the MWM. Days are relative to the last day of kindling (Day 0). VP = visible platform trials, HP = hidden platform trials, P = probe trials

<table>
<thead>
<tr>
<th>Pretraining</th>
<th>Spatial Memory Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>Day 3 to Day 31</td>
</tr>
<tr>
<td>Visible Platform Testing</td>
<td>Acquisition (Day 3)</td>
</tr>
<tr>
<td>6 VP</td>
<td>15 HP, 1 P, 3 HP</td>
</tr>
</tbody>
</table>
Table 2. Schedule for testing in the modified water maze. Days are relative to the last day of rekindling (Day 0'). VP = visible platform trials. OD = object discrimination trials

<table>
<thead>
<tr>
<th>Pretraining</th>
<th>Object Associative Memory Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 2' and 3'</td>
<td>Day 4' to Day 34', 35', or 36'</td>
</tr>
<tr>
<td>Visible Platform</td>
<td>Acquisition (Day 4' to Criterion)</td>
</tr>
<tr>
<td>Testing</td>
<td></td>
</tr>
</tbody>
</table>
2.2.3.3. Modified Water Maze

The schedule for testing in the modified water maze is shown in Table 2. Two days following the last rekindling stimulation, pretraining began, consisting of two days of testing with 2 blocks of 10 VP trials on each day. On the following day, object associative memory testing began with acquisition training on a single object discrimination problem. Training continued with 2 blocks of 10 OD trials per day until rats achieved criterion performance, chosen a priori based on pilot data as 2 consecutive trial blocks with 2 or fewer errors. At both 7 and 28 days after acquisition, retention of the OD problem was tested with 5 OD trials.

2.2.4. Histology

Following behavioral testing, animals were sacrificed with an overdose of sodium pentobarbital and perfused transcardially with 9% saline. Brains were fixed in formalin and then frozen before 60 μm coronal sections were taken through the dHPC. Every section through the electrode track was mounted and stained with cresyl violet. The location of the electrode tips was documented by matching sections with one of eight plates from Swanson (1992).

2.2.5. Data Analysis

Data analysis was completed using the statistical software package SPSS© for Windows™. Distance, latency, direct swim, correct quadrant dwell percentage, crossing preference, and error measures were subjected to analyses with repeated measures ANOVA, and t tests. Distance data from acquisition in the MWM were log 10 transformed for ANOVA to rectify a violation of the homogeneity of variance requirement. Degrees of freedom for t-tests were adjusted when significantly different
variances were exhibited between groups. Because a learning-related difference in
group performance, and hence an interaction between group and trial blocks, was
expected, MWM acquisition data were subjected to further analyses using planned
comparisons between groups within each trial block of testing. In this regard, simple
main effects were examined and were used to provide an additional characterization of
differences in performance between the groups.

2.3. Results

2.3.1. Histology

Electrodes in all kindled rats included in the study were located in the dHPC.
Specifically, in the 11 kindled rats, the lower tip of the kindling electrode was found in
the pyramidal cell layer (1 rat), the stratum radiatum (5 rats), or the stratum lacunosum
moleculare (5 rats) of the CA1 region (see Fig. 3). In these same rats, the electrode not
used for kindling was found in the dHPCCal CA1 region in 9 rats and in the overlying
corpus callosum in 2 rats. In controls, both electrodes were located in the dHPCCal CA1
region in 7 of 11 rats, and at least one electrode was in the dHPCCal CA1 region in all
rats. Extra-HPCal placements were found in the dorsal thalamus (1 rat) or in the
overlying corpus callosum (3 rats). No gross histological changes were noted in the
brains of either kindled or control rats other than gliosis around the electrode track.

2.3.2. Kindling

Kindling data are shown in Table 3. Briefly, at a mean threshold of 25 ± 2.6µA,
stimulation evoked an initial AD of 29.2 ± 3.8 sec in duration. In all rats, secondary AD
was observed, but was quite variable in latency to onset, duration, and intensity. A
Fig. 3. Location of the lower tip of the stimulating electrode in kindled rats. Plates are posterior relative to bregma and were adapted from Swanson (1992). * = right hemisphere placement, ● = left hemisphere placement.
Table 3. Summary of dHPC kindling and rekindling data. Duration and latency data are in seconds. Stage-5 seizure AD and clonus data for kindling are averaged across the final three stage 5 seizures evoked prior to MWM testing. ADT = threshold for evoking afterdischarge, stim. = stimulations.

<table>
<thead>
<tr>
<th></th>
<th>Kindling</th>
<th>Rekindling</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADT (µA)</td>
<td>25 ± 2.6</td>
<td>n/a</td>
</tr>
<tr>
<td>Initial AD duration</td>
<td>29.2 ± 3.8</td>
<td>59.1 ± 10.5</td>
</tr>
<tr>
<td>Stim. to 1st stage-5 seizure</td>
<td>48.8 ± 6.2</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>Total # of stim.</td>
<td>53.2 ± 6.5</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>Stage-5 seizure AD duration</td>
<td>59.0 ± 6.4</td>
<td>64.8 ± 9.8</td>
</tr>
<tr>
<td>Latency to clonus</td>
<td>21.3 ± 4.6</td>
<td>32.4 ± 10.4</td>
</tr>
<tr>
<td>Duration of clonus</td>
<td>33.5 ± 3.0</td>
<td>30.6 ± 8.4</td>
</tr>
</tbody>
</table>
mean of 48.8 ± 6.2 stimulations was required to evoke the first stage-5 seizure, and 53.2 ± 6.5 stimulations were required to meet our kindling criterion of 3 consecutive stage-5 seizures. On these last 3 stimulations, the mean AD duration, latency to clonus, and duration of clonus were 59.0 ± 6.4, 21.3 ± 4.6, and 33.5 ± 3.0 secs, respectively.

Following MWM testing, rats were rekindled until 1 additional stage-5 seizure was evoked. This required a mean of 1.6 ± .5 stimulations. The mean AD duration, latency to clonus, and duration of clonus for this stage-5 seizure were 64.8 ± 9.8, 32.4 ± 10.4, and 30.6 ± 8.4 secs, respectively. These parameters did not differ significantly from those for the last 3 stage-5 seizures evoked during initial kindling (all p’s ≥ .23).

2.3.3. Behavioral Assessment

2.3.3.1. Controls

The yoked control group in this study comprised 6 unstimulated rats and 5 rats that received a single stimulation to determine ADT in each hemisphere, and thus experienced two ADs, one in each hemisphere. Prior to analyses between kindled and control rats, analyses comparing these two control subgroups on all water maze variables discussed below were first completed. I found that performance by stimulated and non-stimulated controls was nearly identical in all cases, with no differences even approaching statistical significance (all p’s ≥ .490). These findings indicate that two ADs do not have a long-lasting effect on any of the behavioral parameters measured in this study. Thus, for subsequent comparisons with kindled rats, stimulated and nonstimulated control rats are grouped together and hereafter will be referred to simply as controls.
2.3.3.2. Morris Water Maze

2.3.3.2.1. Pretraining - Visible Platform Trials

DHPC kindling did not impair performance on the VP task in the MWM. Kindled and control rats were equally proficient in escaping to the VP as shown by comparable average escape distances over the 6 VP trials ($t(12.7) = 1.01, p = .33$; see Fig. 4; note that for this and all subsequent analyses latencies will not be reported but showed a similar pattern of results). These data suggest that DHPC kindling did not produce gross sensorimotor or motivational deficits, nor did it interfere with the acquisition of many of the basic aspects of water maze performance (e.g., goal directed swimming, mounting the platform, and so on).

2.3.3.2.2. Spatial Memory Testing

2.3.3.2.2.1. Acquisition

DHPC kindling impaired acquisition of a constant platform location in the MWM. Compared to controls, DHPC kindled rats employed longer escape routes and were less likely to head directly to the platform across the 6 trial blocks of acquisition training as shown by a significant group effect in terms of both escape distances ($F(1,20) = 5.83, p = .02$; see Fig. 5a; recall that latency data will not be reported but showed a similar pattern of results) and direct swims ($F(1,20) = 5.24, p = .03$; see Fig. 5b). Subsequent planned comparisons between groups during each trial block indicated that, relative to controls, kindled rats performed significantly worse on at least one measure during trial blocks 2 (latency - $t(89) = 2.24, p < .01$, one-tailed; direct swims - $t(100) = 1.56, p < .05$, one-tailed), 3 (latency - $t(89) = 1.56, p < .05$, one-tailed) and 4 (latency - $t(89) = 2.77, p < .01$, one-tailed; direct swims - $t(100) = 2.50, p < .01$, one-
Fig. 4. Mean escape distances on the 6 VP trials during nonspatial pretraining in the MWM.
Fig. 5. A. Mean escape distances on the 6 HP trial blocks during acquisition of a constant platform location in the MWM (3 trials/block). B. Mean probabilities of a direct swim on any given trial during the 6 HP trial blocks of acquisition of a constant platform location in the MWM (3 trials/block). * p < .05. ** p < .01, one-tailed vs controls.
tailed). Kindled rats also tended to perform more poorly on at least one measure on trial blocks 3 (direct swims – t(100) = 1.26, p < .06, one-tailed), 5 (latency - t(89) = 1.14, p < .08, one-tailed; direct swims – t(100) = 0.94, p < .10, one-tailed) and 6 (latency - t(89) = 1.22, p < .06, one-tailed; direct swims – t(100) = 1.26, p < .06, one-tailed), although these analyses failed to reach statistical significance. Kindled and control rats did not differ significantly in their performance during trial block 1 (latency - t(89) = 0.25, p > .10, one-tailed; direct swims – t(100) = 0.32, p > .10, one-tailed).

Further evidence that kindling impaired MWM acquisition was that 2 of the kindled rats were the only subjects that failed to achieve criterion performance in one day of acquisition training. These rats did, however, achieve criteria performance with 1 additional day of training.

In spite of the acquisition deficit indicated by escape distances, dHPC kindling did not affect performance on the P trial. Both kindled and control rats spent more time searching in the quadrant where the platform was located than in the other three quadrants of the pool and repeatedly passed over the platform’s exact location. The groups did not differ in terms of either correct quadrant dwell percentages (see Fig. 6B) or crossing preferences (data not shown; both t(20)'s ≤ 0.11, p ≥ .91). It is noteworthy that the P trial was administered following trial block 5, at which time kindled rats’ submerged-platform performance was already very good and not significantly worse than controls. Introduction of a P trial earlier on in training might have been more sensitive to the acquisition impairment apparent in escape distances and direct swims. Overall, the above results suggest that dHPC kindling produces a significant although small impairment in the acquisition of spatial information necessary for optimal
Fig. 6. **A.** Mean escape distances on the last HP trial block of acquisition and the HP trial blocks at 7 and 28 days retention (3 trials/block). **B.** Correct quadrant dwell % on the P trial between HP trial blocks 5 and 6 of acquisition and the P trials following the HP trial blocks at 7 and 28 days retention.
performance in the MWM, but does not prevent the achievement of accurate performance levels at a slightly slower rate than controls.

2.3.3.2.2.2. Retention

DHPC kindling did not produce an impairment in the retention of spatial information in the MWM. Both kindled and control rats showed good retention of the platform location and utilized fairly direct escape routes on the 3 retention HP trials at both 7 and 28 days following acquisition. Although both groups showed some forgetting as indicated by a trial block effect in terms of both escape distances and direct swims (both F(2,40)'s ≥ 7.96, both p's < .01), they did not differ in terms of escape distances or direct swims at either time interval as shown by the lack of a group effect (both F(1,20)'s ≤ 0.58, both p's ≥ .45) or a trial block by group interaction (both F(2,40)'s ≤ 0.41, both p's ≥ .67; see Fig. 6A; direct swim data not shown). Good retention was also observed on P trials, where both groups exhibited a significant bias for the platform's quadrant and crossed over the platform's precise location repeatedly. The kindled and control groups did not differ in terms of either correct quadrant dwell percentages (see Fig. 6B) or crossing preferences (data not shown), as indicated by the lack of a group effect (both F(1,20)'s < 0.58, p's > .45) or trial block by group interaction (both F(2,40)'s < 0.47, p's > .63). Forgetting was not apparent on P trials, probably because it was preceded by 3 HP trials at each retention interval and these served as an adequate reminder of the platform's location. The absence of forgetting was shown by the lack of a trial block effect in terms of crossing preferences (F(2,40) = 1.19, p = .32) and a significant trial block effect showing improvement in terms of correct quadrant dwell percentages (F(2,40) = 4.17, p = .02).
Fig. 7. Total number of errors on the 40 VP trials during pretraining in the modified water maze.
2.3.3.3. Modified Water Maze

2.3.3.3.1. Pretraining - Visible Platform Trials

Performance by both groups on the 40 VP trials in the modified water maze did not differ. Kindled and control groups made comparable numbers of errors ($t(20) = -1.10, p = .29$; see Fig. 7) and exhibited similar average escape latencies (data not shown; $t(20) = -0.75, p = .46$). These data again indicate that dHPC kindling did not affect any of the basic sensorimotor or motivational processes required for performance in the modified water maze or in the water maze in general.

2.3.3.3.2. Object Associative Memory Testing

2.3.3.3.2.1. Acquisition and Retention

DHPC kindling did not affect acquisition or long-term retention of the OD problem in the modified water maze. To achieve criterion performance in acquisition (2 consecutive trial blocks with < 2 errors), kindled and control groups required comparable numbers of trial blocks (see Fig. 8A) and made comparable numbers of errors (see Fig. 8B; both $t(20)$'s ≤ -1.20, $p$'s ≥ .25). In retention, at both 7 and 28 days after training, kindled and control groups exhibited excellent performance. Forgetting was not apparent, as each group made comparable numbers of errors on the last 5 trials of acquisition as on the 5 trials at both retention intervals, as shown by the absence of a significant trial block effect ($F(2,40) = 0.80, p = .46$; see Fig. 9). Moreover, the groups did not differ from one another as shown by the absence of a group effect ($F(1,20) = 0.03, p = .87$) or a group by trial block interaction ($F(2,40) = 0.61, p = .55$).
Fig. 8. Performance during acquisition of the OD problem in the modified water maze.

A. Total number of errors to criterion. B. Trial blocks to criterion.
Fig. 9. Total number of errors on the last 5 OD trials of OD acquisition and on the 5 OD trials at 7 and 28 days retention.
2.4. Discussion

In the present study, kindling to a criterion of three consecutive fully generalized seizures by stimulation of the dHPC produced a selective impairment in the subsequent within-day acquisition of a constant hidden platform (HP) location in the MWM. This result contrasted with observations that kindling spared performance on all other components of our testing regimen including: i) retention of the HP location tested 7 and 28 days later; ii) performance on a VP control task in the MWM tested prior to HP testing; iii) acquisition, 7 day retention, and 28 day retention of an OD problem in a modified water maze; and iv) performance on a VP control task in the modified water maze tested prior to OD testing.

The first goal of the present study was to investigate the possibility that dHPC kindling produces a non-mnemonic disruption of behavior that might account for impaired performance on spatial tasks. Several observations suggest that this is not the case. First, kindled rats were not impaired on the VP control task in the MWM when tested 48 hours following the last kindling stimulation (i.e., 24 hr prior to HP acquisition). The VP task shares a number of demands with the spatial MWM task, including determining target position, swimming directly to a goal, mounting a platform, and overcoming thigmotaxic tendencies. Furthermore, the tasks utilize the same motivation, escape from cool water. Since testing on the VP task was done prior to spatial testing, intact VP performance provides strong evidence that an impairment of one of the above components of general water maze performance does not account for the HP acquisition deficit. Second, although kindled rats acquired the HP location more slowly than controls, they did all achieve criterion performance and subsequently
retained the platform location as well as controls. This further suggests that kindled rats were relatively unimpaired with respect to the non-mnemonic abilities required for water maze performance, since, once they acquired the necessary spatial information to locate the platform, they were able to utilize and retain this information as efficiently as controls. Lastly, intact performance on the OD task and its control variation also indicate that kindling did not disrupt non-mnemonic functions necessary for general water maze performance.

The second goal of the present study was to investigate the mnemonic specificity of the deficit produced by dHPC kindling. In the MWM, a HP deficit can be readily accounted for by a disruption of spatial cognition, since the platform can be located most accurately through the concurrent use of multiple cues located outside the featureless walls of the pool and thus requires allocentric spatial processing for optimal performance. However, even in the absence of gross nonspecific behavioral disturbances as discussed above, a HP deficit might also be accounted for by a more general cognitive or attentional impairment. To assess this possibility, I also tested rats on the acquisition and retention of an OD problem in a modified water maze. I found that kindled rats performed as well as controls on this task, indicating that at least one form of nonspatial cognitive processing, object associative learning and memory, was unimpaired. In addition, this result indicates that task difficulty alone cannot account for the HP deficit I observed, since the OD task was more difficult than the HP task (i.e., it required more training to acquire). One caveat in comparing HP and OD task performance is that HP testing was preceded by several weeks of kindling, whereas OD was most immediately preceded by only a few days of rekindling. Thus one might argue
that the dissociation of effects on HP and OD task performance results from differences in the preceding kindling regimen. This argument relies on speculation that the neural changes important for cognitive impairments are differentially induced by the weeks of stimulation required for initial kindling than by the few days required for rekindling, and that these changes fade significantly over the period of approximately 35 days that separated the start of OD from the completion of kindling. Such a possibility would also require that the neural changes that contribute to cognitive impairments are distinct from those underlying the kindled state, since the rapid rekindling and similarities in the seizure characteristics I observed during rekindling demonstrate that the kindled state was maintained prior to OD testing. Although I cannot rule such possibilities out, I believe a more parsimonious interpretation of the data is that dHPC kindling preferentially disrupts spatial task performance over OD task performance.

The third goal of the present study was to investigate whether the kindling-induced deficit in spatial cognition resulted in a disruption of learning/STM, LTM, or both. In our acquisition paradigm, rats received 18 HP trials with an intertrial interval of approximately 3 min and 1 probe trial with an interval of 5 min from the preceding and following HP trials. Therefore, successful acquisition required that rats be able to learn the spatial information necessary for locating the platform and retain it over intervals of less than 5 min (i.e., learning/STM). Retention, on the other hand, was tested 7 and 28 days later and required long-term maintenance of the spatial information necessary for locating the platform (i.e., LTM). Therefore, the impairment in acquisition but not retention indicates that dHPC kindling impairs spatial cognition in a manner that disrupts learning/STM but not LTM processes.
In conclusion, Experiment 1a has shown that full dHPC kindling: 1) produces an impairment in the MWM, 2) does not produce non-specific deficits that would be likely to underlie the MWM impairment, 3) does not produce an impairment of object associative memory, and 4) spares LTM but not learning/STM. Collectively, these data suggest that full kindling of the dHPC produces a selective impairment of spatial learning/STM.
3. EXPERIMENT 1B: PRH KINDLING’S EFFECTS ON SPATIAL AND
OBJECT MEMORY

3.1. Introduction

In Experiment 1a, full kindling of the dHPC was found to selectively impair spatial learning/STM. The primary goal of Experiment 1b was to determine whether this result was specific to kindling of the dHPC. Thus, the effects of full kindling of the PRH were examined on behavioral tasks identical to those used in Experiment 1a.

There are several reasons for choosing the PRH cortex as an alternative kindling site for comparison with the effects of dHPC kindling. First, the PRH has intimate connections with the HPCal formation. Second, the PRH is thought to play a critical role in the generalization of limbic seizure activity in kindling. Third, the PRH is thought to play a critical role in at least some types of memory function. Each of these reasons will be discussed in more detail below.

3.1.1. Anatomy of the PRH

The majority of current data about PRH anatomy is derived from studies with monkeys, though studies with rats suggest that considerable homology exists between species. Since the research in this dissertation involves rats, the majority of the following anatomical discussion will focus on rat data but where information is missing monkey data will be used with the assumption that subsequent investigations on rats will
provide substantially similar information.

The PRH is situated in the medial temporal lobe and is characterized by prominent bidirectional connections with polymodal and unimodal association cortices as well as both the HPCal formation and the amygdalar complex (Burwell, Amaral, & Witter, 1995). It consists of two distinct cytoarchitectonic regions, area 35 and laterally adjacent to it, area 36. In rats, the PRH comprises tissue on both sides of the posterior rhinal sulcus though its precise borders with adjacent cortical regions have not been definitively established. Anteriorly, lies the insular cortex, which can be differentiated from rostral area 35 by its distinct isocortical appearance (6 visible layers), which contrasts with area 35’s more bilaminate appearance. The area 36 insular cortical transition is less distinct and will likely be defined precisely by connectional criteria, though a currently useful landmark is the caudal limit of the claustrum. Ventrally, the PRH is bordered by the entorhinal cortex, which can be distinguished by its cell sparse lamina dessicans (layer IV or V). Timm’s stain and parvalbumin immunoreactivity also mark this border by staining the PRH either darker or lighter respectively relative to the entorhinal cortex. Dorsally and caudally, the PRH abuts temporal association cortex (areas Te2 and Te3, probably auditory in function) and postrhinal cortex respectively. These borders are more difficult to define cytoarchitectonically and will likely be defined precisely by connectional criteria.

The afferents of the PRH can be grouped into unimodal association cortices, polymodal association cortices, and subcortical structures. Unimodal inputs representing all senses project to the PRH including strong visual inputs from occipital cortex (areas 18a and 18b) and area TE of the inferotemporal region (which represents
the strongest single input to the primate PRH), auditory inputs from dorsally adjacent
temporal cortical areas Te2 and Te3, somatosensory information from the insular
cortex, and olfactory input from piriform cortex and the periamygdaloid region. A
variety of polymodal association regions project to PRH including the medial prefrontal,
ventrolateral prefrontal, anterior cingulate, retrosplenial, and entorhinal cortices. The
strongest polymodal input, however, arises from the postrhinal cortex which itself
receives a considerable convergence of polymodal and unimodal information.
Subcortical afferents of the PRH originate in the AM (the lateral, basolateral, accessory,
and central nuclei), the thalamus (perigeniculate region, midline thalamic nuclei, and
lateral posterior thalamic nuclei), and the basal forebrain (nucleus accumbens).

Reciprocal connections are made with many of the above regions. For example,
unimodal association cortices including the occipital, piriform, temporal (areas Te2 and
Te3), and insular cortices receive return connections from the PRH, as do polymodal
areas including the cingulate, entorhinal, orbitofrontal, claustral, and infralimbic cortices.
The lateral entorhinal area in particular receives a dense innervation from the PRH
while, notably, no reciprocal projection to the postrhinal cortex is made. The frontal
cortical areas (Fr1, 2 and 3), which include the motor cortex, also receive a heavy
projection from the PRH that originates predominantly from cells in layers V/VI.
Subcortical PRH efferents are also largely reciprocal and include amygdaloid nuclei
(central, lateral and basolateral, various thalamic nuclei (medial geniculate, mediodorsal,
posterior, reuniens, sub- and parafascicularus, and ventral posteromedial nuclei), CA1
region of the HPC, the caudate/putamen and nucleus accumbens, and several others
such as the fundus striati, central grey, and the substantia nigra, pars compacta.
The connections of the PRH cortex are highly compatible with its proposed roles in both mnemonic processes and limbic seizure generalization. The PRH constitutes higher level polymodal association cortex on the grounds that it receives diverse input from unimodal areas and its greatest input is from other polymodal association areas. Given this access to a substantial amount of diverse and highly processed information the PRH would be well suited for memory functions particularly in light of its significant connections with limbic memory-related structures including the HPCal formation, the AM, and the neostriatum. Moreover, these limbic connections coupled with strong efferents to the frontal motor areas also highlight the PRH as a suitable for both the elaboration and propagation of limbic seizure activity.

3.1.2. PRH’s Role in Seizure Generalization

Epileptic activity is initially localized to the stimulation focus. However, as kindling progresses, epileptiform activity spreads to other areas and convulsive responses of increasing complexity emerge. The substrates of this propagation and elaboration of epileptic activity are unknown but it is generally believed that convulsions initiated from a limbic substrate utilize characteristic pathways to access the motor structures that drive the convulsion. There are several lines of evidence that suggest that the PRH may be a critical component of these characteristic pathways (Applegate, 1998; Burchfiel et al., 1998; Kelly & McIntyre, 1996, McIntyre & Kelly, 1998).

First, as discussed above, anatomical considerations suggest that the PRH is well situated to propagate activity originating in the HPC or AM to widespread brain regions including motor cortex (Burwell et al., 1995). Second, the PRH shows very rapid kindling and a short latency to the onset of clonus indicating facile access to the
substrates of motor seizures (personal observations; Kelly & McIntyre, 1996). Third, prolonged direct stimulation to the “unkindled” PRH produces convulsions that closely resemble those observed in limbic kindling (Applegate, 1998; McIntyre & Kelly, 1998). Fourth, generalized seizures produced by AM kindling produce widespread activation of c-fos ipsilateral to the stimulation focus but activation restricted to the PRH contralateral to the stimulation focus (Applegate, 1998). Fifth, infusions of KCl into the contralateral PRH, which produce spreading depression, truncate AD duration and reduce seizure severity to stage 1 or 2 after AM stimulation in fully kindled rats (McIntyre & Kelly, 1998). Finally, in the kindling antagonism paradigm, the PRH exhibits dominance over the AM suggesting preferred access to the motor substrate of convulsions for the PRH (Mohapel & Corcoran, 1996).

3.1.3. The Role of the PRH in Mnemonic Processes

Since the first work by Scoville & Milner (Scoville & Milner, 1957) with the profound amnesiac H.M., the extreme importance of the temporal lobes in memory has been recognized. Although it initially proved difficult to successfully model H.M.’s amnesia in animals, eventually both primate and rodent models were established. Mishkin’s work highlighted the importance of the HPC and AM in object recognition memory in primates (Mishkin, 1978), while the work of O’Keefe and Nadel highlighted the importance of the HPC in spatial memory in rodents (O'Keefe & Nadel, 1978). While both of these bodies of work correctly identified the HPC as central to memory function, they failed to correctly recognize the roles of the AM and neighboring cortical regions in the temporal lobes’ role in memory function.

Further investigation of the HPC and AM model in primates served as a catalyst
for generating a better understanding of the temporal lobes in memory. One troublesome finding was that an exacerbation of the memory deficit produced by HPC lesions by the addition of an AM lesion could not be reproduced in rodents (O'Keefe & Nadel, 1976). Consideration of the lesion techniques in primate versus rodent studies suggested that adjacent cortical regions might be critical since they were often damaged in primate studies where aspiration was used but not in rodent studies that used electrolytic means for lesioning the AM. This observation, plus a finding by Horel and Pytko (1982) that cooling of the temporal pole region including the PRH and parahippocampal cortices impaired recognition memory, served as an impetus for Squire and colleagues to perform a set of studies involving systematically lesioning different components and combinations of the HPC, AM, and neighboring cortex in primates (Zola-Morgan, Squire, Amaral, & Suzuki, 1989; Zola-Morgan, Squire, & Ramus, 1994). These studies showed that the PRH and parahippocampal cortices but not the AM played a critical role in recognition memory and that this role was even greater than that of the HPC itself. This prompted comparable studies in rodents. These have shown that the PRH plays a more important role in object-related mnemonic processes than the HPC but plays a less important role in spatial memory (Duva et al., 1997; Ennaceur, Neave, & Aggleton, 1996; Mumby & Pinel, 1994; Wiig & Bilkey, 1994a; Wiig & Bilkey, 1994c).

In summary, the PRH has extensive reciprocal connections with the HPC, appears to play a role in the generalization of seizure activity originating in the HPC and other limbic structures, and contributes to some of the same memory functions as the HPC. For these reasons, I chose the PRH as a site to kindle to compare the effects with
those of dHPC al kindling.

3.1.4. Design and Goals of Experiment 1b

In Experiment 1b, seizures were kindled using stimulation of the PRH until 3 consecutive fully generalized, or Stage 5, seizures were evoked. In some rats, additional stimulations in the contralateral PRH were administered until an additional Stage 5 seizure was evoked. Forty-eight hours following the last seizure, rats were tested on a nonspatial VP variant of the MWM task. On the next day, rats were tested for acquisition of a constant HP location in the MWM and were subsequently tested for retention 7 and 28 days later. Forty-eight hr following the last MWM retention test, kindling stimulation resumed until 1 Stage 5 seizure was evoked (rekindling). After another 48 hr, rats were tested for two days on a VP variant of the OD task in a modified water maze. On the next several days, rats were tested for acquisition of an OD problem in the modified water maze, followed by retention testing 7 and 28 days later. Since the effects of PRH lesions on this task were unknown, I also tested rats with bilateral radiofrequency lesions of the anterior PRH on a protocol otherwise identical to that described above beginning 1 week after surgery.

The primary objective of Experiment 1b was to assess the specificity of the kindling-induced impairment of spatial cognition observed in Experiment 1a to kindling of the dHPC. This study also addressed several issues regarding the specific effects of PRH kindling on behavior and the effects of kindling in general. These included whether PRH cortex kindling impairs spatial and/or object-related task performance, whether non-mnemonic factors underlie any such impairment, whether any mnemonic impairment is specific to spatial or object-related cognition, and whether learning/STM
and/or LTM processes are affected. Finally, this study provides evidence regarding the relative involvement of the PRH in spatial and object-related memory.

3.2. Methods and Materials

3.2.1. Subjects

Thirty seven male Long-Evans hooded rats (Charles River) weighing 325-400 g at the beginning of the study were used as subjects. Food and water were available ad libitum throughout the experiment. Rats were maintained in pairs in shoebox cages prior to surgery and were housed individually for the remainder of the experiment. All experimental procedures were carried out during the light portion of the 12:12 hour light/dark cycle. All rats were handled each day throughout the experiment except during the first four days following surgery.

Subjects were randomly assigned to either the kindled group (n=10), the kindling control group (n=10), the lesioned group (n=9), or the lesion control group (n=8). Each kindled rat was yoked with one kindled control rat and each lesioned rat was yoked with one lesioned control rat.

3.2.2. Surgery

In preparation for surgery, animals were anaesthetized with Somnotol™ (sodium pentobarbital, 60 mg/kg) and given methyl scopolamine (1 mg/kg) to reduce respiratory congestion. For the kindling groups, rats were placed in a stereotaxic apparatus and bipolar nichrome wire electrodes (127 µm dia.) were implanted bilaterally at the following coordinates relative to bregma: -1.0 mm (AP); ±4.0 mm (ML); - 8.0 mm (DV) with the toothbar set at + 5.0 mm and the manipulator arm angled 13° towards the
skull. Four jeweler’s screws were used to secure the electrode assembly to the skull, with one screw over the anterior cortex serving as the reference electrode. The electrode assembly was affixed to the skull with dental acrylic, and a topical antibiotic/steroid, Topagen™, was applied to the wound. For the lesion groups, rats were placed in a stereotaxic apparatus, the skull was leveled, and a bipolar nichrome wire electrode (127 μm dia.) with 0.4 mm of insulation scraped off the lower tip was lowered to the following coordinates relative to bregma: -2.8 mm (AP); 4.1 mm (ML); 8.6 mm (DV) with the manipulator arm angled 13º towards the skull. Radiofrequency lesions were produced by brief application of a train of RF current (Radionics, Model RFG-4A, Burlington, Mass.) at 6 to 8 mA for 60 sec. This procedure was then completed in the contralateral hemisphere. The wound was sutured and a topical antibiotic/steroid, Topagen™, was applied locally. For both groups, a subcutaneous injection of Anafen (0.5 cc/kg) was given for postsurgical analgesia before returning the rat to a recovery cage.

3.2.3. Kindling

One to two weeks following surgery, the intensity of stimulation required to induce an afterdischarge (AD) in all rats to be kindled was determined as follows. A Grass S8800 stimulator was used to deliver a 1 sec train of balanced biphasic square wave pulses at 60 pps at an initial intensity of 50 μA (base-to-peak). If AD greater than 5 sec in duration was not evoked, intensity was increased step-wise in 50μA increments up to 200μA, 100μA increments up to 1000μA, and 1000μA increments up to 10000μA every 2 minutes until AD was elicited. The minimal intensity triggering AD was arbitrarily defined as ADT. On the following day, the ADT was determined in the
contralateral hemisphere. Subsequently, rats in the kindled group received daily
stimulation in the right (n=6) or left hemisphere (n=4) at ADT until 3 consecutive stage
5 seizures were evoked. In four rats, stimulation was continued in the contralateral
hemisphere until one additional stage 5 seizure was evoked. Control rats were handled
and then placed in the kindling box, but were not stimulated. Behavioral testing
commenced approximately 48 hr following the last evoked seizure.

3.2.4. Behavioral Assessment

All aspects of behavioral assessment were identical to those in Experiment 1a.

3.2.5. Histology

Following behavioral testing, animals were sacrificed with an overdose of
sodium pentobarbital and perfused transcardially with 9% saline. Brains were fixed in
formalin and then frozen before 60 μm coronal sections were taken through sections
approximating 0.4 mm to 4.8 mm posterior to bregma. Every section through the
electrode track or lesion was mounted and stained with cresyl violet. The location of
the electrode tips and lesions were documented by matching sections with
corresponding plates from Swanson (1992). The extent of anterior PRH cortex
lesioned in each subject was calculated as follows. First, the area of the PRH cortex in
each section corresponding to plates 2.12, 2.30, 2.56, 2.80, 3.14, and 3.30 mm
posterior to bregma was determined. Then, the volume of the PRH cortex between any
2 adjacent planes (defined as a “segment”) was calculated according to the formula:
[(area of section 1 + area of section 2)/2 * distance between plates] and the total
volume was calculated by summing the volumes of segments 1, 2, 3 and 4. The volume
of the PRH lesions was calculated in an identical manner and the % completeness of the
lesion was calculated as: \((\text{volume of PRH lesion})/(\text{volume of PRH}) \times 100\%\).

3.2.6. Data Analysis

Data analysis was completed using the statistical software package SPSS\textsuperscript{TM} for Windows\textsuperscript{TM}. Distance, latency, correct quadrant dwell percentage, crossing preference, and error measures were subjected to analyses with repeated measures ANOVA, and t tests. Degrees of freedom for t-tests were adjusted when significantly different variances were exhibited between groups.

3.3. Results

3.3.1. Histology

3.3.1.1. Kindling Groups

Electrodes in all kindled rats included in the study were located in or near the anterior PRH cortex. Specifically, in all ten kindled rats the kindling electrode was found in layers 3 to 6 of the anterior PRH (see Fig.10). In the four bilaterally kindled rats, the contralateral electrode was also found in the deep layers of the PRH. In unilaterally kindled rats, the electrode not used for kindling was found in the deep PRH (2 rats), the ventral caudate nucleus (3 rats), or the entorhinal cortex (1 rat). In controls, both electrodes were located in or near the PRH in five rats, and at least one electrode was in or near the PRH in the remaining five rats. Placements outside the PRH were found in the ventral caudate nucleus (3 rats), the insular cortex (1 rat), and the parietal cortex (1 rat). No gross histological changes were noted in the brains of either kindled or control rats other than gliosis around the electrode track.
Fig. 10. Location of the lower tip of the stimulating electrode in kindled rats. Plates are posterior relative to bregma and were adapted from Swanson (1992). * = right hemisphere placement. ● = left hemisphere placement.
3.3.1.2. Lesion Groups

The lesions were targeted for the anterior PRH, which I defined as the cortex surrounding the rhinal fissure from \(-2.12\) mm to \(-3.3\) mm posterior to bregma (see Campeau & Davis, 1995, Corodimas & LeDoux, 1995, and Rose & Dunwiddie, 1986 for comparable definitions of the anterior PRH). There were six rats not included in this study because their lesions failed to meet my criterion of being at least 20% complete in each hemisphere. A schematic illustrating a typical lesion in coronal plates is provided in Fig. 11, and data summarizing the lesions is provided in Table 4.

3.3.2. Kindling

Kindling data are shown in Table 5. Briefly, at a mean threshold of \(825 \pm 293\) \(\mu\)A, stimulation evoked an initial AD of \(8.9 \pm 0.7\) sec in duration. A mean of \(6.1 \pm 1.0\) stimulations was required to evoke the first stage-5 seizure, and \(10.5 \pm 1.4\) stimulations were required to meet our kindling criterion of 3 Stage-5 seizures. On these last 3 stimulations, the mean AD duration, latency to clonus, and duration of clonus were \(30.9 \pm 5.0, 2.6 \pm 1.1,\) and \(22.4 \pm 1.8\) secs, respectively. In bilaterally kindled rats, \(7.0 \pm 1.3\) stimulations were required to elicit 1 additional seizure.

Following MWM testing, rats were rekindled until 1 additional stage-5 seizure was evoked. This required a mean of \(1.2 \pm 0.2\) stimulations. The mean AD duration, latency to clonus, and duration of clonus for this stage-5 seizure were \(43.7 \pm 8.5, 6.4 \pm 3.3,\) and \(33.9 \pm 6.4\) secs, respectively. These parameters did not differ significantly from those for the last 3 stage-5 seizures evoked during initial kindling (all \(p's \geq .10\)) except duration of clonus, which was slightly longer for rekindling (t(19) = 1.84, \(p = .10\)).
Fig. 11. Schematic diagram illustrating one hemisphere of a typical lesion. Plates were adapted from Swanson (1992) with coordinates posterior to bregma.
Table 4. Summary of data describing the PRH lesions.

1 All range readings are posterior to bregma.

2 Indicates rats excluded from behavioral analyses.

3 Mean values only include rats used in behavioral analyses (n = 11).

<table>
<thead>
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<th>Left</th>
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<td>44</td>
<td>36</td>
<td>40</td>
<td>79</td>
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</table>
Table 5. Summary of PRH kindling and rekindling data. Duration and latency data are in seconds. Stage-5 seizure AD and clonus data for kindling are averaged across the final three stage 5 seizures evoked prior to MWM testing. ADT = threshold for evoking afterdischarge, stim. = stimulations

<table>
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<tr>
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<th>Kindling</th>
<th>Rekindling</th>
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<td>ADT (μA)</td>
<td>825 ± 293</td>
<td>n/a</td>
</tr>
<tr>
<td>Initial AD duration</td>
<td>8.9 ± 0.7</td>
<td>36.5 ± 6.0</td>
</tr>
<tr>
<td>Stim. to 1st stage-5 seizure</td>
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<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Total # of stim.</td>
<td>10.5 ± 1.4</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Stage-5 seizure AD duration</td>
<td>30.9 ± 5.0</td>
<td>43.7 ± 8.5</td>
</tr>
<tr>
<td>Latency to clonus</td>
<td>2.6 ± 1.1</td>
<td>6.4 ± 3.3</td>
</tr>
<tr>
<td>Duration of clonus</td>
<td>22.4 ± 1.8</td>
<td>33.9 ± 6.4</td>
</tr>
</tbody>
</table>
3.3.3. Behavioral Assessment

3.3.3.1. Unilaterally versus Bilaterally PRH Kindled Rats

Prior to analysis of the performance of the kindled group versus controls, the performance of unilaterally and bilaterally kindled rats on all aspects of behavioral testing was compared. These analyses revealed that there was little difference between the performance of either kindled group on any aspect of testing. All statistical tests failed to approach significance (all p’s > .20) and in most cases the group means were nearly identical. Interestingly, in those cases where performance differed, that of the bilaterally kindled tended to be slightly better. Based on these findings, data from the unilaterally and bilaterally kindled rats were grouped and these data will subsequently be referred to simply as the kindled group’s data.

3.3.3.2. Morris Water Maze

3.3.3.2.1. Pretraining - Visible Platform Trials

Neither PRH kindling nor PRH lesions impaired performance on the VP task in the MWM. Kindled and control rats were equally proficient in escaping to the VP as shown by comparable average escape distances over the 6 VP trials ($t(18) = -0.30, p = .77$; see Fig. 12; note that for this and all subsequent analyses latencies will not be reported but showed a similar pattern of results). Similarly, lesioned and their respective control rats were equally proficient in escaping to the VP as shown by comparable average escape distances over the VP trials ($t(15) = 0.40, p = .97$; see Fig. 13). These data suggest that neither PRH kindling nor PRH lesions significantly disrupted general sensorimotor or motivational functions necessary for water maze performance.
Fig. 12. Mean escape distances on the 6 VP trials during nonspatial pretraining in the MWM for kindled and control groups.
Fig. 13. Mean escape distances on the 6 VP trials during nonspatial pretraining in the MWM for lesioned and control groups.
3.3.3.2.2. Spatial Memory Testing

3.3.3.2.2.1. Acquisition

Neither PRH kindling nor PRH lesions disrupted acquisition of a constant HP location in the MWM. Kindled and control rats exhibited good spatial learning and utilized increasingly direct routes to the escape platform as shown by shorter escape distances across trial blocks (F(5, 90) = 34.6, p < .001; see Fig 14). Performance by both groups was comparable as shown by the absence of a group effect (F(1,18) = 1.4, p = .24) or a group by trial block interaction (F(5,90) = 0.46, p = .80). Similar results were obtained for lesioned rats and their respective control rats. There was a significant trial block effect (F(5,75) = 15.1, p < .001; see Fig. 15) indicating improvement in performance across training but neither the group effect (F(1,15) = 0.11, p = .74) nor group by trial block interaction (F(5,75) = 0.25, p = .94) approached significance indicating that both groups performed comparably. Successful spatial learning was also demonstrated by both kindled, lesioned and their respective control groups on the P trial administered during acquisition training. All groups spent more time searching in the quadrant where the platform was located than in the other three quadrants of the pool and repeatedly passed over the platform’s exact location. Performance by both the kindled and lesioned groups was comparable to or better than their respective control group’s performance in terms of both correct quadrant dwell percentage (kindled - t(18) = 2.29, p = .03, lesioned - t(15) = -1.12, p = .28 ; see Figs 16B and 17B) and crossing preference (kindled - t(18) = 0.41, p = .68, lesioned - t(15) = -1.01, p = .33; data not shown).
Fig. 14. Spatial acquisition for kindled and control groups. Mean escape distances on the 6 HP trial blocks during acquisition of a constant platform location in the MWM (3 trials/block).
Fig. 15. Spatial acquisition for lesioned and control groups. Mean escape distances on
the 6 HP trial blocks during acquisition of a constant platform location in the MWM (3
trials/block).
3.3.3.2.2.2. Retention

Neither PRH kindling nor PRH lesions impaired retention of spatial information in the MWM. Both kindled and control rats showed good retention of the platform location and utilized fairly direct escape routes on the 3 retention HP trials at both 7 and 28 days following acquisition. Although both groups showed some forgetting as indicated by a trial block effect (F(2,36) = 5.58, p = .01), they did not differ in terms of escape distances at either time interval as shown by the lack of a group effect (F(1,18) = 1.09, p = .31) or a trial block by group interaction (F(2,36) = 1.21, p = .31; see Fig 16A). Good retention was also observed on P trials, where both groups exhibited a significant bias for the platform's quadrant and crossed over the platform's precise location repeatedly. The kindled and control groups did not differ in terms of either correct quadrant dwell percentages (see Fig. 16B) or crossing preferences (data not shown), as indicated by the lack of group effects (both F(1,18)'s ≤ 2.41, p's ≥ .14) or group by trial block interactions (both F(2,36)'s ≤ 0.76, p's ≥ .48). Forgetting was not apparent on P trials, probably because it was preceded by 3 HP trials at each retention interval and these trials served as an adequate reminder of the platform's location. The absence of forgetting was apparent by improvements in performance across retention testing that just failed to reach significance in terms of trial block effects for both measures (both F(2,36)'s ≥ .290, p's ≤ .07).

A similar pattern of results was observed with PRH lesioned and their respective control rats. Good retention was shown on HP trials, although some forgetting was apparent as shown by a significant trial block effect (F(2,30) = 5.40, p = .01; see Fig 17A). Performance by the groups did not differ as shown by a group effect
Fig. 16. Spatial retention performance for kindled and control groups. A. Mean escape distances on the last HP trial block of acquisition and the HP trial blocks at 7 and 28 days retention (3 trials/block). B. Correct quadrant dwell % on the P trial between HP trial blocks 5 and 6 of acquisition and the P trials following the HP trial blocks at 7 and 28 days retention.
Fig. 17. Spatial retention performance for lesioned and control groups.  

A. Mean escape distances on the last HP trial block of acquisition and the HP trial blocks at 7 and 28 days retention (3 trials/block).  

B. Correct quadrant dwell % on the P trial between HP trial blocks 5 and 6 of acquisition and the P trials following the HP trial blocks at 7 and 28 days retention.
(F(1,15) = 0.53, p = .48) and group by trial block interaction (F(2,30) = 1.26, p = .30) that failed to reach significance. Good retention was also shown on P trials as indicated by trial block effects that failed to reach significance in terms of either correct quadrant dwell percentage or crossing preference (both F(2,30)'s ≤ 3.12, p's ≥ .06; see Fig 17B). Performance by the groups did not differ in terms of either measure as shown by group effects (both F(1,15)'s ≤ 1.68, p's ≥ .21) and group by trial block interactions (F(2,30) ≤ 0.85, p's ≥ .44) that failed to reach significance.

3.3.3.3. Modified Water Maze

3.3.3.3.1. Pretraining - Visible Platform Trials

Neither PRH kindling nor PRH lesions impaired performance on the 40 VP trials in the modified water maze. Kindled and control groups made identical numbers of errors (t(18) = 0.00, p=1.00; see Fig. 18) and exhibited similar average escape latencies (data not shown; t(18) = 2.71, p = .79). Similarly, the lesioned group and their respective control group made comparable numbers of errors (t(15) = 1.08, p = .30; see Fig. 19) and exhibited similar average escape latencies (data not shown; t(15) = 0.45, p = .66). These data again indicate that neither PRH kindling nor PRH lesions had any profound effects on any of the basic sensorimotor or motivational processes required for performance in the modified water maze or the water maze in general.

3.3.3.3.2. Object Associative Memory Testing

3.3.3.3.2.1. Acquisition

PRH kindling did not affect acquisition of an OD problem in a modified water maze. Both the kindled and control group learned to discriminate between the two objects and selectively approach the object associated with the HP. To
Fig. 18. Total number of errors on the 40 VP trials during pretraining in the modified water maze for kindled and control groups.
Fig. 19. Total number of errors on the 40 VP trials during pretraining in the modified water maze for lesioned and control groups.
achieve criterion performance in acquisition (2 consecutive blocks of 10 trials with \( \leq 2 \) errors), kindled and control groups required comparable numbers of trial blocks (\( t(18) = -0.34, p = .74; \) see Fig. 20A) and made comparable numbers of errors (\( t(18) = -0.21, p = .84; \) see Fig 20B). These findings suggest that object associative learning/STM was not affected by PRH kindling.

In contrast, PRH lesions did impair acquisition of the OD problem. To achieve criterion performance, the lesioned group required significantly more trial blocks (\( t(15) = 1.90, p = .04, \) one-tailed; see Fig 21A) and made significantly greater numbers of errors (\( t(15) = 1.97, p = .03, \) one-tailed; see Fig. 21B) than its respective control group. This finding indicates that, unlike PRH kindling, PRH lesions impair object associative learning/STM.

3.3.3.3.2.2. Retention

PRH kindling impaired retention of the OD problem. Analyses of the number of errors made on the last 5 trials of acquisition and on the 5 trials at both retention intervals revealed a significant group by trial block interaction (\( F(2,36) = 3.18, p = .001; \) see Fig. 22). Subsequent analyses of simple main effects, then, suggested that significant forgetting was exhibited by the kindled group (\( F(2,18) = 15, p < .001 \)) but not the control group (\( F(2,18) = 0.37, p = .70 \)). Comparison of performance by the groups at each time point indicated that the kindled group made significantly more errors than the control group at 28 days retention (\( t(18) = 3.49, p = .003 \)), but performed comparable to controls at the end of acquisition and at 7 days retention (both \( t(18)'s \leq 0.82, \) both \( p's \geq 0.42 \)). These findings suggest that PRH kindling impairs long-term object associative memory.
Fig. 20. Performance during acquisition of the OD problem in the modified water maze by kindled and control groups. **A.** Total number of errors to criterion. **B.** Trial blocks to criterion.
Fig. 21. Performance during acquisition of the OD problem in the modified water maze by lesioned and control groups. A. Total number of errors to criterion. B. Trial blocks to criterion. * p < .05 relative to controls
Fig. 22. Total number of errors on the last 5 OD trials of OD acquisition and on the 5 OD trials at 7 and 28 days retention by kindled and control groups. * p < .05 relative to controls
In contrast PRH lesions did not affect retention of the OD problem. At both 7 and 28 days after training, lesioned and control groups exhibited excellent performance. Forgetting was not apparent, as each group made comparable numbers of errors on the last 5 trials of acquisition as on the 5 trials at both retention intervals, as shown by the absence of a significant trial block effect (F(2,40) = 0.80, p = .46; see Fig. 23). Moreover, the groups did not differ from one another as shown by the absence of a group effect (F(1,20) = 0.03, p = .87) or a group by trial block interaction (F(2,40) = 0.61, p = .55). These findings suggest that PRH lesions do not disrupt long-term object associative memory.

3.4. Discussion

In the present study, kindling to a criterion of three consecutive fully generalized seizures by stimulation of the anterior PRH selectively impaired long-term (i.e., 28 day) retention of an OD problem in a modified water maze. This result contrasted with observations that kindling spared performance on all other components of my testing regimen including: i) acquisition, 7 day retention, and 28 day retention of a hidden platform (HP) location in the Morris water maze (MWM); ii) performance on a VP control task in the MWM tested prior to HP testing; iii) acquisition, and 7 day retention of the OD; and iv) performance on a VP control task in the modified water maze tested prior to OD testing. I also found that anterior PRH lesions impaired acquisition of the OD but spared performance on all other aspects of testing.

The primary aim of the present study was to investigate the site-specificity of
Fig. 23. Total number of errors on the last 5 OD trials of OD acquisition and on the 5 OD trials at 7 and 28 days retention by lesioned and control groups.
kindling's effects on spatial cognition as observed in Experiment 1a. In that study, I found that kindling of the dorsal HPC selectively impaired acquisition of a HP location in the MWM using a training protocol identical that used in the present study. The important anatomical links between the PRH and HPC (Burwell et al., 1995; Suzuki, 1996a), their mutual involvement in limbic seizures (Applegate, 1998; Kelly & McIntyre, 1996; McIntyre & Kelly, 1998), and their shared and/or complimentary roles in memory function (Eichenbaum et al., 1994; Squire, 1992) suggested that PRH kindling might be expected to produce similar effects to dHPC kindling. However, the findings of the present study suggest that PRH kindling does not impair spatial cognition since PRH kindling spared performance on all aspects of spatial testing in our study. Importantly, PRH kindling spared spatial performance under identical testing circumstances to those that proved sensitive to the disruptive effects of dHPC kindling. Thus, the present study supports the conclusion that kindling specific to the dHPC disrupts spatial cognition.

Despite the absence of effects on spatial cognition, PRH kindling was found to disrupt performance in the OD task. This task required the rat to discriminate between two similar appearing objects and associate one with a positive outcome (i.e., escape) and the other with a negative outcome (i.e., no escape). Optimal performance on this task required intact object (visual) processing capacity, object associative learning and memory, and a variety of other general functions that are necessary for water maze performance. I found that PRH kindled rats acquired the OD and retained it for up to 7 days normally but showed significantly poorer performance at 28 days retention relative to controls. In the absence of other impairments, the disruption of OD performance is
best accounted for by an impairment of object associative memory. However, due to the inherently diffuse nature of kindling, the presence of non-mnemonic impairments that might also account for the observed impairment must first be seriously considered.

In this regard, observations that PRH kindling spared performance on the VP control task in the modified water maze, some aspects of OD testing, and all components of spatial testing in the MWM suggest that non-mnemonic impairments are unlikely to underlie the OD deficit at 28 days retention. First, the VP task in the modified water maze immediately preceded OD testing and required the rat to locate a remote visual cue, the platform’s black edges above the water’s surface, and approach it. It shares a number of demands with the OD task, including identifying a visual target, swimming directly to it, mounting a platform, and avoiding the use of inefficient search strategies. Furthermore, both tasks utilize the same motivation, escape from cool water. Since testing on the VP task was done prior to OD testing, intact VP performance provides strong evidence that an impairment of one of the above components of general modified water maze performance does not account for the OD deficit. Second, although kindled rats retained the OD for 28 days more poorly than controls, they did achieve criterion performance and retain the OD problem for 7 days as well as controls. This further suggests that kindled rats were relatively unimpaired with respect to the non-mnemonic abilities required for water maze performance, because they were able to perform the OD task normally during these prior phases of testing. Finally, intact performance on the MWM task also indicates that kindling failed to disrupt either the non-mnemonic or general cognitive functions necessary for water maze performance. Thus, the findings of the present study suggest that PRH kindling
specifically disrupts object associative memory and are the first to demonstrate that kindling can disrupt this class of mnemonic function.

The pattern of performance observed across different phases of OD testing indicates that PRH kindling produced a selective but mild impairment of LTM. In our acquisition paradigm, rats received 20 OD trials per day with an inter-trial interval of approximately 4 min and typically required 3 to 5 days or 60 to 80 trials to achieve criterion performance. Therefore, successful acquisition required that rats be able to learn the information necessary for choosing the correct object and retain it for intervals of less than 5 min within sessions (i.e., learning/STM) and for as long as 24 hours between training days (LTM). Intact acquisition performance indicates that PRH kindling did not impair object associative learning, STM, or even LTM over relatively short intervals. Retention, on the other hand, was tested 7 and 28 days later and required longer-term maintenance of the information necessary for choosing the correct object. The impairment at 28 but not 7 days retention indicates that PRH kindling produced a mild disruption of LTM such that performance was only affected at very long retention intervals.

The effects of PRH kindling in the present study are partially consistent with my findings of the effects of similarly placed anterior PRH lesions. I found that anterior PRH lesions disrupted acquisition of the OD problem but spared all other aspects of testing. This finding confirms that the PRH plays an important role in object associative memory and, importantly, demonstrates that the PRH is critical to performance in the same task that proved sensitive to the effects of PRH kindling. This suggests that the effects of PRH kindling could be mediated through kindling-induced changes in function
that are local to the stimulation site.

It should be noted, however, that while PRH lesions disrupted acquisition of the OD problem PRH kindling only disrupted long-term retention of the OD problem. The former finding demonstrates that the PRH is involved in acquisition and initial storage of information necessary for solving the OD problem. The latter suggests that kindling does not disrupt the mechanisms responsible for this initial mnemonic processing but rather interferes with processes involved in later stages of mnemonic processing. There are at least two possible accounts for such a deficit. First, PRH kindling may have selectively affected the mechanisms responsible for long-term consolidation or storage of information necessary for solving the OD problem. Alternatively, PRH kindling may have disrupted the retrieval of information necessary for solving the OD problem through processes related to state-dependent recall. The effects of PRH kindling continue to evolve significantly for some time after kindling is discontinued. This is apparent in that the severity of convulsions elicited by PRH stimulation often increases following a period without stimulation (personal observations). Such changes could produce amnesia if the brain were to be sufficiently altered in the intervals between subsequent testing sessions (e.g., the 3 weeks between 7 and 28 day retention) so that state-dependent recall was disturbed. Whatever the mechanism, the disruption of LTM was specific to object-related memory function since retention in the MWM was not affected even at 28 days following acquisition.

The effects of PRH kindling and PRH lesions in the present study are generally consistent with other studies of the effects of PRH lesions on mnemonic function. PRH lesions have been shown to produce a mild disruption of associative memory in rats in a
variety of tasks (Aggleton, Keen, Warburton, & Bussey, 1997; Bunsey & Eichenbaum, 1993; Myhrer & Wangen, 1996). For example, Myhrer and Wangen (1996) found that PRH lesions disrupted both acquisition and retention of a thirst-motivated simultaneous brightness discrimination problem. Similar results have been obtained in studies with primates (Suzuki, 1996a). PRH lesions have also been shown to disrupt object recognition in rats in several different tasks (Ennaceur & Aggleton, 1997; Ennaceur et al., 1996; Mumby & Pinel, 1994; Wiig & Bilkey, 1995). For example, PRH lesions disrupt performance of a delayed-non-match-to-sample task with objects in an operant chamber and this impairment is more severe than that produced by HPC lesions (Mumby & Pinel, 1994). Again, similar results have been obtained with primates (Zola-Morgan et al., 1994). Taken together, these findings suggest that the PRH plays an especially important role in object-related mnemonic functions.

The observation that both PRH kindling and PRH lesions spared acquisition performance in the MWM is also consistent with prior lesion studies. Some studies have shown that PRH lesions disrupt spatial performance in the MWM, RAM, or in a T-maze DMTP task (Liu & Bilkey, 1998a; Liu & Bilkey, ; Nagahara, Otto, & Gallagher, 1995; Otto, Wolk, & Walsh, 1997; Wiig & Bilkey, 1994a; Wiig & Bilkey, 1994b). However, in most cases these impairments were less severe than those produced by HPCal system lesions (Liu & Bilkey, 1998a; Nagahara et al., 1995; Otto et al., 1997; Wiig & Bilkey, 1994a). Moreover, in other cases PRH lesions have been shown to spare performance in spatial tasks such as the MWM, RAM, and T-maze DMTP tasks (Aggleton et al., 1997; Ennaceur & Aggleton, 1997; Ennaceur et al., 1996). Notably, subtotal PRH lesions, which resemble those in the present study, have been shown to
spare acquisition in the MWM (Wiig & Bilkey, 1994c). The above patterns of findings have led some to suggest that there is a double dissociation of functions between the PRH and HPC which is consistent with the pattern of findings in Experiments 1a and 1b (Ennaceur et al., 1996; Gaffan, 1994). That is, the PRH contributes disproportionately to object-related mnemonic functions whereas the HPC contributes disproportionately to spatial cognition.

In summary, the present study has shown that kindling of the PRH produces a selective impairment of long-term object associative memory. Along with the findings from Experiment 1a, the findings of the present study suggest that spatial cognition is disrupted by kindling specific to the dHPC. Moreover, they support the view that kindling can have highly specific effects on mnemonic function that are not secondary to non-mnemonic effects. Finally, they show that kindling has the potential to disrupt different classes of mnemonic function (e.g., spatial versus object associative memory) and different stages of memory processing (learning/STM versus LTM) in a manner that may depend upon the site being kindled (dHPC versus PRH respectively).
4. EXPERIMENT 2 – THE RELATION BETWEEN EXTENT OF DHPC KINDLING AND DMTP PERFORMANCE

4.1. Introduction

Previous studies have shown that full kindling of the dHPC disrupts performance in spatial tasks such as the RAM and MWM (Gilbert et al., 1996; Leung & Shen, 1991; Leung et al., 1994; Leung et al., 1990; Leung et al., 1996; Sutherland, Leung, Weisend, Schlife, & McDonald, 1997). In Experiments 1a and 1b, I have provided evidence that such disturbances represent a specific disruption of spatial learning/STM and are preferentially induced by kindling with a dHPC focus. The main goal of Experiment 2 was to further our understanding of this effect by exploring the importance of the degree or extent of kindling achieved prior to testing to subsequent spatial task performance.

Kindling is inherently a progressive phenomenon. That is, with repeated kindling stimulations, the brain undergoes progressive changes that are evidenced by altered afterdischarge (AD) characteristics such as increases in the duration and degree of propagation of AD (Racine, 1972a). These are also accompanied by an elaboration of the behavioral responses associated with seizures (Racine, 1972b). These responses progress through a characteristic hierarchy of severity, beginning with little or no behavioral response and eventually plateau in a fully generalized convulsion, and
constitute a standard means for monitoring the progress of kindling. Indeed, the 6-point scale established by Racine is now routinely reported in most studies of kindling. Commonly employed terminology refers to subjects kindled until Stage 5 seizures have been evoked as “fully kindled,” and subjects kindled until only Stage 3 or lower seizures have been evoked as “partially kindled.” In addition to changes in AD characteristics and accompanying behavioral responses, kindling produces a host of other electrophysiological (e.g., Maru & Goddard, 1987a; Maru & Goddard, 1987b; Sutula & Steward, 1986), anatomical (e.g., Cavazos, Golarai, & Sutula, 1991; Hosokawa et al., 1995), and molecular (e.g., Bendotti, A, Tarizzo, & Samanin, 1993; Chiasson, Dennison, & Robertson, 1995; Greenwood, Abdou, Meeker, & Hayward, 1994; Meyerhoff, Bates, & Kubek, 1990; Strecke & Moneta, 1994; Titulaer, Kamphuis, & Lopes da Silva, 1995; Wu, Monno, Schwarcz, & Vezzani, 1995) changes in the brain that vary with the extent of kindling reached. Given these observations, it is reasonable to hypothesize that extent of kindling may be an important variable in determining the mnemonic effects of kindling.

Some evidence suggests that extent of kindling is indeed a critical determinant of the disruptive effects of dHPC kindling on spatial cognition. Full kindling of the dHPC has been shown to disrupt acquisition in naïve subjects in the Morris water maze (MWM; Gilbert et al., 1996; Experiment 1a), retention in subjects trained prior to kindling in the MWM (Gilbert et al., 1996), and retention in subjects trained prior to kindling in the radial arm maze (RAM; Leung et al., 1990). In contrast, partial kindling of the dHPC has been shown to spare acquisition in naïve subjects in both the MWM (Gilbert et al., 1996) and the RAM (Leung et al., 1996), although it too disrupts
retention in subjects trained prior to kindling in both the MWM (Gilbert et al., 1996) and RAM (Leung & Shen, 1991; Leung et al., 1994; Leung et al., 1990; Leung et al., 1996). These data suggest that the production of an anterograde disruption of spatial cognition capable of impairing acquisition performance is dependent upon the extent of dHPC kindling achieved, with full but not partial kindling being effective. However, the effects of different extents of partial dHPC kindling on spatial cognition have not been systematically investigated, nor have the effects of kindling extent on spatial cognition been investigated using a more powerful within-subjects design. Thus, the goal of the present study was to investigate the effects of various degrees of partial kindling up to and including full kindling on spatial cognition using a within-subjects design.

Repeated testing of the same subjects on memory tests poses several problems. In order to compare performance between different testing sessions, the information required for optimal performance in each session must be both unique to that session and equivalent in nature and quantity to that required in other sessions. This can typically be achieved when some task requirements, such as the basic procedural components of the task, are held constant between testing sessions whereas the exact parameters of the most critical information necessary for performance are varied between sessions. Types of tasks that are suitable for multiple testing sessions are those that assess WM. WM was first described by Honig (1978) and later popularized by Olton (Olton, Becker, & Handelmann, 1979). It refers to memory for information that is only useful within a single trial and is differentiated from RM, which refers to memory for information that remains constant, and is therefore useful, across groups of trials.

The standard MWM task, as used in Experiments 1a and 1b, requires RM,
because the location of the platform remains constant across trials. However, the MWM task can be modified to assess WM-like performance by utilizing a DMTP procedure. In this procedure, the subject is given a trial pair consisting of a sample trial, in which the subject must locate the platform in a novel location, and a match trial, in which the subject must return to the same location. This task requires RM for the general requirements of the task and WM to retain the platform’s precise location for any given trial pair. Therefore, to enable repeated testing in the same rats during the progression of kindling, I used a DMTP procedure in the MWM.

The procedure for the present study can be summarized as follows. I implanted electrodes bilaterally into the CA1 region of the dHPC and 1 week later began pretraining rats on the DMTP procedure. Pretraining consisted of 4 days of training with a 10 sec delay between sample and match trials and 2 days of training with a 10 sec and 30 min delay between trials in successive trial pairs. During pretraining and throughout the study, two trial pairs separated by at least 4 hours were administered on each day of DMTP testing. Following pretraining, rats were randomly divided into a kindled and yoked control group. Kindling stimulation was applied up to 5 times daily until each of the following extents of kindling was reached: 1 AD, 6 ADs, 11 ADs, 16 ADs, 1 stage 1 seizure, and 1 stage 5 seizure. Performance was assessed after each extent of kindling with two days of DMTP testing, with one trial pair at each delay on both days. Kindling and DMTP testing were separated by 48 to 72 hrs throughout the study.
4.2. Methods and Materials

4.2.1. Subjects

Eighteen male Long-Evans hooded rats (Charles River) weighing 325-400 g at the beginning of the study were used as subjects. Food and water were available ad libitum throughout the experiment. Rats were maintained in pairs in shoebox cages prior to surgery and were housed individually for the remainder of the experiment. All experimental procedures were carried out during the light portion of the 12:12 hour light/dark cycle. All rats were handled each day throughout the experiment except during the first four days following surgery.

Subjects were randomly assigned to either the kindled group (n=9) or the control group (n=9). The 9 control rats were each yoked to one of the kindled rats.

4.2.2. Surgery

In preparation for surgery, animals were anaesthetized with Somnotol™ (sodium pentobarbital, 60 mg/kg) and given methyl scopolamine (1 mg/kg) to reduce respiratory congestion. Rats were placed in a stereotaxic apparatus, the skull was leveled, and bipolar nichrome wire electrodes (127 μm dia.) were implanted bilaterally at the following coordinates relative to bregma: -3.5 mm (AP); 2.6 mm (ML); -3.1 mm (DV). The electrode tips were separated by 0.4 to 0.5 mm, with the lower tip used as the stimulating electrode. Five jeweler’s screws were used to secure the electrode assembly to the skull, with one screw over the anterior cortex serving as the reference electrode. The electrode assembly was affixed to the skull with dental acrylic, and a topical antibiotic/steroid, Topagen, was applied to the wound. Finally, a subcutaneous injection of Anafen (0.5 cc/kg) was given for postsurgical analgesia.
4.2.3. Kindling

Two or three days after the completion of pretraining, kindling began. In the first kindling session, the stimulus intensity required to evoke an AD was determined in one and only one pseudo-randomly chosen hemisphere of each rat in the kindled group (left \(n=5\), right \(n=4\)). A Grass S8800 stimulator was used to deliver a 1 sec train of balanced biphasic square wave pulses at 60 pps at an initial intensity of 1\(\mu\)A (base-to-peak). If AD greater than 5 sec in duration was not evoked, intensity was increased along the following scale 10, 20, 30, 40, 60, 80, 100, 120 \(\mu\)A every 2 minutes until an AD of 5 sec or greater in duration was elicited. The minimal intensity triggering AD was arbitrarily defined as ADT and was the intensity used for kindling during the remainder of the study. Two days of behavioral testing were conducted 48-72 hrs later. Kindling resumed 48 to 72 hrs after behavioral testing and, in the remainder of the study, consisted of up to 5 stimulations per day with 2 hours between stimulations until each of the following extents of kindling was achieved: 6 ADs, 11 ADs, 16 ADs, 1 stage 1 seizure (consisting of 5 sec or more of continuous chewing), and 1 stage 5 seizure (see Racine, 1972b). Behavioral testing was conducted 48 to 72 hrs following after each extent of kindling with kindling resuming another 38 to 72 hrs later. Each control rat was handled and placed in the kindling box (but not stimulated) according to a schedule yoked to one kindled rat.

4.2.4. Behavioral Assessment

4.2.4.1. Apparatus

Behavioral assessment was conducted in a circular fibreglass pool that was 150 cm in diameter, had 45 cm high featureless white walls, and was filled to a height of 26
cm with 22 ± 1°C water rendered opaque with 1500-2000 ml of skim milk powder. A clear plexiglass platform 23 cm in height with a 10 by 12 cm upper face was used throughout the study. The maze was located in a windowless room with white walls and two doors. Various items placed on the walls provided visual cues. Two overhead ventilation fans and a radio placed on the floor facing away from the pool produced background noise. The movement of rats in the pool was recorded and analysed with a video camera coupled to a microcomputer by an image analyser (Chromotrack, San Diego Instruments). A remote switch was used to start and stop recording.

4.2.4.2. Procedure

Spatial WM was assessed using a DMTP procedure that consisted of a sample trial and a match trial (collectively called a trial pair). The following procedures were common to both trial types: i) the rat was placed in the pool with its head facing the wall of the pool; ii) trials were terminated after the rat found the platform or 90 sec expired; iii) if the rat did not find the platform after 90 sec, it was gently guided to the platform; and iv) after being removed from the platform, the rat was carried to a holding pen 2.5 m from the pool with a 250 W heat lamp located 45 cm above.

Trial pairs were administered as follows. For each trial pair, the platform location was pseudo-randomly chosen from one of eight possible predetermined locations, with the restriction that the location was separated by at least 90° as measured from the center of the pool from the location used during the previous trial pair. Start locations were pseudo-randomly chosen from one of four predetermined locations separated by 90° as measured from the center of the pool, with the restriction that the start location used on any trial was different from the one used on the previous
trial. On sample trials, the rat was released from one of the four starting sites and allowed to swim for 90 seconds or until the platform was found. The rat was left on the platform for 15 seconds and then removed to the holding pen. After either a 10 sec or 30 min delay, the rat was returned to the pool for the match trial. Again, the rat was released from one of the three previously unused starting sites and allowed to swim for 90 seconds or until the platform was found. The rat was then immediately removed to the holding pen. Two measures were recorded on both sample and match trials—escape latency and direct swims. A direct swim was scored each time the rat swam to the platform while remaining within a 25 cm wide alley from the start location to the platform location.

4.2.4.3. Behavioral Testing Schedule

DMTP testing began with 6 days of pretraining. During pretraining and throughout the study, a day of DMTP testing consisted of two trial pairs, which were separated by an interval of 4 hrs. On days 1 to 4, both trial pairs were administered with a delay between sample and matching trials of 10 sec. On days 5 and 6, 1 trial with a 10 sec delay and 1 trial with a 30 min delay were administered with the intra-day order reversed on days 5 and 6 for each rat pair tested. Kindling began between 48 and 72 hrs following the last DMTP trial.

Post-kindling DMTP testing after each extent of kindling took place between 48 and 72 hours following the last kindling stimulation and comprised two days of testing. The pattern of trials administered on these two days was identical to that used on days 5 and 6 of pretraining. Kindling resumed 48–72 hrs following the last DMTP trial and continued until the next extent of kindling was reached.
4.2.5. Histology

Following behavioral testing, animals were sacrificed with an overdose of sodium pentobarbital and perfused transcardially with 9% saline. Brains were fixed in formalin and then frozen before 60 μm coronal sections were taken through the dHPC. Every section through the electrode track was mounted and stained with cresyl violet. The location of the electrode tips was documented by matching sections with one of eight plates from Swanson (1992).

4.2.6. Data Analysis

Data analysis was completed using the statistical software package SPSS\textsuperscript{®} for Windows\textsuperscript{®}. Latency and direct swim data were subjected to analyses with repeated measures ANOVA. Extent of kindling (levels = 6) was the within subjects factor and group (levels = 2) and delay (levels = 2) were the between subjects factor. Group differences were further evaluated with planned comparisons using between subjects t-tests.

4.3. Results

4.3.1. Histology

Electrodes in all kindled rats included in the study were located in the dHPC. Specifically, in the 9 kindled rats, the lower tip of the kindling electrode was found in the pyramidal cell layer (2 rats), the stratum radiatum (4 rats), or the stratum lacunosum moleculare (3 rats) of the CA1 region (see Fig. 3, Experiment 1a for comparable placements). In these same rats, the electrode not used for kindling was found in the dHPCal CA1 region in 8 rats and in the overlying corpus callosum in 1 rat. In controls,
both electrodes were located in the dHPCal CA1 region in 6 of 9 rats, and at least one electrode was in the dHPCal CA1 region in all rats. Extra-HPCal placements were found in the overlying corpus callosum (3 rats). No gross histological changes were noted in the brains of either kindled or control rats other than gliosis around the electrode track.

4.3.2. Kindling

In kindled rats, the mean initial ADT was $31.2 \pm 13.1$ µA. This intensity evoked an average initial AD duration of $35.0 \pm 3.8$ sec and secondary AD that was quite variable in its latency to onset, duration, and intensity. Subsequent kindling data are shown in Table 6.

4.3.3. DMTP Testing

The delay between the sample and matching trials did not affect performance in the DMTP task. Both kindled and control groups performed comparably on matching trials at both delays, as shown by the absence of a delay effect or a group by delay interaction in terms of either direct swims or escape distances (all F(1,28)'s $\leq 1.48$, all p's $> .24$). Thus, for all subsequent analyses, trials were collapsed across delays and analyzed in blocks of 4 trials, which comprised the full number of trials administered after each extent of kindling.

However, kindling did affect performance in the DMTP task. Overall, kindled rats were less likely to swim directly to the platform and employed longer average escape routes than control rats on matching trials during the post-kindling phases of the study. This impairment was evident in group effects in terms of both escape distances (see Fig. 24; F(1,14) = 13.49, p = .003) and direct swims (see Fig. 25; F(1,14) = 10.81,
Table 6. Summary of Kindling Data. Dur = duration, Stim = stimulations, AD = afterdischarge

<table>
<thead>
<tr>
<th>Kindling Extent</th>
<th>Stim. to Reach Kindling Criterion</th>
<th>Mean AD Dur. Over Final 5 Stim. (sec)</th>
<th>AD Dur. on Final Stim. (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADT</td>
<td>N/A</td>
<td>N/A</td>
<td>35.0 ± 3.8</td>
</tr>
<tr>
<td>6 ADs</td>
<td>6</td>
<td>32.3 ± 2.6</td>
<td>35.4 ± 4.4</td>
</tr>
<tr>
<td>11 ADs</td>
<td>11</td>
<td>30.1 ± 2.5</td>
<td>31.3 ± 3.3</td>
</tr>
<tr>
<td>16 ADs</td>
<td>16</td>
<td>34.8 ± 2.4</td>
<td>34.8 ± 3.4</td>
</tr>
<tr>
<td>1 Stage 1 Seizure</td>
<td>32.5 ± 2.9</td>
<td>68.6 ± 11.9</td>
<td>73.9 ± 13.0</td>
</tr>
<tr>
<td>1 Stage 5 Seizure</td>
<td>48.9 ± 5.6</td>
<td>57.0 ± 4.3</td>
<td>63.0 ± 8.6</td>
</tr>
</tbody>
</table>
p = .005). Subsequent analyses of performance after each extent of kindling suggested a tri-phasic pattern to the impairment. Kindled rats performed more poorly relative to controls during the early phases of the study (i.e., after 1, 6, or 11 ADs), performed comparably to controls during the middle phases of the study (i.e., after 16 ADs or 1 stage 1 seizure), and then performed more poorly relative to controls again during the final phase of the study (i.e., after 1 stage 5 seizure). T-tests comparing performance between groups after each extent of kindling indicated an impairment in terms of direct swims after 1, 6, and 11 ADs and after 1 stage 5 seizure (all t(14)'s ≥ 1.81, p's < .05, one-tailed) and in terms of escape distances after 6 and 11 ADs and after 1 stage 5 seizure (all t(14)'s ≥ 1.83, p's < .05, one-tailed).

It is important to note that kindling did not affect performance on sample trials and that the kindled and control groups did not differ in their performance during pretraining. On sample trials throughout all phases of post-kindling testing, both groups were equally effective in searching for the platform in a novel location in terms of both direct swims and escape distances. This was evident in the absence of a group effect (both F(1,14)'s ≤ 2.31, both p's ≥ 0.15) or group by trial block interaction (both F(5, 70)'s ≤ 1.10, both p's ≥ 0.37) on either measure. Performance on both sample and matching trials during the last trial block of pretraining (4 trials) was also comparable between kindled and control groups. The groups did not differ in terms of either direct swims or escape distances on sample (both t(14)'s ≤ 0.58, both p's ≥ 0.57) or matching (both t(14)'s ≤ 0.47, both p's ≥ 0.65) trials.
Fig. 24. Escape distances on sample and matching trials. Data are averaged across 4 trials at each testing phase. * p < .05 relative to controls
Fig. 25. Direct swims on sample and matching trials. Data are averaged across 4 trials at each testing phase. * p < .05 relative to controls
Number of Direct Swims

Extant of Kindling

- - - Sample Trials ○ Control
- - Match Trials ■ Kindled

Pre K 1 AD 6 ADs 11 ADs 16 ADs 1 St.1 Seizure 1 St.5 Seizure
4.4. Discussion

Kindling of the dHPC disrupted performance on matching but not sample trials in a DMTP task in the MWM. This impairment was not sensitive to the delay between matching and sample trials, either 10 sec or 30 min, but was related to the extent of kindling attained prior to each phase of testing. As little as 1 AD produced a mild disruption of performance, whereas both 6 and 11 ADs produced a more severe impairment. With continued kindling the impairment abated such that the disruption was no longer significant during testing after 16 ADs or 1 stage 1 seizure. However, 1 stage 5 seizure again produced a moderate impairment.

The present results indicate that performance in spatial WM tasks, similar to performance in spatial RM tasks, is sensitive to the disruptive effects of dHPC kindling. This is consistent with previous reports that, in rats trained prior to kindling, both partial and full dHPC kindling disrupt performance in the standard RAM task (Leung & Shen, 1991; Leung et al., 1994; Leung et al., 1990) and partial dHPC kindling disrupts performance in a spatial WM version of the MWM (Sutherland et al., 1997) similar to that used in the present study. The disruption of spatial WM performance by partial kindling with as little as 1 AD in the present study and as little as 10 ADs in the studies above (Leung et al., 1996) suggests that spatial WM may be exquisitely sensitive to the disruptive effects of kindling.

However, in all of these studies (the present study; Leung & Shen, 1991; Leung et al., 1994; Leung et al., 1990; Leung et al., 1996; Sutherland et al., 1997) subjects were trained prior to kindling (the present study; Leung & Shen, 1991; Leung et al.,
1994; Leung et al., 1990; Sutherland et al., 1997), allowing for an alternative interpretation of the observed impairments. All WM tasks necessarily rely on both WM and RM for optimal performance. In spatial WM tasks, RM demands typically include memory for both the layout of the testing environment and the general task procedures, whereas WM demands typically include memory for areas that have been recently visited. In protocols in which subjects are trained prior to kindling, the RM components of the task are acquired during this training phase and must be retained during kindling to be used during subsequent post-kindling testing. Therefore, any subsequent impairments could be attributed to either an anterograde disruption of spatial WM (e.g., STM for which area as been recently visited) or a retrograde disruption of RM (e.g., LTM for the general layout of the maze, the reward contingencies of the task, or other procedural variables).

Several considerations suggest that the deficit in the DMTP task observed in the present study following the early phases of kindling (i.e., after 1, 6, and 11 ADs) might indeed be due to a retrograde disruption of RM. First, partial dHPC kindling (15 ADs) has been shown to disrupt retention performance in pretrained rats but not acquisition performance in naive rats on a version of the RAM with distinct RM and WM components (Leung et al., 1996). This pattern of results can best be explained by concluding that partial dHPC kindling produces a retrograde impairment of RM but not an anterograde impairment of either WM or RM. Second, full kindling of the dHPC or dentate gyrus has been shown to disrupt RM but not WM performance in rats trained prior to and during kindling on a version of the RAM with distinct WM and RM components (Feasey-Truger, Kargl, & ten Bruggencate, 1993; Lopes Da Silva, Gorter,
& Wadman, 1986). This suggests that even full kindling in the dHPC may not disrupt WM. Third, partial dHPC kindling fails to produce an anterograde disruption of spatial cognition in the standard MWM task (Gilbert et al., 1996; Sutherland et al., 1997), although this task only requires RM. Nonetheless, this result suggests that partial dHPC kindling does not produce an anterograde effect on at least one form of spatial cognition. Fourth, partial dHPC kindling has been shown to produce retrograde disruptions of spatial RM in both the standard MWM task (Gilbert et al., 1996) and on a 2-arm spatial discrimination task in the RAM (Laurent-Demir & Jaffard, 1997). These findings clearly demonstrate that partial dHPC kindling is capable of producing a retrograde disruption of RM. Taken together, the above observations suggest the present results after the early phases of partial dHPC kindling are most parsimoniously interpreted as a retrograde impairment of RM. They further suggest that long-term retention of information acquired prior to kindling, rather than WM per se, is exquisitely sensitive to the disruptive effects of kindling.

Interpretation of the deficit observed following full dHPC kindling phase (i.e., 1 stage 5 seizure) is more complicated for several reasons. First, by this stage of testing, extensive training had already been administered and the accurate performance observed after 16 ADs and 1 stage 1 seizure suggests that the RM components of the task had been well learned by this point. Evidence from studies on retrograde amnesia suggests that well learned material is more resistant to retrograde disruption by seizures (McGaugh, 1966). Second, full dHPC kindling has been shown to disrupt acquisition by naïve rats in the standard MWM task (Gilbert et al., 1996; Experiment 1a). This finding shows that full dHPC kindling is capable of producing an anterograde disruption
of spatial cognition, although only RM has been assessed. Thus, a stronger argument can be made that the DMTP deficit observed following 1 stage 5 seizure is due to an anterograde disruption of spatial WM, although, even in this case, a retrograde disruption of RM cannot be definitively ruled out.

The primary purpose of the present study was to investigate the relation between extent of dHPC kindling and effects of spatial cognition. The present findings strongly argue that disturbed spatial cognition does not show a simple relation to kindling extent whereby a progressively more severe impairment is observed with greater extents of kindling. Rather, a triphasic pattern of results is exhibited. The early phases of partial kindling (1, 6, and 11 ADs) produce a moderate impairment that is likely to represent a retrograde disruption of RM. The later phases of partial kindling (16 ADs and 1 stage 1 seizure) do not appear to produce any greater impairment, and in fact a recovery of performance is observed. This suggests that the mechanisms responsible for the retrograde disruption of RM during the early phases of kindling are not progressive during these phases or, at least, can be adequately compensated for by the amount of training received by this point in the study. The final phase of kindling in this study (i.e., full kindling; 1 stage 5 seizure) was associated with the re-emergence of a moderate impairment that may have constituted an anterograde disruption of spatial WM. This finding suggests that full kindling, in contrast to partial kindling, is associated with a novel or more striking induction of mechanisms capable of compromising spatial WM function.

There are several questions that emerge from the present study that could be addressed in future studies. First, whether dHPC kindling is capable of producing an
anterograde disruption of spatial WM could be determined more convincingly. To this end, the effects of various extents of dHPC kindling on a version of the RAM with distinct WM and RM components could be explored. A finding of a WM impairment in the absence of a RM impairment would provide the most convincing evidence that spatial WM was specifically affected. Alternatively, a spatial WM task using various delays between responses could be used. In this case, a finding of a delay-dependent impairment would argue in favor of a WM rather than a RM impairment. Unfortunately, in the present study, my delay procedure did not appear to affect performance in either the kindled or control group. This finding suggests that a learning impairment was produced that may have been secondary to a retrograde disruption of RM during the early phases of partial kindling or an anterograde disruption of WM during the full kindling phase of the study. Second, the basis of recovered performance during the middle phases of partial kindling could be further investigated. This could be achieved by a between groups analysis of performance after a few ADs, 1 stage 1 seizure, and 1 stage 5 seizure. Impairments after each extent of kindling would confirm the results of the present study and suggest that the recovery I observed during the late phases of partial kindling was due to relearning. Alternatively, impairments after a few ADs and 1 stage 5 seizure but not 1 stage 1 seizure would suggest that the neural mechanisms responsible for the early phase deficit are either compensated for or reversed by neural changes that occur at the onset of stage 1 seizures.
5. EXPERIMENT 3A - DHPC KINDLING'S EFFECTS ON ANXIETY, ACTIVITY, OBJECT MEMORY, AND SPATIAL MEMORY

5.1. Introduction

A variety of studies have indicated that kindling can disrupt spatial cognition (Leung & Shen, 1991; Leung et al., 1994; Leung et al., 1990; Leung et al., 1996; Lopes Da Silva et al., 1986; Sutherland et al., 1997). In Experiments 1a and 1b, I have provided evidence that such disturbances represent a specific disruption of spatial learning/STM and are preferentially induced by kindling with a dHPC focus. In Experiment 2, I have provided evidence that full kindling is probably required to induce this effect. In Experiment 3a, I have attempted to further investigate the following issues related to the effects of dHPC kindling on spatial cognition: 1) the role of non-mnemonic effects, particularly changes in anxiety-related behaviors, in spatial task impairments, 2) the specificity of the mnemonic effects to spatial cognition, and 3) the stage of spatial memory processing that may be affected - learning or STM.

In addition to effects on spatial cognition, kindling has been shown to disrupt several other classes of mnemonic function. These include object associative memory (Experiment 1b) and both aversive and non-aversive conditioning (Becker et al., 1992; Becker, Letzel, Letzel, & Grecksch, 1997; Boast & McIntyre, 1977; McIntyre & Molino, 1972; Peele & Gilbert, 1992; Robinson et al., 1989; Rosen et al., 1996; Stone
& Gold, 1988). Although many of these effects have been observed following kindling in sites other than the dHPC, such as the AM, dHPC kindling has been shown to alter aversive conditioning acquisition in a Y-maze brightness discrimination task (Becker et al., 1997). Collectively, the above observations raise questions regarding the mnemonic specificity of the effects of kindling to spatial cognition. One possibility is that kindling produces effects restricted to processes or circuitry involved in normal HPCal functions and thus produces a selective disruption of spatial cognition and other behaviors that are hippocampally mediated. Alternatively, kindling may produce effects that impact processes and circuitries involved in the normal mnemonic functions of a variety of brain systems and thus should produce effects on a variety of both hippocampally and non-hippocampally mediated mnemonic functions. In Experiment 1a, I have shown that full kindling of the dHPC spares both acquisition and retention of an OD problem in a modified water maze thus providing some evidence the effect may indeed be selective to hippocampally mediated functions. However, this important question warrants further investigation.

Kindling has also been shown to alter a variety of non-mnemonic functions. For example, kindling has been shown to alter both motor behavior (Caldecott-Hazard, 1988; Ehlers & Koob, 1985) and sensory-evoked potentials (Tsuru & Shimada, 1984). Moreover, one of the most well established behavioral effects of kindling is a change in anxiety-related behaviors (Adamec, 1990a; Adamec, 1998; Adamec & Stark-Adamec, 1983; Depaulis, Helfer, Deransart, & Marescaux, 1997; Kalynchuk, Pinel, & Treit, 1998a; Kalynchuk et al., 1998b; Kalynchuk, Pinel, Treit, & Kippin, 1997; Pinel et al., 1998). Of particular interest for the present study, dHPC kindling has been shown to
alter anxiety-related behaviors in the elevated-plus maze (Kalynchuk et al., 1998a) and defense-related behaviors on several different tests (Pinel, Treit, & Rovner, 1977). These findings highlight the importance of determining the contribution of non-mnemonic effects, and changes in anxiety-related behaviors in particular, to the effects of dHPC kindling on spatial cognition.

Previously, the Corcoran lab has shown that dHPC kindling disrupts spatial cognition in the MWM (Gilbert et al., 1996). In Experiment 1a, I dissociated effects on spatial learning/STM and LTM by using a procedure where all acquisition training took place within a 1-hour period and retention was tested 7 and 28 days later. I found that dHPC kindling impaired acquisition but not retention suggesting that the impairment in spatial cognition disrupted learning/STM but not LTM processes. In the present study, I attempted to dissociate effects on learning and STM by using a DMTP procedure in the MWM with variable delays of 0.25, 1, and 4 minutes. In this case, a delay-dependent impairment would suggest that STM was affected whereas a delay-independent impairment would suggest that learning was affected.

The design of Experiment 3a was as follows. Rats were fully kindled with twice daily stimulation of the dHPC until a criterion of 3 stage 5 seizures was reached. One week later, to examine anxiety-related behaviors, rats were tested in both an elevated plus maze and, on the next day, an open field task. The next phase of testing was designed to assess object recognition memory and began the day following open field testing. Rats were habituated for two additional days in the open field and then given a day off. The next day, rats were tested in a two-trial object exploration task modified from that of Ennaceur and colleagues (Ennaceur et al., 1996). The final phase of testing
was designed to assess delay-dependent changes in spatial WM related performance and began two days following object exploration testing. Rats were pretrained on a 4-trial DMTP task for 1 day using a VP. Rats then received 3 additional days of training with an inter-trial delay of 0.25 seconds. Delay testing then began, with rats tested with inter-trial delays of .25, 1, 4, 0.25, 1, and 4 minutes on successive days.

5.2. Methods and Materials

5.2.1. Subjects

Twenty-six male Long-Evans hooded rats (Charles River) weighing 300-375 g at the beginning of the study were used as subjects. Food and water were available ad libitum throughout the experiment. Rats were maintained in pairs in shoebox cages prior to surgery and were housed individually for the remainder of the experiment. All experimental procedures were carried out during the light portion of the 12:12 hour light/dark cycle. All rats were handled each day throughout the experiment except during the first four days following surgery. Subjects were randomly assigned to either the kindled group (n=13) or the control group (n=13). Each control rat was yoked to one of the kindled rats.

5.2.2. Surgery

In preparation for surgery, animals were anaesthetized with Somnotol™ (sodium pentobarbital, 60 mg/kg) and given methyl scopolamine (1 mg/kg) to reduce respiratory congestion. Rats were placed in a stereotaxic apparatus, the skull was leveled, and a bipolar nichrome wire electrode (127 μm dia.) was implanted in either the right (n=6) or left (n=7) HPC using the following coordinates relative to bregma: -3.5
mm (AP); 2.6 mm (ML); - 3.1 mm (DV). The electrode tips were separated by 0.4 to
0.5 mm, with the lower tip used as the stimulating electrode. Five jeweler's screws
were used to secure the electrode assembly to the skull, with one screw over the
anterior cortex serving as the reference electrode. The electrode assembly was affixed
to the skull with dental acrylic, and a topical antibiotic/steroid, Topagen™ was applied
to the wound. Finally, a subcutaneous injection of Anafen (0.5 cc/kg) was given for
postsurgical analgesia.

5.2.3. Kindling

Approximately 7 days following surgery kindling was initiated. In the first
kindling session, the stimulus intensity required to evoke an afterdischarge (AD) was
determined. A Grass S8800 stimulator was used to deliver a 1 sec train of balanced
biphasic square wave pulses at 60 pps at an initial intensity of 1 μA (base-to-peak). If
AD greater than 4 sec in duration was not evoked, intensity was increased along the
following scale 10, 20, 40, 80, 120, 160, 250 μA every 2 minutes until 4 sec or greater
AD was elicited. The minimal intensity triggering AD was arbitrarily defined as ADT
and was the intensity used for kindling during the remainder of the study. Subsequently,
rats were kindled twice daily with stimulations separated by at least 4 hrs until a
criterion of 3 stage 5 seizures was achieved. At this point, rats were considered fully
kindled and behavioral testing was started 7 or 8 days later. Each control rat was yoked
to a specific kindled rat with which it received identical treatment except that it was not
stimulated.
5.2.4. Behavioral Assessment

5.2.4.1. Testing Environment

All testing took place in a rectangular windowless room with one door. The walls were painted an off-white color and were hung with numerous posters. Background noise was produced by an overhead ventilation fan. During all testing, the experimenter remained within the room at a computer station set up in one corner. For data acquisition, an overhead video camera coupled to a microcomputer by an image analyzer (Chromotrack, San Diego Instruments; EthoVision, Noldus) was used to track movement of rats in the various mazes. A remote switch was used to start and stop tracking and a VCR was used to videotape most trials.

5.2.4.2. Apparatus

5.2.4.2.1. Elevated Plus Maze

The elevated plus maze was constructed from 19 mm thick plywood and corrugated plastic, which was used to line all areas of the maze that would be exposed to a rat during a trial. It consisted of two sets of perpendicular interlocking arms 110 cm in length and 10 cm in width. The interlocking central region bisected the maze into two pairs of arms, one with 45 cm high walls, the closed arms, and one without walls, the open arms. The entire maze was elevated on legs that were 45 cm high.

5.2.4.2.2. Open Field

The open field was made of a white industrial plastic and was painted white. It was circular in shape with 45 cm high walls and a diameter of 150 cm. Two identical objects, glass 500 ml beakers with rings of black and white tape, were placed in the center of adjacent quadrants of the open field at a distance of 40 cm from the wall. For
analysis purposes, the open field was divided into various regions – an outer ring (0 to 15 cm from the wall), a middle ring (15 to 40 cm from the wall), an inner ring (40 to 75 cm from the wall), and two object zones (30 cm in diameter centered on each object).

5.2.4.2.3. Object Exploration Task in the Open Field

The maze was identical to that used for the open field task except that novel objects, constructed from Lego™, were used. Each object was approximately 8 cm high and 4 cm wide and was composed of pieces of three of the following different colors – red, blue, yellow, black, white. For the first exploration trial, two identical objects were used. On the second exploration trial, an identical copy of the first object pair and a novel object were used.

5.2.4.2.4. Morris Water Maze

The Morris water maze was made of a white industrial plastic and was painted white. It was circular in shape with 45 cm high walls and a diameter of 200 cm. The maze was filled to a height of 26 cm with 22 ± 1°C water rendered opaque with 1500-2000 ml of skim milk powder. A clear plexiglass platform 23 cm in height with 10 by 12 cm upper face was used throughout the study. On VP trials, a black-sided attachment was added to the platform, which caused the platform’s upper face to protrude 3 cm above the water’s surface.

5.2.4.3. Procedure

5.2.4.3.1. Elevated Plus Maze

Activity (i.e., exploratory behaviors) and anxiety-related behaviors were assessed in an elevated plus maze (Pellow, Chopin, File, & Briley, 1985; Rodgers & Dalvi, 1997). Rats were brought to the testing room and tested individually. Prior to
each trial the maze was cleaned thoroughly with a solution of 60% alcohol. The trial began with the rat being placed in the central region of the maze facing an open arm and continued for 5 minutes, at which point the rat was promptly removed and returned to the housing colony. Data were obtained using tracking software and supplemented by experimenter observations, either live or from videotape. The following measures were taken. The time spent in each of the five regions of the maze (the 2 open arms, the 2 closed arms, and the central region) was recorded. For this purpose, the rat’s position was determined according to the location of its center of gravity as indicated by the central point in the pixels representing the rat. Entries into each of the arms were recorded. For this purpose, the rat’s entry to any of the 4 arms was counted each time all four paws crossed from the central region into an arm. Finally, the total distance traveled was recorded. Measures of activity included overall distance moved and total number of arm entries with higher values on each measure indicating higher levels of activity. Measures of anxiety included dwell ratio, the ratio of dwell time in the open arms to the dwell time in all four arms, and entry ratio, the ratio of open arm to closed arm entries, with lower values indicating higher levels of anxiety.

5.2.4.3.2. Open Field

Activity/exploration and anxiety-related behaviors were assessed in a modified open field task (Walsh & Cummins, 1976; Williams & Russel, 1972). This task also served as preparation for object exploration testing as described below. On each of three consecutive days, rats were brought to the testing room and tested individually. Prior to each trial, the maze and the objects were thoroughly cleaned with a solution of 60% alcohol. The trial began with the rat being placed in the maze at the opposite end
from the objects facing the wall and continued for 5 minutes, at which point the rat was promptly removed and returned to the housing colony. Data were obtained using the tracking software and supplemented by observations by the experimenter either live or from videotape. The following measures were taken. The time spent in each of the five regions of the maze – the 3 rings of the maze (outer, middle, and inner rings), and the 2 object areas, was recorded. For this purpose, the rat’s position was determined according to the location of its center of gravity as indicated by the central point in the pixels representing the rat. Entries into the object regions were recorded. For this purpose, entries were counted each time both of the rat’s front paws crossed the region’s border subsequent to being completely outside of the region. Rears were recorded each time the rat raised both front paws off the ground. Finally, the total distance traveled was recorded. Measures of activity included overall distance moved, time investigating the objects, and rears, with larger values on each measure indicating higher levels of activity. Measures of anxiety included the entries to and amount of time spent in the central region of the maze, with lower values indicating higher levels of anxiety.

5.2.4.3.3. Object Exploration Task in the Open Field

An object exploration task in the open field modified from Ennaceur and colleagues (Ennaceur et al., 1996) was used to assess object recognition memory. The three days of open field testing described above served as an opportunity for the rats to become habituated to the testing environment. After one day off, the critical trials were administered. Rats were brought to the testing room and tested individually. Prior to each trial, the maze and objects were thoroughly cleaned with a solution of 60%
alcohol. The first exploration trial began with the rat being placed in the maze at the opposite end from the objects facing the wall and continued for 5 minutes, at which point the rat was promptly removed and returned to the housing colony. The objects on this trial were identical. After 15 minutes, the rat was returned to the testing room for the second object exploration trial. It was identical to the first trial except that its duration was only 3 minutes and the objects were replaced by one identical copy and a novel object. On this trial, rats normally show a bias towards exploration of the novel object (Ennaceur et al., 1996). This, of course, requires that the rat recognizes the familiar object, and therefore represents a test of object recognition memory. Data were obtained using the tracking software and were supplemented by observations by the experimenter either live or from videotape. The following measures were taken. The time spent in each of the five regions of the maze – the 3 rings of the maze (outer, middle, and inner rings), and the 2 object areas, was recorded. For this purpose, the rat’s position was determined according to the location of its center of gravity as indicated by the central point in the pixels representing the rat. Entries into the object regions were recorded. For this purpose, entries were counted each time both of the rat’s front paws crossed the region’s border subsequent to being completely outside of the region. Rears were recorded each time the rat raised both front paws off the ground. Finally, the total distance traveled was recorded. Measures of activity included overall distance moved, time investigating the objects, and rears. Measures of object recognition included dwell ratio, the time spent in the region of the novel versus the familiar object, and entry ratio, the entries into the region of the novel versus the familiar object, with larger values of each measure indicating better object recognition.
5.2.4.3.4. DMTP Testing in the MWM

Spatial WM was assessed using a DMTP procedure consisting of 10 consecutive days of testing. On each day, one trial group, consisting of a sample trial and 3 match trials, was administered with the platform location selected randomly without replacement from 1 of 10 possibilities. On the first day of testing, the VP was used, whereas, on subsequent days, the HP was used. The delays between trials were constant within days and were as follows across the 10 days of testing – 0.25, 0.25, 0.25, 0.25, 0.25, 1, 4, 0.25, 1, and 4 minutes. The first 4 days of testing were considered the “acquisition phase” of the task on the basis of pilot data that had shown that rats’ performance asymptoted by the fifth day of testing using the above protocol. The following 6 days were considered the “delay phase” of testing and were used to assess the impact of varying the inter-trial interval on performance. For analysis purposes, data from each of the two trial groups at the same delay during this phase of testing were averaged.

Throughout testing, rats were brought to the testing room and tested in pairs (1 kindled and 1 yoked control rat). On the sample trial, the rat was placed on the platform and allowed to remain there for a period of 20 seconds, after which it was removed to a holding pen with a 250 W red heat lamp in the corner of the testing room. Previous work has shown that rats are able to acquire considerable information about a spatial location simply by viewing the environment from that location (Sutherland & Linggard, 1982). On the matching trials, the rat was returned to the pool and gently placed into the water facing the edge of the pool at a constant starting location (a spot on the “southernmost” wall of the pool proximal to the computer station) and allowed
to swim until it found the platform or until 90 seconds elapsed, after which it was gently guided to the platform. The rat was allowed to remain there for 20 seconds and was then returned to the holding pen. Two additional matching trials were administered as above except that on the last trial the rat was removed from the platform immediately after the trial was completed. Each matching trial was started at a delay of 0.25, 1, or 4 minutes following the removal of the rat from the platform on the preceding trial. On day 1, the VP was used. On days 2 to 10, each trial group was preceded by a “free swim” in which no platform was present. The rat was placed in the pool at the usual starting position and permitted to swim until it passed over the exact location of the platform on the previous day of testing or until 30 seconds expired at which point the experimenter removed the rat from the pool and returned it to the holding pen. Five minutes later the DMTP trial group was started. In pilot work, this procedure was found to enhance performance by decreasing the likelihood that a rat would search for the platform in its location on the previous day of testing on subsequent training that day.

Data were obtained using the tracking software and were supplemented by observations by the experimenter either live or from videotape. The following measures were taken. The latency to escape and the directness of the swim path to the platform were recorded. A direct swim was recorded if the rat remained within a 25 cm alley between the start location and the platform.

5.2.4.4. Kindling and Behavioral Testing Schedule

Starting approximately one week after surgery, rats were kindled twice daily
until 3 stage 5 seizures were evoked. Seven days later, rats were tested in the elevated plus maze (day 7). The next 3 days, rats were tested in the open field (days 8 to 10). Following one day off, rats were tested in the object exploration task in the open field (day 12). After another day off, rats began 10 days of testing on the DMTP task (days 14 to 23). On day 1 of DMTP testing, training was completed using the VP and an inter-trial delay of 0.25 min. On days 2 to 4, task acquisition was completed using the submerged platform and an inter-trial delay of 0.25 min. On days 5 to 10, memory testing was completed using the submerged platform and an inter-trial delay of 0.25, 1, 4, 0.25, 1, and 4 min on successive days.

5.2.5. Histology

Following behavioral testing, animals were sacrificed with an overdose of sodium pentobarbital or chloroform and perfused transcardially with 9% saline. Brains were fixed in formalin and then frozen before 60 μm coronal sections were taken through the dHPC. Every section through the electrode track was mounted and stained with cresyl violet. The location of the electrode tips was documented by matching sections with one of 4 plates from Swanson (1992).

5.2.6. Data Analysis

Data analysis was completed using the statistical software package SPSS® for Windows™. Dependent measures from each of the behavioral tasks were subjected to analyses with repeated measures ANOVA and t tests. Planned comparisons were made using t-tests. One-tailed tests were used when directional hypotheses guided analyses.
5.3. Results

5.3.1. Histology

Electrodes in all kindled rats included in the study were located in the dHPC and electrodes in all control rats included in the study were found in or near the dHPC (see Fig. 3, Experiment 1a for similar placements). No gross histological changes were noted in the brains of either kindled or control rats other than gliosis around the electrode track.

5.3.2. Kindling

Kindling data were as follows (see Table 7). At a mean threshold of $35.4 \pm 3.3 \mu A$, stimulation evoked an initial AD of $22.9 \pm 1.5$ sec in duration. In all cases, secondary AD was observed, although it was highly variable in its latency to onset and duration. A mean of $45.8 \pm 5.3$ stimulations was required to evoke the first stage-5 seizure, and $49.0 \pm 5.2$ stimulations were required to meet our kindling criterion of 3 Stage-5 seizures. For the 3 stage 5 seizures, the mean AD duration, latency to clonus, and duration of clonus were $59.0 \pm 6.4$, $18.3 \pm 4.8$, and $24.8 \pm 1.4$ secs, respectively.

5.3.3. Behavioral Assessment

5.3.3.1. Elevated Plus Maze

Full kindling of the dHPC did not significantly affect either activity- or anxiety-related behaviors in the elevated plus maze. Kindled and control rats exhibited comparable levels of activity as shown by similar distances traveled, total arm entries, and rears between groups (see Fig. 26; all $t(24)'s < 1.09$, $p's > .29$). Also, kindled and control rats exhibited comparable levels of anxiety as shown by similar open arm dwell ratios and entry ratios (see Fig. 27; both $t(24)'s = 0.83$, $p's = .41$).
Table 7. Summary of dHPC kindling data. Duration and latency data are in seconds.

Stage-5 seizure AD and clonus data for kindling are averaged across the final three stage 5 seizures evoked prior to MWM testing. ADT = threshold for evoking afterdischarge, stim. = stimulations

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADT (µA)</td>
<td>35 ± 3.3</td>
</tr>
<tr>
<td>Initial AD duration</td>
<td>22.9 ± 1.5</td>
</tr>
<tr>
<td>Stim. to 1st stage-5 seizure</td>
<td>45.8 ± 5.3</td>
</tr>
<tr>
<td>Total # of stim.</td>
<td>49.0 ± 5.2</td>
</tr>
<tr>
<td>Stage-5 seizure AD duration</td>
<td>59.0 ± 6.4</td>
</tr>
<tr>
<td>Latency to clonus</td>
<td>18.3 ± 4.8</td>
</tr>
<tr>
<td>Duration of clonus</td>
<td>24.8 ± 1.4</td>
</tr>
</tbody>
</table>
**Fig. 26.** Measures of activity/exploration in the elevated plus maze. Total entries = total number of entries to all arms in the maze.
Fig. 27. Measures of anxiety in the elevated plus maze. Entry ratio = number of open arm entries / number of closed arm entries. Dwell ratio = time in open arms / trial duration.
5.3.3.2. Open Field

Similar to results in the elevated plus maze, full kindling of the dHPC did not significantly affect anxiety-related behaviors in the open field (see Fig. 28). Kindled and control rats exhibited comparable levels of anxiety as shown by similar dwell times in \( t(24) = 0.58, p = .57 \) and numbers of entries to \( t(24) = 1.49, p = .15 \) the central ring of the open field.

However, in contrast to results in the elevated plus maze, some evidence suggests that full kindling of the dHPC did impact activity-related behaviors as assessed in the open field (see Fig. 29). Kindled rats spent significantly greater amounts of time exploring the objects \( t(24) = 1.72, p < .05, \text{ one-tailed} \) and reared more frequently \( t(24) = 1.89, p < .05, \text{ one-tailed} \) compared to control rats. However, kindled rats did not show significantly greater amounts of ambulation as shown by comparable overall distance traveled by kindled and control rats \( t(24) = 0.72, p = .48 \).

5.3.3.3. Object Exploration in the Open Field

Full kindling of the dHPC did not significantly affect object recognition memory as assessed in the object exploration in an open field task. On the first trial, with two copies of a novel object, kindled rats exhibited comparable amounts of activity- (distance traveled, \( t(24) = 0.72, p = .48 \); rears \( t(24) = 1.17, p = .25 \)) and anxiety-related behaviors (central ring dwell time, \( t(24) = 0.59, p = .56 \)) relative to controls. Importantly, both groups also exhibited similar amounts of object exploration as shown by comparable dwell times in \( t(24) = 0.02, p = .98 \) and entries to \( t(24) = 1.66, p = .11 \) the object regions. These data suggest that both groups had comparable opportunities to become familiar with the object used.
Fig. 28. Measures of anxiety in the open field task. Entries = the number of entries to the central ring of the open field. Dwell time = time spent in the central ring of the open field.
Fig. 29. Measures of activity/exploration in the open field task. Object dwell times = time spent exploring the two objects placed in the open field. * p < .05 relative to controls
On the second trial, object recognition memory was assessed by replacing the objects from trial 1 with one identical copy of these objects and a novel object. Recognition memory should be reflected in a preference for exploring the novel object (Ennaceur et al., 1996). Kindled rats’ performance was comparable to controls’ in terms of both the ratio of novel to familiar object area entries ($t(24) = 0.52, p = .61$) and the ratio of novel to familiar object area dwell times ($t(24) = 1.80, p = .08$) (see Fig. 30). Moreover, both groups approached the novel object more frequently than the familiar object as shown by entries ratios that were biased towards the novel object, although only kindled rats’ performance was significantly greater than chance on this measure (i.e., greater than 1; kindled – $t(12) = 2.25, p = .02$, one-tailed; control – $t(12) = 1.385, p = .11$, one-tailed). Control rats also spent a significantly greater amount of time investigating the novel object as suggested by a dwell times ratio that was significantly biased towards the novel object (i.e., greater than 1; $t(12) = 3.29, p = .003$, one-tailed). However, this measure failed to reach statistical significance for kindled rats ($t(12) = 0.26, p = .40$, one-tailed). Kindled rats’ performance on all other measures, including distance traveled, rears, central ring dwell time, total object area dwell times, and total object area entries, did not significantly differ from that of control rats (all $t(24)$’s $< 1.01$, $p$’s $>.32$). Collectively, these data suggest that kindling did not significantly disrupt object recognition memory.

5.3.3.4. Delayed-Match-to-Place in the MWM

DHPC kindling did not affect performance on the DMTP task during the initial phase of testing (i.e., acquisition of the task). Kindled and control rats performed comparably across days of acquisition in terms of both escape latencies and direct swims
Fig. 30. Performance on measures of object recognition memory in the object exploration open field task. Dashed line represents chance levels of performance. Entries Ratio = number of bouts of exploration of the novel object / number of bouts of exploration of the familiar object. Dwell Time Ratio = time spent exploring the novel object / time spent exploring the familiar object. # = p < .05 relative to chance performance
(data not shown). This was evidenced by a lack of group effects (both F(1,24)'s < 0.06, both p's > .59), group by trial interactions (both F(2,48)'s < 2.32, both p's > .11), group by day interactions (both F(2,48)'s < 0.03, both p's > .97), and group by trial by day interactions (both F(4,96)'s < 0.07, both p's > .659) on either measure (data not shown). These results suggest that the groups did not differ in their initial abilities to perform the task.

However, dHPC kindling did significantly disrupt performance on the DMTP task during the delay phase of testing. Overall, kindled rats required longer latencies to escape and were less likely to take a direct swim path to the platform compared to controls as shown by significant group effects in terms of both escape latencies (see Fig. 31; F(1,24) = 5.67, p = .03) and direct swims (see Fig. 32; F(1,24) = 13.36, p = .001). Both groups did show significant improvements across trials as indicated by a highly significant trial effect in terms of both escape latencies (F(1,4,34.6) = 131.62, p < .001) and direct swims (F(2,48) = 108.94, p < .001). However, there was some evidence that the groups differed in their performance across trials as shown by a significant group by trials interaction in terms of direct swims (F(2,48) = 3.27, p < .05) but not escape latencies (F(1.4, 34.6) = 2.08, p = .15). Analysis of simple main effects of group within trials in terms of direct swims indicated that kindled rats performed more poorly than controls on trials 2 and 3 (both F(1,24)'s > 5.01, both p's < .04) but not trial 1 (F(1,24) = 0.50, p = .49). For comparison, the same analyses were performed on the escape latency data and a similar pattern of results was found (trials 2 and 3 - both F(1,24)'s > 11.39, both p's < .003; trial 1 - F(1,24) = 0.01, p = .98). Although both groups tended to perform better at shorter delays, the delay effect.
Fig. 31. Latency to escape to the hidden platform on matching trials 1 (A), 2 (B), and 3 (C) during delay phase of testing on the DMTP task in the MWM. Data are averaged across 2 days of testing at each delay. * = p < .05 relative to control performance.
Fig. 32. Probability of making a direct swim to the hidden platform on matching trials 1 (A), 2 (B), and 3 (C) during delay phase of testing on the DMTP task in the MWM. Data are averaged across 2 days of testing at each delay. A direct swim was scored if the rat remained within a 25 cm alley from the start position to the platform. * = p < .05 relative to control performance.
was not significant in terms of either escape latencies ($F(2,48) = 2.19, \ p = .12$) or direct swims ($F(2,48) = 2.58, \ p = .09$), nor were the delay by group (both $F(2,48)$'s < 0.54, both $p$'s > .59), delay by trial, or delay by group by trial interactions significant in terms of either measure (all $F(4,96)$'s < 1.36, all $p$’s > .26). Planned comparisons between groups suggested that kindled and control rats performed comparably on trial 1 at each delay (direct swims - all $t(24)$’s < 0.68, all $p$’s > .10, one-tailed; latency - all $t(24)$’s < 0.61, all $p$’s > .10, one-tailed) and on trial 3 at the 60 second delay, at least in terms of escape latency ($t(24) = 0.78, \ p > .10$, one-tailed). In contrast, kindled rats performed significantly worse than controls on trial 2 at all delays (direct swims - all $t(24)$’s > 2.29, all $p$’s < .01, one-tailed; latency - all $t(24)$’s > 1.77, all $p$’s < .05, one-tailed) and on trial 3 at the 15 and 240 sec delays (direct swims - all $t(24)$’s > 1.63, all $p$’s < .05, one-tailed; latency - all $t(24)$’s > 1.85, all $p$’s < .05, one-tailed). The difference between the groups on trial 3 at the 60-second delay also approached significance, at least in terms of direct swims ($t(24) = 1.06, \ p < .10$, one-tailed).

5.4. Discussion

In experiment3a, the effects of full kindling of the dHPC on anxiety- and activity-related behaviors, object recognition memory, and spatial cognition were assessed in the elevated plus and open field mazes, an open field object exploration task, and a DMTP task in the MWM respectively. Kindling did not affect anxiety-related behaviors in either the elevated plus maze or the open field but did lead to an increase in some exploratory behaviors, although only in the open field. Kindling did not significantly disrupt performance of the object recognition task relative to controls,
although the kindled group did not show the expected bias for the novel object on one measure in this task. Finally, kindling produced a significant impairment of DMTP performance in the MWM, which was not related to the delay between trials.

This is the first study to show that full kindling of the dHPC does not affect anxiety-related behaviors in either the elevated plus maze or an open field task. Kindled rats explored both open arms of the elevated plus maze and the central region of the open field as freely as control rats. Both of these are well-validated measures of anxiety or fearfulness in rodents and the consistent finding of no effect across the tasks argues strongly that full dHPCal kindling did not affect anxiety-related behaviors, or emotionality in general.

This finding contrasts with previous research suggesting that dHPCal kindling may increase anxiety/emotionality in rats. Pinel et al. (Pinel et al., 1977) found that kindling in either the dHPC or AM but not the caudate nucleus increased resistance to capture from an open field and reactivity to tail tap scores above pre-kindling baseline levels. These data were originally interpreted to indicate an increase in emotionality or aggression, although subsequent research from the same laboratory has suggested that kindling-induced changes are better characterized as an increase in anxiety-related/defensive behaviors (Pinel et al., 1998). More recently, Kalynchuk et al. (Kalynchuk et al., 1998a) replicated these results and extended the analysis to include the elevated plus maze and an open field task. They found that kindling in either the dHPC or AM but not the caudate nucleus increased resistance to capture from an open field, decreased square crossings in an open field, and increased open arm entries and dwell times in the elevated plus maze. This profile was interpreted to indicate increased
anxiety/emotionality, although the elevated plus maze findings are the opposite of those conventionally interpreted as reflecting anxiogenesis. The authors reconcile this apparent contradiction by convincingly arguing that the increased open arm exploration reflected increased attempts to escape the maze and therefore the extreme end on the continuum of emotional behavior (i.e., flight) (Kalynchuk et al., 1997). The major difference between the present study and the above findings is the extent of kindling. In the present study, rats were fully kindled with approximately 45 stimulations and experienced a total of 3 stage 5 seizures whereas, in the above studies, rats were kindled with 99 stimulations and presumably experienced a concomitantly greater number of stage 5 seizures (although this number was not reported). Thus, the data suggest that extended kindling of the dHPC is required to elicit anxiogenic effects. This hypothesis is generally consistent with observations that greater extents of AM kindling produce increasingly greater effects on anxiety/emotionality (Kalynchuk et al., 1997). It should be noted, however, that, unlike dHPC kindling, full AM kindling appears to be capable of producing anxiogenic effects (Adamec, 1990a; Adamec, 1998; Helfer, Deransart, Marescaux, & Depaulis, 1996; Kalynchuk et al., 1997; Nieminen et al., 1992), suggesting that the AM is more sensitive to the anxiogenic effects of kindling than the dHPC. This possibility is consistent with observations that extended kindling of the AM produces consistently more extreme scores on measures of anxiety than extended kindling of the HPC, although these differences have not reached statistical significance (Kalynchuk et al., 1998a).

The present study is also the first to show that full dHPCal kindling affects activity/exploration. Kindled rats reared more frequently and spent more time exploring
objects in the open field relative to control rats. However, they did not show increased activity/exploration on other measures in the open field or on any measure in the elevated plus maze. The discrepancy of the effect between tasks might be explained by the observation that richer environments typically elicit greater amounts of activity/exploration and hence are more likely to be sensitive to small changes in such behaviors (Hall, 1956). In our case, the open field had a much larger surface area and contained a greater variety of stimuli (e.g., the objects) than the elevated plus maze and thus may have been more sensitive to a slight effect of kindling on activity/exploration. Increased activity/exploration is generally consistent with the pattern of effects of HPCal lesions on open field behavior (see O’Keefe & Nadel, 1978) and thus could indicate a disruption of HPCal function. This interpretation would, of course, be consistent with the hypothesis that HPCal dysfunction also underlies the disruption of spatial cognition following kindling (Leung & Shen, 1991; Leung et al., 1994; Leung et al., 1990; Leung et al., 1996; Lopes Da Silva et al., 1986; Sutherland et al., 1997; Experiments 1a and 2) and presents a possible account for dHPC kindling’s disruptive effects on brightness discrimination task performance (Becker et al., 1997). Since this task is sensitive to the effects of HPCal lesions (McLamb, Mundy, & Tilson, 1988; Munoz & Grossman, 1981), this kindling-induced effect may also be a result of a direct alteration of HPCal function.

Although the effects of dHPC kindling on open field behavior have not been previously reported, the effects of kindling in other sites have been investigated yielding variable results. In a small circular open field, full AM kindling was found to produce a decrease in activity/exploration that was interpreted, along with elevated plus maze
results, to indicate increased anxiety (Nieminen et al., 1992). In a small square open field that contained objects, full ventral HPCal kindling was found to be without effect (Holmes et al., 1993). Finally, in a small square open field, extended kindling of either the perforant path or AM was found to increase activity/exploration (Cammisuli et al., 1997). These findings suggest that the effects of kindling on open field behavior can be quite variable and may depend upon the site and extent of kindling as well as the particular attributes of the open field being used. This also underscores the general point that open field behavior must be interpreted cautiously in the absence of a broader behavioral profile obtained on additional tasks. However, these studies do suggest that under some circumstances, the open field may be sensitive to some of the behavioral changes induced by kindling.

The present study also provides further evidence that other forms of cognition besides spatial cognition are spared by full dHPCal kindling. Kindled rats' performance was not significantly different than controls on either measure of object recognition as assessed in the open field object exploration task (Ennaceur et al., 1996). This suggests that object recognition memory is unaffected by kindling and is consistent with our previous finding in Experiment 1a that full dHPCal kindling does not disrupt object associative memory. Together these findings indicate that object-related memory in general is unaffected by dHPCal kindling. The present results are also consistent with the finding that HPCal system lesions (e.g., fimbria/fornix transections) do not disrupt performance on the object exploration task (Ennaceur & Aggleton, 1997). One caveat in the present result should be noted however. The kindled group failed to show the expected bias for the novel object in terms of the relative time spent exploring the novel
and familiar objects (i.e., the dwell ratio was not significantly greater than 1). This result could indicate the presence of a very mild impairment of object recognition memory, a subtle change in some other aspect of behavior involved in exploring novelty, or simply a spurious finding. Further investigation is required to differentiate these possibilities.

The main finding of the present study is that full kindling of the dHPC disrupted DMTP performance in the MWM. Kindled rats were significantly slower in escaping to the HP and utilized less direct routes on matching trials 2 and 3 at all three delays tested (0.25, 1, and 4 minutes). This result could indicate that full dHPC kindling produces an anterograde impairment of spatial WM function. However, it could also indicate an impairment of other mnemonic functions or non-mnemonic functions, which could have resulted in a secondary disruption of spatial task performance. Several considerations argue against this possibility. First, both kindled and control rats rapidly improved on the task and did not differ during the acquisition phase of testing. Second, during the delay phase, kindled and control rats performed comparably on the first matching trial at all delays and both groups improved significantly across trials. The impairment reflected the fact that kindled rats' performance did not improve as quickly as controls, not that they did not improve at all. Fourth, I have previously shown that dHPC kindled rats perform normally on a VP control task in the MWM (Gilbert et al., 1996), even when tested prior to any other water maze experience (Experiment 1a). Finally, kindled rats performed normally on most other aspects of performance in other tasks assessed in the present study suggesting that their basic behavioral repertoires were unaffected by kindling. In particular, the failure of kindling to alter anxiety-related behaviors in either
the elevated plus maze or open field eliminates anxiety-related factors as a basis for altered DMTP performance.

The present results provide further evidence that the deficit produced by dHPCal kindling shows some specificity in the class of mnemonic function that is affected. In Experiment 1a, I found that kindling disrupted spatial RM but not object associative memory. In the present study, I have found that kindling disrupted spatial WM but not object recognition memory. Collectively, these findings indicate that the properties of the information to be processed (spatial vs object-related) may be a critical determinant of the susceptibility of the task to disruption by dHPC kindling. Other dimensions of the mnemonic demands, such as the WM versus RM component, may be of less significance since kindling has been observed to disrupt both spatial RM tasks (e.g., Experiment 1a) and spatial WM tasks (Experiment 2 and the present findings).

The absence of a relation between the inter-trial delay and the deficit suggests that the impairment is more likely one of spatial learning than STM. If spatial learning were intact and STM affected, one would predict that performance should deteriorate more quickly relative to controls across longer delays since these would place progressively greater demands on STM function. The absence of a delay-dependent deficit is thus not consistent with the presence of a STM deficit and suggests, in the absence of a non-mnemonic basis for the deficit, that learning was disrupted. However, given that the shortest delay used was 15 seconds, it is still possible that an impairment in STM underlies the observed effect, but it would have to be one that reaches asymptotic levels within that time-period.

An additional point that can be derived from the present DMTP findings is that
the kindling-induced impairment of spatial cognition is quite long-lasting. Testing on the DMTP task began 14 days after the completion of kindling and the delay phase of testing took place over a period of 6 days from 18 to 23 days following the completion of kindling. This is the longest interval between dHPCal kindling and testing at which an anterograde impairment of spatial cognition has been detected. Thus, the anterograde disruption of spatial cognition produced by full dHPC kindling lasts for at least approximately 3 weeks following kindling, although it is not clear whether the deficit lasts longer. Partial dHPCal kindling has been shown to impair performance on a WM MWM task at 7 but not 28 days following kindling suggesting that impaired spatial cognition may resolve by 4 weeks following kindling (Sutherland et al., 1997). However, subjects in this study were trained prior to kindling and thus it is not clear whether the impairment reflects an anterograde or retrograde deficit. Moreover, the intervening training prior to the 28 day testing confounds recovery with relearning and the fact that partial kindling was used prevents direct comparison with results following full kindling as in the present study. Thus, there is no evidence that the impairment induced by full dHPC kindling is not permanent, although surprisingly little investigation of this issue has been undertaken.

In summary, the present study has shown that full kindling of the dHPC produces a profile of behavioral effects that is consistent with a mild disruption of HPCal function. That is, kindling produces a disturbance of spatial cognition and an increase in activity/exploration without affecting object recognition memory or anxiety-related behaviors. I also determined that kindling’s effects on spatial cognition likely reflect a disruption of learning rather than STM-related processes and are long-lasting
up to a period of at least 16 days following the completion of kindling. These results further highlight the selective effects of dHPC kindling on spatial cognition and further characterize this model as a useful means to study epilepsy-related mnemonic dysfunction.
6. EXPERIMENT 3B – PRH KINDLING’S EFFECTS ON ANXIETY, ACTIVITY, OBJECT MEMORY, AND SPATIAL MEMORY

6.1. Introduction

The PRH has direct and reciprocal anatomical connections with the HPC (Burwell et al., 1995), plays an important role in the generalization of limbic seizure activity (Kelly & McIntyre, 1996; McIntyre & Kelly, 1998), and contributes significantly to temporal lobe memory function (Eichenbaum et al., 1994; Squire, 1992). For these reasons, I expected that PRH kindling might produce similar effects to dHPC kindling. However, in experiments 1a and 1b, I found that the effects of full dHPC and PRH kindling could be doubly dissociated. DHPC kindling impaired spatial learning/STM whereas PRH kindling impaired long-term object associative memory. In Experiment 3a, I found that full dHPC kindling significantly impaired performance of a DMTP task in the MWM but did not impair performance on an object recognition task in an open field. Therefore, Experiment 3b sought to further explore the site specificity of these effects by examining the impact of full PRH kindling on a different spatial memory task than that used in Experiment 1b, a DMTP task that required WM as well as spatial cognition, and also on a different object memory task than that used in Experiment 1b, an object exploration task that required object recognition learning and STM. The present study had the additional goal of determining the contribution of non-mnemonic
effects to changes in spatial and object-related cognition by assessing activity- and anxiety-related behaviors in the elevated plus maze and the open field.

The design of Experiment 3b was the same as that of Experiment 3a. In summary, rats were fully kindled with twice-daily stimulation of the PRH until a criterion of 3 stage 5 seizures was reached. One week later, to examine activity- and anxiety-related behaviors, rats were tested in an elevated plus maze one day and an open field task the next. The following phase of testing was designed to assess object recognition memory. Rats were habituated for two additional days in the open field and then given a day off. The next day, rats were tested in a two-trial object exploration task modified from Ennaceur and colleagues (Ennaceur et al., 1996). The final phase of testing was designed to assess delay-dependent changes in spatial WM related performance. Rats were pretrained on a 4-trial DMTP task for 1 day using a VP. Rats then received 3 additional days of training with an inter-trial delay of 0.25 minutes. Delay testing then began, with rats tested on consecutive delays with inter-trial delays of .25, 1, 4, 0.25, 1, and 4 minutes.

6.2. Methods and Materials

6.2.1. Subjects

Twenty-six male Long-Evans hooded rats (Charles River) weighing 300-375 g at the beginning of the study were used as subjects. Food and water were available ad libitum throughout the experiment. Rats were maintained in pairs in shoebox cages prior to surgery and were housed individually for the remainder of the experiment. All experimental procedures were carried out during the light portion of the 12:12 hour
light/dark cycle. All rats were handled each day throughout the experiment except
during the first four days following surgery. Subjects were randomly assigned to either
the kindled group (n=13) or the control group (n=13). The 13 control rats were each
yoked to one of the kindled rats.

6.2.2. Surgery

In preparation for surgery, animals were anaesthetized with Somnotol™
(sodium pentobarbital, 60 mg/kg) and given methyl scopolamine (1 mg/kg) to reduce
respiratory congestion. Rats were placed in a stereotaxic apparatus, the skull was
leveled, and a bipolar nichrome wire electrode (127 μm dia.) was implanted in either the
right (n=7) or left (n=6) PRH with the manipulator arm angled 13° towards the skull at
the following coordinates relative to bregma: -3.0 mm (AP); 4.0 mm (ML); 8.8 mm
(DV). Five jeweler’s screws were used to secure the electrode assembly to the skull,
with one screw over the anterior cortex serving as the reference electrode. The
electrode assembly was affixed to the skull with dental acrylic, and a topical
antibiotic/steroid, Topagen™ was applied to the wound. Finally, a subcutaneous
injection of Anafen (0.5 cc/kg) was given for post-surgical analgesia.

6.2.3. Kindling

Approximately 7 days following surgery kindling was initiated. In the first
kindling session, the stimulus intensity required to evoke an afterdischarge (AD) was
determined. A Grass S8800 stimulator was used to deliver a 1 sec train of balanced
biphasic square wave pulses at 60 pps at an initial intensity of 50μA (base-to-peak). If
AD greater than 3 sec in duration was not evoked, intensity was increased along the
following scale 100, 200, 300, 500, 700, 1000, 2000, 3000, 5000 μA every 2 minutes
until 3 sec or greater AD was elicited, or greater than 2 sec AD was elicited at consecutive intensities. The minimal intensity triggering AD was arbitrarily defined as ADT and was the intensity used for kindling during the remainder of the study.

Subsequently, rats were kindled twice daily with stimulations separated by at least 4 hrs until a criterion of 3 stage 5 seizures (Racine, 1972b) was achieved. At this point, rats were considered fully kindled and behavioral testing was started 7 or 8 days later. Each control rat was yoked to a specific kindled rat and received identical treatment except that it was not stimulated.

6.2.4. Behavioral Assessment

All aspects of behavioral testing were identical to those used in Experiment 3a.

6.2.5. Histology

Following behavioral testing, animals were sacrificed with an overdose of sodium pentobarbital or chloroform and perfused transcardially with 9% saline. Brains were fixed in formalin and then frozen before 60 μm coronal sections were taken through the full extent of the PRH. Every section through the electrode track was mounted and stained with cresyl violet. The location of the electrode tips was documented by matching sections with one of 4 plates from Swanson (1992).

6.2.6. Data Analysis

Data analysis was completed using the statistical software package SPSS© for Windows™. Dependent measures from each of the behavioral tasks were subjected to analyses with repeated measures ANOVA and planned comparisons using t-tests.
6.3. Results

6.3.1. Histology

Electrodes in all kindled rats included in the study were located in the PRH. Specifically, in the 13 kindled rats, the electrode was found in layers 3 to 6 of the anterior PRH (for similar placements see Fig. 10, Experiment 1b). Electrodes in all control rats included in the study were found in or near the PRH. Specifically, in 13 control rats, the electrode was found in the deep layers of the PRH (7 rats), the superficial layers of the PRH (4 rats), or the external capsule (2 rats). No gross histological changes were noted in the brains of either kindled or control rats other than gliosis around the electrode track.

6.3.2. Kindling

Kindling data are shown in Table 8. Briefly, at a mean threshold of 1226 ± 602 μA, stimulation evoked an initial AD of 3.6 ± 0.2 sec in duration. A mean of 7.2 ± 0.8 stimulations was required to evoke the first stage-5 seizure, and 12.3 ± 1.4 stimulations were required to meet our kindling criterion of 3 Stage-5 seizures. On these last 3 stimulations, the mean AD duration, latency to clonus, and duration of clonus were 14.0 ± 1.5, 1.2 ± 1.1, and 12.9 ± 1.2 secs, respectively.

6.3.3. Behavioral Assessment

6.3.3.1. Elevated Plus Maze

Full kindling of the PRH significantly altered anxiety- but not activity-related behaviors in the elevated plus maze. Kindled rats were less likely to enter and spent less time exploring open arms relative to controls as shown by significantly lower open arm dwell ratios (t(25) = 2.59, p = .02) and entry ratios (t(25) =2.62, p= 0.02) (see Fig.
Table 8. Summary of PRH kindling data. Duration and latency data are in seconds.

Stage-5 seizure AD and clonus data for kindling are averaged across the final three stage 5 seizures evoked prior to MWM testing. ADT = threshold for evoking afterdischarge, stim. = stimulations

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<tr>
<td>ADT (μA)</td>
<td>1226 ± 602</td>
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<tr>
<td>Initial AD duration</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>Stim. to 1st stage-5 seizure</td>
<td>7.2 ± 0.8</td>
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<tr>
<td>Total # of stim.</td>
<td>12.3 ± 1.4</td>
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<tr>
<td>Stage-5 seizure AD duration</td>
<td>14.0 ± 1.5</td>
</tr>
<tr>
<td>Latency to clonus</td>
<td>1.2 ± 1.1</td>
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<tr>
<td>Duration of clonus</td>
<td>12.9 ± 1.2</td>
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Fig. 33. Measures of anxiety in the elevated plus maze. Entry ratio = number of open arm entries / number of closed arm entries. Dwell ratio = time in open arms / trial duration. * p < .05 relative to controls
However, both kindled and control rats exhibited comparable levels of activity as shown by similar distances traveled, total arm entries, and rears between groups (all t(25)'s < 1.90, p's > .05; see Fig. 34). Together, these data suggest that PRH kindling produced selective anxiogenic effects in the elevated plus maze.

6.3.3.2. Open Field

Similar to results in the elevated plus maze, full kindling of the PRH significantly altered anxiety- but not activity-related behaviors in the open field. Kindled rats were less likely to enter and spent less time exploring the central ring of the open field relative to control rats as shown by significantly lower central ring entries (t(25) = 2.42, p = 0.02) and lower central ring dwell times that just failed to achieve significance levels (t(25) = 1.80, p = .08) (see Fig 35). In contrast, kindled and control rats did not differ on several measures of activity in the open field including total distance traveled (t(25) = 0.72, p = .48), time spent exploring the objects (t(25) = 0.82, p = .42), and numbers of rears (t(25) = 0.42, p = .89) (see Fig. 36). Together, these data suggest that PRH kindling produced selective anxiogenic effects in the open field.

6.3.3.3. Object Exploration in the Open Field

Full kindling of the PRH significantly disrupted object recognition memory as assessed in the object exploration in an open field task. On the first trial, with two novel objects, kindled rats exhibited comparable amounts of activity- (distance traveled, t(25) = 1.12, p = .27; rears t(25) = 0.25, p = .80) and anxiety-related behaviors (central ring dwell time, t(25) = 0.71, p = .48) relative to controls. Importantly, both groups also exhibited similar amounts of object exploration as shown by comparable dwell times in (t(25) = 0.66, p = .51) and entries to (t(25) = 1.66, p = .11) the object
Fig. 34. Measures of activity/exploration in the elevated plus maze. Total entries = total number of entries to all arms in the maze.
Fig. 35. Measures of anxiety in the open field task. Entries = the number of entries to the central ring of the open field. Dwell time = time spent in the central ring of the open field. * p < .05 relative to controls
Fig. 36. Measures of activity/exploration in the open field task. Object dwell times =
time spent exploring the two objects placed in the open field.
regions. These data suggest that both groups had comparable opportunities to become familiar with the appearance of the object used.

On the second trial, object recognition memory was assessed by replacing the objects from trial 1 with an identical copy and a novel object. Recognition memory should be reflected in a preference for exploring the novel object (Ennaceur et al., 1996). Kindled rats’ performance indicated a significant disruption of object recognition memory. Kindled rats failed to approach the novel object more frequently than the familiar object as shown by a ratio of novel to familiar object area entries that was significantly lower than that of control rats ($t(25) = 2.36, p = .01$, one-tailed) and was not significantly biased towards the novel object (i.e., greater than 1; $t(12) = -0.48, p = .64$) (see Fig. 37). In contrast, control rats showed a significant entry ratio bias for the novel object ($t(13) = 3.91, p = .002$). Moreover, kindled rats also failed to spend a greater amount of time investigating the novel object compared to the familiar object as suggested by a ratio of novel to familiar object area dwell times that was significantly lower relative to that of control rats ($t(25) = 2.07, p = .02$, one-tailed) and was not significantly biased towards the novel object (i.e., greater than 1; $t(12) = -0.14, p = .89$). Again, in contrast, control rats showed a significant dwell ratio bias for the novel object ($t(13) = 3.36, p < .01$). Notably, kindled rats’ performance on all other measures, including distance traveled, total rears, central ring dwell time, total object area dwell times, and total object area entries, did not significantly differ from that of control rats (all $t(25)$’s $\leq 1.01$, all $p$’s $\geq .32$) suggesting that the difference in performance was restricted to the relative degree of preference of the novel to the familiar object between groups. Overall, these data suggest that PRH kindling either severely disrupted
Fig. 37. Performance on measures of object recognition memory in the object exploration open field task. Dashed line represents chance levels of performance. Entries Ratio = number of bouts of exploration of the novel object / number of bouts of exploration of the familiar object. Dwell Time Ratio = time spent exploring the novel object / time spent exploring the familiar object. * = p < .05 relative to controls
object recognition memory or impaired some other aspect of cognition that mediates enhanced exploration of novelty.

6.3.3.4. Delayed-Match-to-Place in the MWM

PRH kindling did not affect performance on the DMTP task during either the initial phase (i.e., acquisition of the task) or the delay phase of testing. Kindled and control rats performed comparably across days of acquisition in terms of both escape latencies and direct swims (data not shown). This was evidenced by a lack of group effects (both F(1,23)'s ≤ 0.07, both p's ≥ .80), group by trial interactions (both F(2,46)'s ≤ 0.04, both p's ≥ .97), group by day interactions (both F(2,48)'s ≤ 0.01, both p's ≥ .99), and group by trial by day interactions (both F(4,96)'s ≤ 1.38, both p's ≥ .25) on both measures. These results suggest that the groups did not differ in their ability to acquire the basic procedural components of the task.

Kindled and control rats also performed comparably on the DMTP task during the delay phase of testing. Overall, both groups required comparable latencies to escape and were equally likely to take a direct swim path to the platform as shown by the absence of significant group effects in terms of either escape latencies (F(1,23) = 0.25, p = .62; see Fig 38) or direct swims (F(1,23) = 0.19, p = .67; see Fig 39). Moreover, both groups showed significant improvements across trials as indicated by a highly significant trial effect in terms of both escape latencies (F(2,46) = 67.65, p < .001) and direct swims (F(1.5,34.7) = 125.06, p < .001) and did not differ in their trial-related performance as indicated by the absence of a significant group by trial (latency – F(1.5,34.7) = 2.27, p = .13; direct swims – F(2,46) = 0.81, p = .45) or group by trial by delay interaction (latency – F(3.2,73.5) = 0.84, p = .48; direct swims...
Fig. 38. Latency to escape to the hidden platform on matching trials 1 (A), 2 (B), and 3 (C) during delay phase of testing on the DMTP task in the MWM. Data are averaged across 2 days of testing at each delay.
Fig. 39. Probability of making a direct swim to the hidden platform on matching trials 1 (A), 2 (B), and 3 (C) during delay phase of testing on the DMTP task in the MWM. Data are averaged across 2 days of testing at each delay. A direct swim was scored if the rat remained within a 25 cm alley from the start position to the platform.
- $F(4,92) = 0.51, p = .73$). Both groups also showed superior performance at shorter inter-trial delays as shown by a significant delay effect (latency - $F(2,46) = 8.15, p = .001$; direct swims - $F(2,46) = 4.62, p = .02$) but did not differ in their delay-related performance as indicated by the absence of a significant group by delay (latency – $F(2,46) = .08, p = .92$; direct swims – $F(2,46) = 0.04, p = .96$) or group by trial by delay interaction (latency – $F(3.2,73.5) = 0.84, p = .48$; direct swims – $F(4.92) = 0.51, p = .73$). The delay effect appeared to show some relation to the trial of testing as indicated by a trial by delay interaction that was significant in terms of escape latencies ($F(3.2,73.5) = 2.64, p < .05$) but not direct swims ($F(4.92) = 1.98, p = .11$).

Subsequent analyses of simple main effects of delay within trials for latency data suggested that performance was worse at longer delays on trial 1 ($F(2,46) = 10.04, p < .001$) but not trials 2 or 3 (both $F(2,46)$'s $\leq 2.12$, both $p$'s $\geq .13$). For comparison, the same analyses were performed on the direct swims data and a similar pattern of results was found (trial 1 - $F(2,46) = 5.54, p < .01$; trials 2 and 3 - both $F(2,46)$'s $\leq 2.11$, both $p$'s $\geq .13$). Finally, planned comparisons between the groups on each trial at each delay further suggested that kindled and control rats performed comparably throughout testing (direct swims - all $t(23)$'s $\leq 1.06$, all $p$'s $>.10$; latency - all $t(24)$'s $\leq 1.67$, all $p$'s $>.05$).

6.4. Discussion

In Experiment 3b, the effects of full kindling of the PRH on anxiety- and activity-related behaviors, object recognition memory, and spatial cognition were assessed in the elevated plus and open field mazes, an open field object exploration task,
and a DMTP task in the MWM respectively. Kindling produced anxiogenic effects in both the elevated plus maze and the open field but did not affect activity/exploration in either task. Kindling also significantly disrupted performance of the object recognition task. Kindling did not, however, affect performance of the DMTP task in the MWM.

The present study is the first to show that full kindling in an area outside of the AM is capable of altering anxiety-related behaviors. PRH kindled rats explored the open arms in the elevated plus maze and the central region of the open field less frequently than controls. Based on standard interpretations (Lister, 1990; Rodgers & Dalvi, 1997; Walsh & Cummins, 1976; Williams & Russel, 1972), both results indicate an increase in anxiety and lead me to conclude that PRH kindling is anxiogenic. The strength of this conclusion is enhanced by the consistency of results across both tasks and the concomitant lack of effect of PRH kindling on measures of activity/exploration in either task. The latter observation is important because changes in these-types of behaviors can also affect the measures used to assess anxiety in these tasks (Rodgers & Dalvi, 1997; Walsh & Cummins, 1976).

Although little previous research has directly implicated the PRH in anxiety-related behaviors, several observations are consistent with a role for this region in emotional behavior. First, the PRH has strong reciprocal links with the AM (Amaral, Price, Pitkanen, & Carmichael, 1992; Burwell et al., 1995; Suzuki, 1996a; Suzuki, 1996b), and the AM has been strongly implicated in the regulation of a wide variety of emotional behaviors including anxiety (Davis, 1998; LeDoux, 1992; Ninan, 1999; Rosen & Schulkin, 1998). In fact, the PRH serves as one of the major sources of multi-modal sensory input to the AM (Turner & Zimmer, 1984). Second, the PRH has been
implicated in fear-related behaviors using the fear-potentiated startle paradigm.

Specifically, post-training PRH lesions have been shown to disrupt fear conditioning to either visual, auditory, or contextual stimuli (Corodimas & LeDoux, 1995; Falls, Bakken, & Heldt, 1997; Rosen et al., 1992), although lesions prior to training are without effect (Campeau & Davis, 1995; Phillips & LeDoux, 1995). This suggests that the PRH is part of the normal circuitry participating in associative learning about fearful stimuli, although its participation is not essential. It should be noted however, that studies of immediate-early gene activation following exposure to the elevated plus maze have not shown activation in the PRH (Duncan, Knapp, & Breese, 1996; Hinks, Brown, Field, Poat, & Hughes, 1996; Silveira, Sandner, & Graeff, 1993). This raises the possibility that the effects of PRH kindling on anxiety/emotionality may be mediated by functional changes produced in other brain regions.

The second major finding of the present study is that PRH kindling disrupted performance of the object exploration task in the open field. Kindled rats failed to show the normally observed bias for the novel object on the second of two open field trials separated by 15 minutes in terms of either the relative number of approaches made to or the time spent exploring the novel object relative to the familiar one. This result has been interpreted to indicate an impairment of recognition memory since comparable exploration of both the novel and previously used object suggests that the rat considers both objects equally unfamiliar (Aggleton et al., 1997; Ennaceur et al., 1996). However, because PRH kindling also increased anxiety, it is worth considering whether this effect, rather than an object recognition memory impairment, could be responsible for the object exploration task result. I consider this unlikely for several reasons. First,
although kindled rats exhibited increased anxiety in the elevated plus maze and prior open field testing, they no longer exhibited signs of increased anxiety on either the sample or matching trials of the object exploration task. Second, kindled rats showed comparable amounts of object exploration as controls but failed to distribute their exploration between the objects in a manner indicative of the recognition of one but not the other object. Third, kindled rats performed normal on the DMTP task in the MWM suggesting that any changes in anxiety that were still present were not sufficient to disturb other forms of cognitive function or behavioral performance. Finally, as will be discussed in Experiment 3c, AM kindling is also anxiogenic but does not affect object recognition memory performance.

The finding of an impairment of object recognition memory following PRH kindling is consistent with the literature indicating a specific role for this structure in object-related mnemonic function. Previously, several studies have reported that lesions of the PRH impair performance on the open field object exploration task used in the present study (Aggleton et al., 1997; Ennaceur & Aggleton, 1997; Ennaceur et al., 1996). Likewise, PRH lesions have been shown to disrupt object recognition memory using delayed-non-match to sample tasks in rats (Mumby & Pinel, 1994; Wiig & Bilkey, 1995) and a variety of tasks in primates (Eacott, Gaffan, & Murray, 1994; Meunier, Bachevalier, Mishkin, & Murray, 1993; Nakamura & Kubota, 1996; Zola-Morgan et al., 1989). In Experiment 1b, I found that PRH kindling also impairs long-term retention of an OD problem. Similarly, lesion studies have indicated that PRH lesions can also produce an impairment on visual discrimination tasks in both rats (Myhrer & Wangen, 1996; Experiment 1b) and primates (Buckley & Gaffan, 1998a; Buckley & Gaffan,
1998b; Buckley & Gaffan, 1998c). These impairments reflect a disruption of object associative memory. Together with the present finding, these results suggest that PRH kindling may produce a general defect in object-related memory function. Indeed, it has been suggested that PRH lesions produce a general defect in object identification, which manifests itself in performance deficits on a variety of object-related tasks if the task places sufficient demands on determining or discriminating the identity of objects (Buckley & Gaffan, 1998c).

One difference between PRH kindling’s effects on object associative memory as assessed in Experiment 1b and object recognition memory as assessed in the present study is that LTM but not learning/STM was affected in the former whereas learning/STM was affected in the latter. This finding could reflect fundamental differences in the stage of memory processing susceptible to disruption by kindling for these different classes of object-related memory function. This would imply that distinct mechanisms underlie kindling’s effects on each of these mnemonic functions to the extent that it is unlikely identical mechanisms could be responsible for both the initial and long-term storage of object-related information. Alternatively, the observed pattern of effects could be accounted for by differences in the sensitivities of the two tasks to a mild disruption of object-related mnemonic function. The object recognition task may place sufficient demands on object-related mnemonic function such that a mild impairment is revealed even at the least demanding level of the task (i.e., initial learning and STM). The object associative task may be easier, perhaps due to the redundant involvement of other systems at shorter retention durations, such that the kindling-induced impairment is only revealed when the task is made more difficult by increasing
the retention interval and challenging the mechanisms responsible for LTM. It should be noted that the effects of PRH kindling on long-term object recognition memory have not been investigated. Results of such a study could help differentiate between the above possibilities. Also, it should be noted that the lesion literature indicates that object associative memory tasks and object recognition memory tasks may differ in their sensitivity to the effects of PRH lesions. Recognition tasks are consistently affected whereas associative memory tasks are only affected when the task is made more challenging by increasing the inter-trial delays, the retention testing interval, or the number of objects in the set to be learned (Buckley & Gaffan, 1998a; Buckley & Gaffan, 1998b; Eacott et al., 1994; Zola-Morgan et al., 1989).

Full kindling of the PRH did not affect any aspect of performance in the DMTP task in the MWM. This result corroborates the finding in Experiment 1b that PRH kindling does not disrupt acquisition or retention of the standard MWM task and suggests that PRH kindling spares spatial cognition. This finding is also consistent with a recently emerging picture from the lesion literature suggesting that the PRH has a much smaller role in spatial cognition than the HPC. For example, even relatively large PRH lesions produce a mild impairment of the MWM task (Wiig & Bilkey, 1994a) relative to the effects of HPCal system lesions (Hansson & Skelton, 1998; Morris, Schenk, Tweedie, & Jarrard, 1990; Moser, Moser, Forrest, Andersen, & Morris, 1995) and smaller lesions, which probably resemble more closely the volume of tissue directly affected by kindling, produce no effect (Wiig & Bilkey, 1994c; Exp. 1b).

In summary, full kindling of the PRH produces two distinct effects on the tasks employed in the present study. Kindling increased anxiety as measured in both the
elevated plus maze and an open field task and disrupted object recognition learning/STM in an open field task. These effects of kindling showed some selectivity because spatial WM-like performance on a DMTP task in the MWM was not affected. This pattern of effects is generally consistent with the effects of PRH lesions on similar tasks suggesting that the effects of PRH kindling may be mediated by changes local to the kindling site. They also underscore the capacity of kindling to produce enduring and selective disruptions of mnemonic function, the specific nature of which appear to depend upon the site being kindled.
7. EXPERIMENT 3C – AM KINDLING’S EFFECTS ON ANXIETY, ACTIVITY, OBJECT MEMORY, AND SPATIAL MEMORY

7.1. Introduction

In Experiment 3a and 3b, I obtained further evidence that the effects of full dHPC and PRH kindling can be dissociated. DHPC kindling disrupts spatial cognition whereas PRH kindling disrupts object recognition memory and enhances anxiety, but does not affect spatial cognition. To further investigate the relation between some of the behavioral effects observed on different tasks in experiments 3a and 3b and the importance of the site of kindling to these effects, in Experiment 3c I investigated the effects of kindling in a third site, the AM, on a behavioral protocol identical to that used in experiments 3a and 3b.

There are several reasons for choosing the AM as a third site to examine the behavioral effects of kindling. First, the AM has played a prominent role in kindling research (Goddard et al., 1969) and continues to be one of the most heavily used sites in kindling studies (Cain, 1992). Therefore, the behavioral effects of AM kindling are of general interest to generating a broad characterization of kindling from this structure.

Second, the AM is highly susceptible to kindling, as shown by rapid kindling rates and low AD thresholds (Goddard et al., 1969; Mohapel, Kelly, Dufresne, & McIntyre, 1996), and undergoes changes in response to kindling from a variety of
different structures (Le Gal La Salle, 1982) including both the HPC (e.g., Kairiss, 
Racine, & Smith, 1984; Shimizu, Morimoto, Kuroda, & A, 1995) and PRH (Buchanan 
& Bilkey, 1997; Mohapel & Corcoran, 1996). Thus, the important role of the AM in 
kindling raises the possibility that changes in this structure could play a role in 
behavioral effects of kindling. This possibility can be most directly investigated with 
kindling using AM stimulation.

Third, the AM is relatively closely linked anatomically to both the HPC and PRH 
(Amaral et al., 1992). The AM is situated in the temporal lobes proximal to both the 
PRH and HPC and has extensive reciprocal connections with the PRH directly and with 
the HPC primarily via interconnections with the entorhinal cortex (Amaral et al., 1992; 
Stefanacci, Suzuki, & Amaral, 1996). Thus, the intimacy of communication between 
these structures suggests that kindling in any of them might produce partly overlapping 
behavioral effects.

Lastly, kindling of the AM has in fact already been shown to produce a variety 
of different behavioral effects. The most notable of these is that AM kindling reliably 
alters anxiety-related behaviors (Adamec, 1990a; Adamec, 1998; Depaulis et al., 1997; 
Kalynchuk et al., 1997; Pinel et al., 1977; Pinel et al., 1998). In the elevated plus maze, 
kindling of several different AM nuclei has been repeatedly shown to increase anxiety 
(Adamec, 1990a; Adamec, 1999; Adamec & Morgan, 1994; Kalynchuk et al., 1998a; 
Kalynchuk et al., 1998b; Kalynchuk et al., 1997; Nieminen et al., 1992). It should be 
noted, however, that some evidence suggests that AM kindling is also capable of being 
anxiolytic in the elevated plus maze if the kindling focus is found in very specific regions 
of the AM defined precisely in terms of their anterior-posterior, hemispheric, and
nuclear position (Adamec, 1998; Adamec & Morgan, 1994). Further evidence that AM kindling may alter anxiety-related functions or emotionality comes from studies by Pinel showing alterations of a variety of defense-related behaviors after kindling (Kalynchuk et al., 1997; Pinel et al., 1977; Pinel et al., 1998). For example, AM kindled rats have been found to show an increased sensitivity to tail tap, greater resistance to capture, and increased expression of defensive behavior in the resident-intruder paradigm (Pinel et al., 1977; Pinel et al., 1998).

Some evidence suggests that AM kindling can also alter cognitive function as assessed in several different aversive conditioning tasks. The most frequently investigated of these tasks has been passive avoidance. Boast and McIntyre (1977) were the first to show that full bilateral AM kindling impairs performance on a step-out passive avoidance task, a finding subsequently replicated by Peele and Gilbert (1992) on a 2 chamber passive avoidance task. In each of these studies, performance was measured against that of fully unilaterally AM kindled rats and not unkindled controls. Subsequently, Stone and Gold (1988) have shown that full unilateral AM kindling can also disrupt passive avoidance performance relative to unkindled controls in a two chamber task. In addition, full AM kindling, when coupled with a lesion of the contralateral unkindled AM, has been shown to disrupt acquisition of a conditioned emotional response in a lever press task (McIntyre & Molino, 1972), and full AM kindling alone has been shown to impair 24 hour retention of a brightness discrimination in a Y-maze (Becker & Grecksch, 1992; Becker et al., 1992). Interpretation of changes in performance on aversive tasks must be done cautiously, since non-mnemonic factors such as differences in sensitivity to the aversive stimulus, changes in sensorimotor
function, or general emotionality are readily able to affect performance (Bammer, 1982). The latter factor is of particular importance because kindling of the AM, as discussed above, is well known to affect anxiety related behaviors (Adamec, 1998; Depaulis et al., 1997; Kalynchuk et al., 1997), and emotional state has been shown to correlate with performance on aversively motivated tasks (e.g., Ribeiro et al., 1999). Although the role of such non-mnemonic factors has not been directly investigated, it is unlikely that they are the sole basis of the impairments observed following kindling, since a consistent pattern of impairment is not observed across all tasks investigated. For example, differences in shock sensitivity, emotionality, or motor function are unlikely to account for a deficit in one aversive task when performance in other aversive conditioning tasks is spared, as is seen with the disruptive effects of AM kindling on passive avoidance performance, brightness discrimination retention, or conditioned emotional response acquisition but not brightness discrimination acquisition or active avoidance performance (Becker et al., 1992).

The effects of AM kindling on object memory and spatial memory are not well known. To our knowledge, no study has previously investigated the effects of AM kindling on any task requiring object-related cognition while the effects of AM kindling on spatial memory have been investigated in only a few studies. In these studies, full AM kindling has been shown to spare acquisition performance in both the RAM (Letty et al., 1995) and the MWM (McNamara et al., 1992; Nieminen et al., 1992) while extended AM kindling has been shown to disrupt acquisition in the MWM (Cammisuli et al., 1997). While these results might suggest that unlike full dHPC kindling, full AM kindling may spare spatial cognition, it should be noted that the effects of full dHPC
kindling on RAM acquisition are unknown (see Hannesson & Corcoran, 2000) and the
effects of full dHPC and AM kindling on MWM acquisition have not been investigated
using identical protocols. Thus, it is possible that the effects of full AM kindling on
spatial cognition have not been assessed under suitably sensitive circumstances.

In Experiment 3c, I determined to investigate the effects of full AM kindling on
an identical behavioral protocol to that used to assess the effects of full dHPC and full
PRH kindling in experiments 3a and 3b respectively. In addition to further determining
the site specificity of any behavioral effects observed, this experiment also provided an
opportunity to determine the behavioral specificity of any effect observed (i.e., the
extent to which a behavioral effect on one task may co-vary with an effect on another
task). For example, in Experiment 3b PRH kindling was found to both enhance anxiety
and disrupt object recognition memory. Since, AM kindling is also known to enhance
anxiety (Adamec, 1998; Depaulis et al., 1997; Kalynchuk et al., 1997), the present study
can help determine whether such an effect is necessarily accompanied by a disruption of
object recognition memory. The present study had the additional goal of determining
the contribution of non-mnemonic effects to changes in spatial and object-related
cognition by assessing activity- and anxiety-related behaviors in the elevated plus maze
and the open field.

The design of Experiment 3c was the same as that of experiments 3a and 3b. In
summary, rats were fully kindled with twice-daily stimulation of the basolateral AM
until a criterion of 3 stage 5 seizures was reached. One week later, to examine activity-
and anxiety-related behaviors, rats were tested in an elevated plus maze on one day and
in an open field task on the next. The following phase of testing was designed to assess
object recognition memory. Rats were habituated for two additional days in the open field and then given a day off. The next day, rats were tested in a two-trial object exploration task modified from Ennaceur and colleagues (Ennaceur et al., 1996). The final phase of testing was designed to assess delay-dependent changes in spatial WM related performance. Rats were pretrained on a 4-trial DMTP task for 1 day using a VP. Rats then received 3 additional days of training with an inter-trial delay of 0.25 minutes. Delay testing then began, with rats tested on consecutive delays with inter-trial delays of 0.25, 1, 4, 0.25, 1, and 4 minutes.

7.2. Methods and Materials

7.2.1. Subjects

Twenty-seven male Long-Evans hooded rats (Charles River) weighing 300-375 g at the beginning of the study were used as subjects. Food and water were available ad libitum throughout the experiment. Rats were maintained in pairs in shoebox cages prior to surgery and were housed individually for the remainder of the experiment. All experimental procedures were carried out during the light portion of the 12:12 hour light/dark cycle. All rats were handled each day throughout the experiment except during the first four days following surgery. Subjects were randomly assigned to either the kindled group (n=13) or the control group (n=14). The 14 control rats were each yoked to one of the kindled rats.

7.2.2. Surgery

In preparation for surgery, animals were anaesthetized with Somnotol™
(sodium pentobarbital, 60 mg/kg) and given methyl scopolamine (1 mg/kg) to reduce respiratory congestion. Rats were placed in a stereotaxic apparatus, the skull was leveled, and a bipolar nichrome wire electrode (127 \mu m\, dia.) was implanted in either the right (n=7) or left (n=6) basolateral AM at the following coordinates relative to bregma: -2.7 \, mm\, (AP); 4.6\, mm\, (ML); 8.8\, mm\, (DV). Five jewelers' screws were used to secure the electrode assembly to the skull, with one screw over the anterior cortex serving as the reference electrode. The electrode assembly was affixed to the skull with dental acrylic, and a topical antibiotic/steroid, Topagen\textsuperscript{TM} was applied to the wound. Finally, a subcutaneous injection of Analgin (0.5 cc/kg) was given for post-surgical analgesia.

7.2.3. Kindling

Approximately 7 days following surgery kindling was initiated. In the first kindling session, the stimulus intensity required to evoke an afterdischarge (AD) was determined. A Grass S8800 stimulator was used to deliver a 1 sec train of balanced biphasic square wave pulses at 60 pps at an initial intensity of 20 \mu A (base-to-peak). If AD greater than 3 sec in duration was not evoked, intensity was increased along the following scale 40, 60, 80, 120, 160, and 200 \mu A every 2 minutes until 3 sec or greater AD was elicited. The minimal intensity triggering AD was arbitrarily defined as ADT and was the intensity used for kindling during the remainder of the study. Subsequently, rats were kindled twice daily with stimulations separated by at least 4 hrs until a criterion of 3 stage 5 seizures (Racine, 1972b) was achieved. At this point, rats were considered fully kindled and behavioral testing was started 7 or 8 days later. Each control rat was yoked to a specific kindled rat and received identical treatment except that it was not stimulated.
7.2.4. Behavioral Assessment

All aspects of behavioral testing were identical to those used in Experiment 3a.

7.2.5. Histology

Following behavioral testing, animals were sacrificed with an overdose of sodium pentobarbital or chloroform and perfused transcardially with 9% saline. Brains were fixed in formalin and then frozen before 60 μm coronal sections were taken through the full extent of the AM. Every section through the electrode track was mounted and stained with cresyl violet. The location of the electrode tips was documented by matching sections with one of 6 plates from Swanson (1992).

7.2.6. Data Analysis

Data analysis was completed using the statistical software package SPSS® for Windows™. Dependent measures from each of the behavioral tasks were subjected to analyses with repeated measures ANOVA and planned comparisons using t-tests.

7.3. Results

7.3.1. Histology

Electrodes in all kindled rats included in the study were located in the AM. Specifically, in the 13 kindled rats, the electrode was found in or near the basolateral AM (see Fig. 40). Electrodes in all control rats included in the study were also found in or near the basolateral AM. Specifically, in the 13 control rats, the electrode was found in the basolateral AM (7 rats), the central AM (2 rats), dorsolateral AM (2 rats) and the medial AM (2 rats). No gross histological changes were noted in the brains of either kindled or control rats other than gliosis around the electrode track.
Fig. 40. Location of the tip of the stimulating electrode in kindled rats. Plates are posterior relative to bregma and were adapted from Swanson (1992). * = right hemisphere placement, • = left hemisphere placement.
7.3.2. Kindling

Kindling data are shown in Table 9. Briefly, at a mean threshold of 92.9 ± 8.3 μA, stimulation evoked an initial AD of 5.1 ± 0.5 sec in duration. A mean of 17.0 ± 1.4 stimulations was required to evoke the first stage-5 seizure, and 19.9 ± 1.5 stimulations were required to meet our kindling criterion of 3 Stage-5 seizures. On these last 3 stimulations, the mean AD duration, latency to clonus, and duration of clonus were 38.4 ± 3.6, 6.4 ± 1.2, and 27.3 ± 2.5 secs, respectively.

7.3.3. Behavioral Assessment

7.3.3.1. Elevated Plus Maze

Full kindling of the AM significantly altered some anxiety-related but not activity-related behaviors in the elevated plus maze. Kindled rats were relatively less likely to enter open arms than control rats as shown by a significantly lower open arm entry ratio (t(25) = 2.04, p = .03, one-tailed; see Fig. 41). However, although kindled rats did spend relatively less time exploring open arms compared to controls, the difference between the groups on this measure of anxiety failed to reach statistical significance (t(25) = 1.07, p = .15, one-tailed). The differences between kindled and control groups on measures of anxiety did not result from differences in activity levels since the groups scored comparably on any direct measures of activity in the elevated plus maze. Kindled and control rats exhibited similar total distance traveled, total arm entries, and rears (all t(25)'s < 0.82, p's > .10; see Fig. 42). Together, these data suggest that AM kindling produced a selective but mild anxiogenic effect in the elevated plus maze.
Table 9. Summary of AM kindling data. Duration and latency data are in seconds.

Stage-5 seizure AD and clonus data for kindling are averaged across the final three stage 5 seizures evoked prior to MWM testing. ADT = threshold for evoking afterdischarge, stim. = stimulations

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<tr>
<td><strong>ADT (µA)</strong></td>
<td>93 ± 8.9</td>
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<tr>
<td><strong>Initial AD duration</strong></td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td><strong>Stim. to 1st stage-5 seizure</strong></td>
<td>17.0 ± 1.4</td>
</tr>
<tr>
<td><strong>Total # of stim.</strong></td>
<td>19.9 ± 1.5</td>
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<tr>
<td><strong>Stage-5 seizure AD duration</strong></td>
<td>38.4 ± 3.6</td>
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<tr>
<td><strong>Latency to clonus</strong></td>
<td>6.4 ± 1.2</td>
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<tr>
<td><strong>Duration of clonus</strong></td>
<td>27.3 ± 2.5</td>
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Fig. 41. Measures of anxiety in the elevated plus maze. Entry ratio = number of open arm entries / number of closed arm entries. Dwell ratio = time in open arms / trial duration. * $p < .05$ relative to controls
Fig. 42. Measures of activity/exploration in the elevated plus maze. Total entries = total number of entries to all arms in the maze.
7.3.3.2. Open Field

Similar to results in the elevated plus maze, full kindling of the AM altered some anxiety-related but no activity-related behaviors in the open field. Kindled rats spent less time exploring the central ring of the open field relative to control rats as shown by significantly lower central ring dwell times \((t(25) = 1.96, p = .03, \text{one-tailed})\) (see fig. 43). However, although kindled rats were relatively less likely to enter the central ring compared to controls the difference between the groups on this measure of anxiety failed to reach statistical significance \((t(25) = 1.17, p = .13, \text{one-tailed})\). The differences between kindled and control groups on measures of anxiety were not due to differences in activity levels since the groups did not differ on any direct measures of activity in the open field. Kindled and control rats exhibited comparable levels of activity as shown by similar total distances traveled \((t(25) = 0.30, p = .77)\), time spent exploring the objects \((t(25) = 0.38, p = .71)\), and numbers of rears \((t(25) = 1.59, p = .14)\) (see Fig. 44).

Together, these data suggest that AM kindling produced a mild but selective anxiogenic effect in the open field.

7.3.3.3. Object Exploration in the Open Field

Full kindling of the AM failed to affect object recognition memory as assessed in the object exploration in an open field task. On the first trial, with two novel objects, kindled rats exhibited comparable amounts of activity- (distance traveled, \(t(2) = 0.82, p = .42\); rears \(t(25) = 0.14, p = .89\)) and anxiety-related behaviors (central ring dwell time, \(t(25) = 1.34, p = .19\)) to controls. Importantly, both groups also exhibited similar amounts of object exploration as shown by comparable dwell times in \((t(24) = 0.50, p = .62)\) and entries to \((t(24) = 0.15, p = .88)\) the object regions. These data suggest that
**Fig. 43.** Measures of anxiety in the open field task. Entries = the number of entries to the central ring of the open field. Dwell time = time spent in the central ring of the open field. * p < .05 relative to controls
Fig. 44. Measures of activity/exploration in the open field task. Object dwell times = time spent exploring the two objects placed in the open field.
both groups had comparable opportunities to become familiar with the object used.

On the second trial, object recognition memory was assessed by replacing the objects from trial 1 with an identical copy of these objects and a novel object. Recognition memory should be reflected in a preference for exploring the novel object (Ennaceur et al., 1996). Kindled rats’ performance indicated intact recognition. Kindled rats approached the novel object more frequently than the familiar object as shown by a ratio of novel to familiar object area dwell times and entries that were not significantly different from that of control rats (both t(25)’s ≤ 0.60, both p’s ≥ .55) and were significantly biased towards the novel object (i.e., greater than 1; both t(12)’s > 1.85, p < .45, one-tailed) (see Fig. 45). Notably, kindled rats’ performance on all other measures, including distance traveled, rears, central ring dwell time, total object area dwell times, and total object area entries, did not significantly differ from that of control rats (all t(25)’s ≤ 1.72, all p’s ≥ .10) suggesting that both groups performed comparably in all respects on this task. These data suggest that full kindling of the AM does not affect object recognition memory.

7.3.3.4. Delayed-Match-to-Place in the MWM

AM kindling did not affect performance on the DMTP task during either the initial phase (i.e., acquisition of the task) or the delay phase of testing. Kindled and control rats performed comparably across days of acquisition in terms of both escape latencies and direct swims (data not shown). This was evidenced by the lack of group effects (both F(1.27)’s ≤ 2.52, both p’s ≥ .12), group by trial interactions (both F(2,54)’s ≤ .02, both p’s ≥ .98), group by day interactions (both F(2.54)’s ≤ .08, both p’s ≥ .70), and group by trial by day interactions (both F(4,108)’s ≤ .92, both p’s ≥
Fig. 45. Performance on measures of object recognition memory in the object exploration open field task. Dashed line represents chance levels of performance.

Entries Ratio = number of bouts of exploration of the novel object / number of bouts of exploration of the familiar object. Dwell Time Ratio = time spent exploring the novel object / time spent exploring the familiar object.
.454) on both measures. These results suggest that the groups did not differ in their ability to acquire the basic procedural components of the task.

Kindled and control rats also performed comparably on the DMTP task during the delay phase of testing. Overall, both groups required comparable latencies to escape and were equally likely to take a direct swim path to the platform as shown by the absence of significant group effects in terms of either escape latencies (F(1,25) = 0.50, p = .49; see Fig. 46) or direct swims (F(1,25) = 2.00, p = .17; see Fig. 47). Moreover, both groups showed significant improvements across trials as indicated by a highly significant trial effect in terms of both escape latencies (F(1.6,39) = 129.11, p < .001) and direct swims (F(2,50) = 116.21, p < .001) and did not differ in their trial-related performance as indicated by the absence of a significant group by trial (latency – F(1.6,39) = 0.70, p = .47; direct swims – F(2,50) = 0.93, p = .40) or group by trial by delay interaction (latency – F(4,100) = 0.33, p = .86; direct swims – F(4,100) = 0.63, p = .64). Both groups also showed superior performance at shorter inter-trial delays as shown by a significant delay effect (latency – F(2,50) = 13.47, p < .001; direct swims – F(2,50) = 4.02, p = .02) but did not differ in their delay-related performance as indicated by the absence of a significant group by delay (latency – F(2,50) = 1.42, p = .25; direct swims – F(2,50) = 0.12, p = .89) or group by trial by delay interaction (latency – F(4,100) = 0.33, p = .86; direct swims – F(4,100) = 0.63, p = .64). The delay effect appeared to show some relation to the trial of testing as indicated by a trial by delay interaction that approached significance in terms of both escape latencies (F(4,100) = 2.40, p = .06) and direct swims (F(4,100) = 2.24, p = .07). To further explore this interaction, subsequent analyses of simple main effects of delay within trials suggested
Fig. 46. Latency to escape to the hidden platform on matching trials 1 (A), 2 (B), and 3 (C) during delay phase of testing on the DMTP task in the MWM. Data are averaged across 2 days of testing at each delay.
Fig. 47. Probability of making a direct swim to the hidden platform on matching trials 1 (A), 2 (B), and 3 (C) during delay phase of testing on the DMTP task in the MWM. Data are averaged across 2 days of testing at each delay. A direct swim was scored if the rat remained within a 25 cm alley from the start position to the platform.
that performance was worse at longer delays on trial 1 on both measures (both F(2,50)'s ≥ 5.37, both p's ≤ .01), on trial 2 in terms of latency (F(2,50) = 6.15, p = .004) but not direct swims (F(2,50) = 1.11, p = .34), and not on trial 3 in terms of either measure (both F(2,50)'s ≤ 2.58, both p's ≥ .09). Finally, planned comparisons between groups on trials 1 to 3 at each delay failed to detect any significant differences (direct swims - all t(25)'s ≤ 1.42, all p's > .05; latency - all t(24)'s ≤ 1.43, all p's > .05), further underscoring the comparability of kindled and control rats’ DMTP performance.

7.4. Discussion

In Experiment 3c, the effects of full kindling of the AM on anxiety- and activity-related behaviors, object recognition memory, and spatial cognition were assessed in the elevated plus and open field mazes, an open field object exploration task, and a DMTP task in the MWM respectively. Kindling produced anxiogenic effects in both the elevated plus maze and the open field but did not affect activity/exploration in either task, performance of the object exploration task in the open field, or performance of the DMTP task in the MWM.

The major finding of the present study is that full kindling of the AM selectively increased anxiety without affecting exploratory behavior, object recognition memory, or spatial WM. In the elevated plus maze, AM kindled rats were significantly less likely to enter open arms than controls, although the difference in time spent exploring the open arms was not significantly different between the groups. Conversely, in the open field, AM kindled rats spent significantly less time exploring the central region, although the difference in entries to the central region was not significantly different between the
groups. Because scores on both measures in both tasks reflected increased anxiety according to standard interpretations (Lister, 1990; Pellow et al., 1985; Rodgers & Dalvi, 1997; Walsh & Cummins, 1976; Williams & Russel, 1972) and the non-significant effects on each task were on opposite measures (i.e., dwell time in the elevated plus and entries in the open field), I view the discrepant results on the two measures of anxiety used in each task as reflecting statistical variation associated with a mild effect rather than a pattern in the type of anxiety-related behaviors specifically affected by AM kindling. Thus, the overall pattern of results in both tasks indicates a mild increase in anxiety and leads us to conclude that AM kindling is anxiogenic. The strength of this conclusion is enhanced by the consistency of results across both tasks and the concomitant lack of effect of AM kindling on measures of activity/exploration in either task. The latter observation is important because changes in these-types of behaviors can also affect the measures used to assess anxiety in these tasks (Rodgers & Dalvi, 1997; Walsh & Cummins, 1976).

The present finding that full AM kindling is anxiogenic is consistent with previous findings from several different laboratories. Anxiogenic effects in the elevated plus maze have been observed after partial (Helfer et al., 1996), full (Adamec, 1990a; Adamec, 1998; Adamec & Morgan, 1994; Adamec & McKay, 1993; Helfer et al., 1996; Nieminen et al., 1992), and extended (Kalynchuk et al., 1998a; Kalynchuk et al., 1998b; Kalynchuk et al., 1997) kindling of the AM. Increases in emotionality or defensiveness on other tasks, including the open field, have also been observed after full (Helfer et al., 1996; Henke & Sullivan, 1985; Kalynchuk et al., 1997; Nieminen et al., 1992) and extended (Kalynchuk et al., 1998a; Kalynchuk et al., 1998b; Kalynchuk et al., 1997;
Pinel et al., 1998; Pinel & Rovner, 1978) kindling of the AM. However, there is also some evidence that AM kindling may be anxiolytic or without effect on anxiety-related behaviors under some circumstances. For example, full kindling of the AM has been shown to be anxiolytic in the elevated plus maze (Adamec & Morgan, 1994; Adamec & McKay, 1993) and without effect on the acoustic startle response (Ebert & Koch, 1996). One possible resolution of these contradictory findings has been proposed by Adamec (1998) who suggested that the precise nuclear, hemispheric, and anterior-posterior position of the stimulating electrode may account for differences in the observed effects of AM kindling on anxiety. For example, Adamec has suggested that left hemisphere placements in the lateral or central nuclei, with the possible exception of the basolateral nucleus, may be generally anxiolytic whereas right hemisphere placements, particularly anterior ones, may be generally anxiogenic. In the present study, the majority of electrode placements were located in or near the basolateral nucleus of the AM with the hemisphere balanced across rats (right = 7, left = 6). No differences were observed between rats kindled in different hemispheres supporting the suggestion that kindling of the basolateral nucleus of the AM may be anxiogenic regardless of the hemisphere.

The anxiogenic effects of AM kindling are consistent with the lesion literature indicating an important role for the AM in emotional behavior, including anxiety (Davis, 1998; LeDoux, 1992; Rosen & Schulkin, 1998). These data, then, suggest that the effects of AM kindling on anxiety may be mediated by local changes within the AM or in immediately connected circuitry.

The present study found that AM kindling did not affect memory related
performance in either the object exploration task in the open field or the DMTP task in the MWM. Kindled rats exhibited the expected bias for the novel object on the second of two open field trials separated by 15 minutes suggesting that object recognition memory and other functions involved in performance were not altered by AM kindling. Likewise, kindled rats escaped quickly and directly to the HP location on each of the 3 matching trials administered with the platform in a different location on subsequent days and with different inter-trial intervals suggesting that spatial cognition and other functions involved in performance were not altered by AM kindling. This is the first study to show that full AM kindling does not affect object recognition memory whereas the present study adds to previous ones that have also found that full AM kindling does not affect spatial cognition. For example, AM kindling has been shown to spare acquisition of the standard MWM task (McNamara et al., 1992; Nieminen et al., 1992) and the RAM (Letty et al., 1995). However, the present study is the first to show that AM kindling does not alter spatial cognition under identical testing conditions to those that were sensitive to the effects of kindling in another site – the dHPC (Experiment 3a).

The effects of AM kindling on object recognition memory and spatial cognition in the present study might suggest that AM kindling does not affect mnemonic function in general. However, previous studies have shown that AM may affect several forms of aversive conditioning. For example, full kindling of the AM has been shown to disrupt performance of several passive avoidance tasks (Boast & McIntyre, 1977; Peele & Gilbert, 1992; Stone & Gold, 1988), acquisition of a conditioned emotional response (McIntyre & Molino, 1972), and retention of a brightness discrimination in a Y-maze (Becker & Grecksch, 1992; Becker et al., 1992). Thus, AM kindling appears to be
selective in the class of mnemonic function it may affect, with aversive conditioning being susceptible but object-related and spatial cognition spared. This pattern of effects mirrors the sensitivity of some forms of aversive conditioning to the effects of AM lesions (Davis, 1998; Gallagher & Holladn, 1994; Ono, Nishijo, & Uwano, 1995; Yamamoto, Shimura, Sako, Yasoshima, & Sakai, 1994) and the relative insensitivity of object-related (Gaffan, 1994; Mumby & Pinel, 1994) and spatial (Decker, Curzon, & Brioni, 1995; McDonald & White, 1993) cognition to the effects of AM lesions. Collectively, these observations suggest that the mnemonic effects of AM kindling may be mediated local to the kindling site.

One point worth noting is that the behavioral effects of AM kindling may depend upon the extent of kindling achieved prior to testing. In the present study, full kindling produced only a mild effect in the elevated plus maze and the open field. More potent anxiogenic effects appear to be produced by extended AM kindling (Kalynchuk et al., 1997). Similarly, in the present study, full AM kindling spared spatial cognition in the MWM. However, previous research has shown that extended kindling of the AM produces a mild disruption of acquisition in the standard MWM task (Cammisuli et al., 1997). Thus, the effects of AM kindling may be more severe and more general following greater extents of kindling than that used in the present study.

In summary, the present study has shown that full kindling of the AM produces a mild increase in anxiety-related behaviors in both the elevated plus maze and the open field but spares exploratory behavior, object recognition, and spatial cognition in these same and other tasks. Overall, this pattern of results is consistent with previous research on the effects of full kindling of the AM and lends further support to the notion
that kindling, despite being a phenomenon involving widespread brain regions, can produce highly specific effects. Notably, the pattern of effects produced by AM kindling resembles those of AM lesions suggesting that the behavioral effects of AM kindling may be mediated by changes local to the stimulation site or within closely linked circuitry.
8. DISCUSSION

8.1. Summary of Results

The studies constituting this dissertation investigated the lasting effects of kindling on a variety of behaviors. The main findings of these experiments can be summarized as follows. Full kindling of the dHPC disrupted acquisition of a constant platform location in the MWM, disrupted performance of two versions of a DMTP task in the MWM, and mildly increased some measures of activity/exploration in an open field task, but did not affect either 7 or 28-day retention of a constant platform location in the MWM, performance on a VP task in the MWM or a modified water maze, acquisition, 7 or 28-day retention of an OD problem in a modified water maze, anxiety-related behaviors in an elevated plus maze or an open field task, or object recognition memory in an open field object exploration task. Full kindling of the PRH disrupted 28 day retention of an OD problem in a modified water maze, disrupted object recognition memory in an open field object exploration task, and increased anxiety-related behaviors in both the elevated plus maze and an open field, but did not affect acquisition or 7-day retention of an OD problem in a modified water maze, performance on VP tasks in the MWM and modified water maze, acquisition, 7 or 28-day retention of a constant platform location in the MWM, performance on a DMTP task in the MWM, or activity/exploratory behaviors in the elevated plus maze or an open field task. Full
kindling of the AM increased anxiety-related behaviors in both the elevated plus maze and an open field task but did not affect activity/exploration in these tasks, object recognition memory performance in an open field object exploration task, or performance on a DMTP task in the MWM.

The primary purpose of these experiments was to address several questions related to the effects of kindling on spatial cognition. First, does kindling produce a specific disruption of spatial cognition? This was sub-divided into two more specific questions: 1) does kindling disrupt performance on tasks that require allocentric spatial processing, and 2) can such deficits be accounted for by alterations in sensorimotor function, motivational/emotional state, or general aspects of cognitive function (e.g. attention)? Second, if kindling disrupts spatial cognition, what stage of mnemonic processing (e.g., learning, STM and/or LTM) is affected? And third, how do kindling’s effects on spatial cognition relate to several kindling-related variables including the site of kindling, the extent of kindling, and the duration between kindling and testing? In addressing these questions, I hoped to determine a reasonable starting point for subsequent investigations aimed at determining the mechanisms of kindling’s effects on spatial cognition and mnemonic function in general.

8.2. Does Kindling Disrupt Spatial Cognition?

Before examining the data to determine whether kindling specifically impacts tasks that require spatial cognition, it may be worthwhile to briefly consider several important distinctions between classes of spatial behavior (see O’Keefe & Nadel, 1976). The animal that must move through its environment to a particular location has several
kinds of information at its disposal. First, the animal has proprioceptive information regarding its own body position, which enables movements defined relative to the animal's body axis. Second, the animal has information about its position relative to single landmarks within the environment, which enables movements relative to these landmarks. Third, the animal has information about the relative positions of landmarks in the environment, which enables movements relative to a "cognitive map" of the environment. The former two types of information constitute egocentric means for determining spatial locations, since places are defined relative to the animal's own position. This type of information supports orientation and guidance hypotheses respectively (termed taxon hypotheses collectively) according to the terminology of O'Keefe and Nadel (1978). The latter type of information constitutes an allocentric means for determining spatial locations, since places are determined relative to a framework that is external to the animal. This type of information supports place or locale hypotheses according to the terminology of O'Keefe and Nadel (1978).

Separate neural systems are required for the use of different types of spatial information. Of particular importance, the HPCal system is specifically involved in the use of allocentric spatial information. Thus, tasks that require allocentric spatial information for optimal performance, and hence the use of locale hypotheses, have proven to be particularly susceptible to impairments produced by disruptions of function within the HPC or related structures (Barnes, 1988; Jarrard, 1993; O'Keefe & Nadel, 1978). In fact, such tasks have become such an important part of research on spatial function that most allocentric spatial tasks are simply referred to as spatial tasks and any resultant impairments are referred to as spatial impairments. Indeed, throughout the
literature, the use of the terms spatial and non-spatial tasks simply denotes tasks that require allocentric spatial processing and those that do not. I will continue this somewhat dubious tradition, but remind the reader that the use of the term spatial carries with it the distinction between allocentric and egocentric means for defining spatial behavior and not necessarily the distinction between any type of spatial behavior and truly non-spatial behavior.

Several widely used tasks place heavy demands on allocentric spatial processing for optimal performance. These include the radial-arm maze (RAM; Olton & Samuelson, 1976) and the Morris water maze (MWM; Morris, 1984), which are the only two tasks that have been used to assess kindling's affects on spatial cognition. Although investigations on the effects of kindling on spatial cognition have been limited to these two tasks, several versions of each of them (see Introduction) have been used. The first question, then, is whether kindling disrupts performance on these tasks.

8.2.1. Does Kindling Disrupt Performance On Tasks That Require Allocentric Spatial Processing?

In the present dissertation, three different MWM tasks were used to assess spatial cognition. In Experiment 1a, full dHPC kindling was found to disrupt one-day acquisition of a constant HP location in the standard water maze task in naïve rats. In Experiment 2, different extents of dHPC kindling were found to produce a triphasic pattern of effects on performance of a 2-trial DMTP task in the MWM in rats trained prior to kindling. Early stages of kindling (1 to 11 ADs) produced a mild impairment (probably a retrograde effect), middle stages (16 ADs, 1 stage 1 seizure) produced no impairment, and full kindling (1 stage 5 seizure) again produced an impairment
(probably an anterograde effect). In Experiment 3a, full dHPC kindling was found to disrupt performance on a 4 trial DMTP task in the MWM in naïve rats. Although differing delays of 15, 60, or 240 seconds were used between trials, the deficit did not clearly relate to the length of the inter-trial delay.

Previous work in the Corcoran lab and other labs is consistent with the above findings showing that kindling can disrupt MWM performance. Full kindling of the dHPC has been shown to disrupt across-days acquisition of a constant HP location in the standard MWM task in naïve rats (Gilbert et al., 1996). Both partial and full kindling of the dHPC have been shown to disrupt retention of a constant HP location in the standard MWM tasks when the location was acquired by rats trained prior to kindling (Gilbert et al., 1996). Partial dHPC kindling has also been shown to disrupt performance on a 4 trial DMTP task in the MWM in rats trained prior to kindling (Sutherland et al., 1997). Finally, extended kindling of either the perforant path or AM has been shown to disrupt across-days acquisition of a constant HP location in the standard MWM task in naïve rats (Cammisuli et al., 1997).

Findings in the RAM are consistent with those in the MWM, with several studies showing that kindling is capable of impairing performance on a variety of RAM tasks. Extended kindling of the olfactory bulbs has been shown to disrupt acquisition of the RM component of a combined RM/WM version of the RAM in naïve rats (Sutula et al., 1995). Similarly, a disruption of the RM component of a combined RM/WM version of the RAM has been demonstrated after full kindling of either the dentate gyrus or the dHPC (Feasey-Truger, Kargl, & ten Bruggencate, 1993; Lopes Da Silva et al., 1986). However, these results were obtained in rats that were trained prior to and during
kindling. Partial kindling of the dHPC has also been shown to disrupt performance of a combined RM/WM version of the RAM, although again these findings were obtained in rats trained prior to kindling (Leung et al., 1996). Like the RM/WM version of the RAM, the standard WM version of the RAM has also been shown to be sensitive to kindling-induced impairments, with both partial and full kindling of the dHPC having been shown to disrupt performance on this task in rats trained prior to kindling (Leung & Shen, 1991; Leung et al., 1994; Leung et al., 1990). Finally, partial kindling of the dHPC has been shown to disrupt retention of a RM task in the RAM involving 2-arm place discriminations acquired prior to kindling (Laurent-Demir & Jaffard, 1997).

Based on observations in the MWM and RAM, it is clear that under a variety of circumstances kindling is capable of disrupting performance on allocentric spatial tasks. One obvious interpretation is that such impairments are accounted for by a kindling-induced disruption of spatial cognition. However, alternatively, a non-specific impairment resulting from a kindling-induced disruption of sensorimotor, motivational/emotional, or general cognitive processes could underlie these impairments. Thus, the possible contribution of non-specific impairments to changes in spatial task performance must first be considered first before such impairments can be attributed to a specific disruption of spatial cognition.

8.2.2. Can Spatial Task Impairments Be Accounted For By Generalized/Non-Specific Impairments?

Before proceeding to explore the relevant data, two important points need to be made. First, there is evidence that at least some of the non-specific behavioral effects of kindling may relate to both the site and extent of kindling employed (Kalynchuk et al.,
Thus, both of these kindling parameters need to be considered in making comparisons between studies on the effects of kindling on spatial and non-spatial performance. Second, there is an important distinction between the nature and interpretation of strictly anterograde and confounded anterograde/retrograde effects of kindling. Namely, anterograde effects, as observed in rats trained only at some time after the cessation of kindling, can strictly be attributed to brain changes associated with the kindled state, or other enduring after-effects of seizures, and are of primary interest in the present dissertation. Conversely, confounded anterograde/retrograde effects, as observed in rats trained prior to and/or during kindling, can be attributed to the directly disruptive retrograde effects of seizures (McGaugh, 1966), the anterograde effects of brain changes associated with the kindled state, or both. Thus, in comparing the effects of kindling on spatial and non-spatial control tasks their relative susceptibility to anterograde and retrograde effects must also be considered.

As discussed at the beginning of this dissertation, kindling results in a progressive elaboration and propagation of AD that eventually involves much of the brain and thus may be expected to induce alterations in the activity of widespread areas of the brain. From this perspective, the possibility that kindling alters a variety of behaviors and that these alterations may contribute to changes in the performance of both spatial and non-spatial tasks needs to be carefully considered. Indeed, it has been shown that kindling can produce motoric disturbances that are most severe in the immediate post-ictal period but may also endure for some time (e.g., hours to days) after the last seizure (Caldecott-Hazard, 1988; Ehlers & Koob, 1985). Kindling has also
been shown to produce changes in sensory evoked potentials (Ono, Nakatsuka, & Baba, 1981; Tsuru & Shimada, 1984), and it is well established that kindling can alter anxiety-related behaviors (Adamec, 1990a; Adamec, 1990b; Depaulis et al., 1997). Unfortunately, the role of these and other potential non-specific changes in spatial task deficits has received only limited direct attention in other studies, although investigation of this issue was one of the major purposes of the present dissertation.

To the extent that general functions are involved in a wide range of behaviors, I can infer their contribution to spatial task impairments based on the selectivity with which effects are observed on tasks differing in their spatial and non-spatial demands. A variety of non-spatial behaviors were investigated in the studies constituting this dissertation. The results suggest that the same kindling treatment that disrupted spatial cognition (i.e., full dHPC kindling) had limited effects on other behaviors. In the MWM, a commonly employed control task involves using a platform that is visible above the surface of the water. This task uses motivation (i.e., escape from water) and motor skills (e.g., goal-directed swimming, mounting the platform) similar to those required for the spatial MWM task and also shares some of the same sensory demands (e.g., processing of remote visual information). In Experiment 1a, full dHPC kindled rats were tested on this task prior to any other water maze training and failed to show any impairment. This suggests that the MWM impairment observed was not a result of a disruption of any component of water maze performance shared between the VP and HP tasks. Also of note, this finding shows that non-spatial pretraining does not eliminate the spatial task impairment observed following kindling in contrast to the findings of some studies on the effects of selected amnestic drugs (e.g. NMDA
agonists) on spatial cognition (Cain, 1998; Saucier & Cain, 1995). There are also several additional observations that suggest that sensorimotor processes were not sufficiently compromised by dHPC kindling to disrupt spatial task performance. First, although full dHPC kindling disrupted the rate of acquisition in the MWM, kindled rats eventually reached control levels of performance and performed at control levels on subsequent retention tests 7 and 28 days later. Thus, kindled rats did possess sufficient sensorimotor abilities to support very accurate spatial performance, at least by those points in training. Second, full dHPC kindling did not disrupt performance on a VP task or an OD task in a modified water maze. These tasks again require many of the same sensorimotor functions as the spatial MWM task. Thus, spared performance on these tasks suggests that components of water maze behavior shared by the different tasks were not impaired by kindling. Third, full dHPC kindling did not disrupt performance of an open field OD task. This provides evidence that the sensorimotor processes necessary for the recognition and exploration of visuo-tactile stimuli were not impaired by kindling. Collectively, these observations suggest that sensorimotor functions required for MWM performance are unlikely to have been sufficiently impaired by kindling to compromise spatial water maze performance and hence account for the kindling-induced impairments observed.

Several observations suggest that motivational and emotional processes were also not affected by full dHPC kindling. First, the variety of non-spatial water maze tasks that were tested in the studies constituting this dissertation, as mentioned above, all relied on escape from cool water as motivation and were not impaired by kindling. Thus, it seems unlikely that motivational factors or an emotional response to being
placed in cool water could have selectively disrupted the spatial water maze tasks. Second, full dHPC kindling failed to affect anxiety-related behaviors in either the elevated plus maze or an open field task. This also suggests that emotionality was not notably affected by kindling and would be unlikely to impact spatial task performance. The observation that both full PRH and full AM kindling increased anxiety in both the elevated plus maze and an open field task yet failed to disrupt spatial cognition further underscores the point that kindling-induced changes in emotionality are unlikely to underlie full dHPC kindling’s effects on spatial task performance.

Several observations from studies in the present dissertation also suggest that general cognitive functions were not impaired by full dHPC kindling. Kindled rats acquired and retained an OD problem in a modified water maze as well as controls. In addition to demonstrating intact general cognitive functions (e.g., attention), this finding also rules out task difficulty as an explanation for selective spatial task impairments since this task was more difficult to acquire (i.e., required more training trials) than any of the spatial tasks used in the present studies. In further support of these points, full PRH kindling was found to produce the opposite pattern of effects (i.e., spared spatial performance and impaired object-related performance). This double dissociation of effects argues against both a generalized impairment underlying either effect and task difficulty as an explanation of the pattern of results. Kindled rats also showed normal object recognition memory in an open field OD task, furthering supporting the hypothesis that object-related memory functions in particular are not compromised by full dHPC kindling. The observation that both of these tasks were impaired by full PRH kindling establishes that these tasks were sufficiently sensitive to detect kindling-induced
impairments and strengthens the conclusion that dHPC kindling did not impair general cognitive functions or object-related cognition in particular. In conclusion, intact performance on several learning tasks that lacked a notable spatial component strongly suggests that general cognitive functions including attention and at least some forms of non-spatial learning were not impaired by kindling. Thus generalized cognitive dysfunction is unlikely to underlie spatial task impairments.

Results from several other studies also suggest that full dHPC kindling does not produce non-specific impairments that are likely to compromise spatial task performance. Previous research in our laboratory found that rats fully kindled with dHPC stimulation performed normally on a VP task when tested following HP testing (Gilbert et al., 1996). This result is consistent with the findings of Experiment 1a. Also of interest, one study using the combined WM/RM version of the RAM found that full dHPC kindling selectively impaired RM (Lopes Da Silva et al., 1986). Similar results have also been obtained following full kindling of the dentate gyrus (Feasey-Truger et al., 1993). The sparing of WM performance in these studies provides some evidence that most general behavioral functions required for spatial task performance must be intact, since many of the same sensorimotor and motivational functions are required for both RM and WM performance. However, it should be noted that the motivational salience of baited arms is greater than unbaited arms, and it is the latter on which most RM errors are made. Given this difference in valence, it is possible that performance on unbaited arms, and hence RM performance, may be more susceptible to the disruptive influence of non-specific impairments.

The failure of full kindling in many sites outside of the dHPC to disrupt spatial
task performance (Holmes et al., 1993; Letty et al., 1995; McNamara et al., 1992; McNamara et al., 1993; Nieminen et al., 1992; Robinson et al., 1993; Sutula et al., 1995; Experiments 1a, 2, and 3a) also provides some basis for arguing that kindling-induced non-specific effects do not underlie spatial impairments when observed. First, the sparing of spatial cognition despite a comparable number of previously induced stage 5 seizures in these studies and those that have observed spatial deficits after dHPC kindling immediately rules out any of the after-effects of generalized seizures per se as an explanation of the deficit. Second, full kindling, in the sites used in the studies above, produces generalized seizures that are both qualitatively and quantitatively similar, although not identical, to those produced by dHPC kindling. Thus, it is believed that a common circuitry is responsible for the propagation of seizure activity to motor structures during kindling regardless of the site of stimulation (Applegate, 1998; Burchfiel et al., 1998; McIntyre & Kelly, 1998). Indeed, the patterns of activation throughout the brain seen after a generalized seizure are remarkably similar regardless of the stimulation site (e.g., Sato, Yamada, Morimoto, Uemura, & Kuroda, 1998). Thus, the widespread propagation of seizure activity to sites that are more intimately involved in sensorimotor and motivational functions than limbic kindling sites might be expected to be comparable regardless of the kindling site and therefore might be expected to produce comparable changes in sensorimotor and motivational function. The failure of kindling in several sites outside of the dHPC to disrupt spatial cognition thus suggests that such effects are either not produced, are in fact uniquely or more severely produced by dHPC kindling, or are not of sufficient severity to impact spatial performance.
One final behavioral observation is worth noting. Full dHPC kindling was found to produce a mild increase in activity/exploration on some measures in the open field in the present dissertation. At present, it is not certain whether such changes contribute to spatial impairments or are themselves a result of spatial impairments. However, O'Keefe and Nadel (1978) have argued quite convincingly that changes in activity/exploration and spatial cognitive function may share a common etiology in HPCal dysfunction. Thus, alterations in activity/exploration may be an independent consequence of kindling that, at the same time, is in part both a contributor and consequence of spatial cognitive dysfunction.

Taken together, the above observations provide some evidence that non-specific impairments do not account for kindling-induced changes in spatial task performance and thus suggest that kindling can specifically impair spatial cognition. This conclusion, however, should be restricted to studies showing anterograde spatial impairments following full kindling of the dHPC because kindling's non-specific behavioral effects may relate to both the site and extent of kindling and may vary in their relative sensitivity to anterograde and retrograde effects. Thus, although extended kindling of the perforant path, AM, and olfactory bulbs have all been shown to produce anterograde disruptions of spatial task performance (Cammisuli et al., 1997; Sutula et al., 1995), the spatial nature of these deficits cannot be assured based on the considerations presented in the preceding section. Few investigations have made been made into the range of behavioral effects that may result from extended kindling. However, some evidence suggests that non-specific behavioral changes, such as those relating to anxiety, may be more pronounced following extended kindling (Kalynchuk et
al., 1997). Therefore, interpretation of the behavioral effects of extended kindling may warrant particular caution. Nonetheless, it is worth noting that the effects of extended olfactory bulb kindling were specific to RM in a combined RM/WM version of the RAM and thus suggest that general behavioral functions required for task performance, as discussed above, may be preserved.

Also, the effects observed following partial or full dHPC kindling and full DG kindling on spatial task performance in rats trained prior to and/or during kindling may result from both anterograde and retrograde effects. The discussion in the preceding section cannot provide conclusive evidence that at least some of the behavioral functions learned and used normally following kindling are not susceptible to retrograde disruption by kindled seizures themselves. However, again, there is some evidence that some degree of specificity to spatial cognitive function is shown under these circumstances. Leung and colleagues (Leung et al., 1996) found that partial dHPC kindling did not disrupt performance of a cue-based WM/RM version of the RAM acquired prior to kindling. In order to strengthen the conclusion that any particular kindling protocol produces selective effects on spatial cognition, future studies should be vigilant in monitoring a diverse set of behaviors in the same rats tested on the spatial task or at least in rats that have received an identical kindling protocol.

In summary, the previous two sections have explored the question – does full dHPC kindling specifically disrupt spatial cognition. In the first section, I reviewed studies that show that dHPC kindling does disrupt performance on at least two spatial tasks, the MWM and the RAM. In the second section, I reviewed results that indicate that non-specific impairments are unlikely to account for at least some of these effects.
Based on these considerations, then, I conclude that full dHPC selectively disrupts spatial cognition and move on to consider the stage of mnemonic processing that may be affected.

8.3. The Stage of Spatial Mnemonic Processing Affected by Kindling

An important question regarding the effects of kindling on spatial cognition is which stage or stages of mnemonic processing are affected. Before proceeding to examine this question and the relevant kindling data, however, I will briefly consider the general topic of stages of mnemonic processing.

Mnemonic functions can be divided into a number of temporally distinct sequential stages. The first step is learning, the process by which experience is translated into enduring alterations in nervous system function that are capable of manifesting themselves in changes in subsequent behavior. The second stage has been termed STM and represents a transient stage that according to different researchers may last anywhere from 30 seconds or less without rehearsal to several hours or even days. The third widely recognized stage is termed LTM and represents an enduring stage that according to different definitions may take from several hours to several days to be established and may last indefinitely. Several researchers have posited an intermediate stage between STM and LTM termed intermediate-term memory (Eichenbaum et al., 1994; McGaugh, 1966; Rosenzweig, Bennet, Columbo, Lee, & Serrano, 1993), which supports memory-related performance in an interval from several minutes up to 24 hours. For the purposes of the present dissertation, STM will be used to indicate a temporary memory buffer with a duration of at least several minutes and LTM will be
used to indicate an enduring memory that becomes important at retention intervals of 24 hours or longer. Intermediate-term memory will be presumed to support retention over the intervening duration.

In Experiment 1a, I attempted to dissociate the effects of kindling on learning/STM and LTM. Previous work had shown that full dHPC kindling impaired acquisition of a constant HP location in the MWM (Gilbert et al., 1996). However, the protocol used in that study involved training spread over 7 consecutive days and thus placed significant demands upon both learning/STM (between trials within the same day) and LTM (between trials across days). Therefore, I employed a protocol whereby acquisition was achieved in one training session of 18 trials with inter-trial intervals of less than 5 minutes and retention was tested subsequently at 7 and 28 days later. The results showed that kindled rats acquired the platform location more slowly than controls but subsequently retained the platform location as well as controls. This finding suggested that kindling’s anterograde disruption of spatial cognition was most likely manifest in an impairment of spatial learning/STM and not LTM.

In experiment 3, I attempted to dissociate kindling’s effects on learning and STM. Dissociating effects on learning and memory can prove difficult since learning cannot be demonstrated without memory. Therefore, the presence of memory impairments accompanied by intact learning can be determined with some certainty but learning impairments with intact memory cannot. The assessment of performance on tasks with varying delays between training and testing trials has proven quite useful in this regard. On such tasks (e.g., delayed-match-to-sample tasks with objects), a delay-dependent deficit provides evidence that learning is relatively intact and suggests that
the deficit is primarily due to impaired STM. That is, the more that the deficit depends upon the duration over which the learned information must be retained the more likely it is that impaired memory processes rather than learning underlie the performance impairment. However, it should be noted that, in some cases, different types of learning might be able to support comparable performance. In these cases, if the different types of learning show variations in their normal rates of decay (i.e., forgetting), an apparent delay-dependent memory deficit may in fact be secondary to a learning impairment (i.e., the use of alternative learning strategies). In Experiment 3a, I tested the effects of full dHPC kindling on performance of a DMTP task with inter-trial delays of 15, 60, and 240 seconds. Kindling impaired performance at all delays and the severity of the impairment did not increase at longer delays. This result is most consistent with an impairment of learning.

Based on the above findings, I will continue my discussions of the nature of kindling’s effects on spatial cognition while using the working hypothesis that these anterograde effects are manifest in an impairment of spatial learning.

8.4. The Relation Between Kindling Parameters and Kindling’s Effects on Spatial Cognition

The preceding sections have argued that the results presented in this dissertation, as well as those obtained in other studies, indicate that full kindling of the dHPC produces a selective anterograde disruption of spatial learning. The following sections will consider the significance of several procedural variables related to kindling that may be critical in producing this effect including the site of kindling, the extent of kindling.
and the interval between kindling and testing.

8.4.1. Kindling Site

Several observations suggest that the anterograde effects of full kindling on spatial cognition depend critically upon the site being kindled. Full kindling of the dHPC has been shown to disrupt performance in both the standard (Gilbert et al., 1996; Experiment 1a) and WM (Experiments 2 and 3a) versions of the MWM. Using identical training protocols, I have found that full PRH kindling does not affect performance of the standard MWM task (Experiment 1b) and neither full PRH nor AM kindling affects performance of a WM MWM task (Experiments 3b and 3c). In other studies, full kindling of the lateral septum, AM, or ventral HPC has been found to spare performance in the standard MWM (Holmes et al., 1993; McNamara et al., 1992; Nieminen et al., 1992), whereas full perforant path kindling has been found to produce a mild and probably non-spatial impairment that is restricted to the first day of MWM testing (McNamara et al., 1992). Further evidence that full kindling in sites other than the dHPC does not produce a significant anterograde disruption of spatial cognition comes from studies that have shown that full kindling of the AM, perforant path, or olfactory bulb does not impair acquisition in the RAM (Letty et al., 1995; Robinson et al., 1993; Sutula et al., 1995). It should be noted, however, that the effects of full dHPC kindling on RAM acquisition have not yet been investigated. Nonetheless, all of the above data are consistent with the hypothesis that an anterograde disruption of spatial cognition by full kindling requires a dHPC stimulation site.

The site specificity of effects on spatial cognition observed with full kindling does not appear to be observed with either partial or extended kindling. For example,
there is no evidence that partial kindling in any site can produce an anterograde disruption of spatial cognition. Partial kindling of even the dHPC fails to impair acquisition in either the RAM or MWM (Gilbert et al., 1996; Leung et al., 1996; Sutherland et al., 1997), and partial kindling in the only other site tested, the olfactory bulb, is without effect on RAM acquisition (Sutula et al., 1995). Conversely, evidence suggests that extended kindling in several sites can produce an anterograde disruption of spatial cognition. In the only sites tested, extended kindling of either the AM or perforant path has been shown to impair MWM acquisition (Cammisuli et al., 1997), and extended olfactory bulb kindling has been shown to impair RAM acquisition (Sutula et al., 1995). Notably, the same effects are not observed following full kindling in any of these sites (McNamara et al., 1992; Nieminen et al., 1992; Sutula et al., 1995).

Interestingly, there is no evidence that the retrograde effects of kindling on spatial cognition show any degree of site specificity, although only limited relevant data are available. Both partial and full dHPCal kindling have been shown to disrupt performance by previously trained subjects on both versions of the RAM and MWM (Gilbert et al., 1996; Leung & Shen, 1991; Leung et al., 1994; Leung et al., 1990; Leung et al., 1996; Sutherland et al., 1997; Experiment 2) and on a two-arm spatial discrimination task (Laurent-Demir & Jaffard, 1997). In the only other site tested, the dentate gyrus, full kindling has been shown to disrupt RM performance on the combined WM/RM version of the RAM (Feasey-Truger et al., 1993).

It is worth noting that the effects of kindling on object-related cognition may also be sensitive to the site being kindled. I found that full kindling of the PRH disrupts performance on an OD task (Experiment 1b) and an object recognition memory task
(Experiment 3b). Using identical training protocols, OD performance was not impaired by full dHPC kindling (Experiment 1a) and object recognition performance was not impaired by either full dHPC or full AM kindling (Experiments 3a and 3c). Thus, the available data are consistent with the hypothesis that an anterograde disruption of object-related cognition by full kindling requires a stimulation site in the PRH. Along with the site-specific effects of dHPC kindling on spatial cognition, these results suggest that the nature of the mnemonic effects of kindling may in general be dependent upon the site being kindled, at least with full kindling.

8.4.2. Extent of Kindling

The impact of the extent of kindling in effects on mnemonic-function received only moderate direct investigation in the present dissertation (Experiment 2) and very little direct investigation in other studies. Before reviewing the relevant data, however, I would like to point out that extent of kindling could be assessed in terms of any of a variety of variables including: the number of seizures of a given severity elicited (e.g., partial, full, and extended kindling which indicate 0, 1-30, and greater than 30 stage 5 seizures, respectively), the total number of stimulations administered, the maximum severity of seizure evoked, the maximum duration of AD elicited, the cumulative duration of AD elicited, or whether seizures have been induced unilaterally, bilaterally, or in more than one structure. Although any of these variables may eventually prove to be most directly related to mnemonic outcome, I will consider extent of kindling primarily in terms of the number of stage 5 seizures evoked and thus will distinguish between partial, full, and extended kindling. There are several reasons for my choice. First, this measure is widely used to describe extent of kindling and is reported fairly
consistently across studies. Second, it has been varied systematically in attempts to
determine the causes and consequences of kindling, and has been varied across studies
of the effects of kindling on mnemonic function. Finally, this measure allows kindling in
different sites to be roughly equated in terms of extent of kindling according to a
standardized, reliable, easily measured, and clinically important behavioral variable.

The available data suggest that the anterograde effects of kindling on spatial
cognition show a distinct relation to extent of kindling. Kindling of the dHPC produces
anterograde disruption of spatial cognition only if full kindling is achieved. Full
dHPCal kindling impairs acquisition in both the standard and WM version of the MWM
(Gilbert et al., 1996; Experiments 1a, 2, and 3a), whereas partial dHPCal kindling fails
to impair acquisition performance in either the RAM or standard MWM task (Gilbert et
al., 1996; Leung et al., 1996; Sutherland et al., 1997).

It is noteworthy that kindling in other sites may produce a significant
anterograde disruption of spatial cognition but only after extended kindling. Extended
but not partial or full kindling of the olfactory bulb impairs acquisition in the RAM
(Sutula et al., 1995). Similarly, extended kindling of the perforant path or AM
effectively impairs MWM acquisition (Cammisuli et al., 1997), whereas full kindling of
either structure produces little or no effect (McNamara et al., 1992; Niemenen et al.,
1992). Effects that require extended kindling should be viewed cautiously in terms of
their specificity to spatial cognition, however, since non-mnemonic behavioral changes
are likely to be more pronounced following extended kindling (e.g., Kalynchuk et al.,
1998b), and the possible contributions of such changes to spatial task performance
following extended kindling have received only a limited assessment. Nonetheless,
these observations suggest that an interesting interaction between the site and extent of kindling may exist such that kindling in a sensitive region (e.g., the dHPC) requires only full kindling whereas kindling in less sensitive sites (e.g., the AM, PP, or olfactory bulbs) requires extended kindling for an anterograde disruption of spatial cognition to be produced.

There is, however, an alternative measure of extent of kindling that may be relevant to the above pattern of findings. There is a notable difference in the total number of stimulations required to achieve full kindling in the dHPC (approximately 50 stimulations; Experiments 1a, 2, and 3a) compared to the AM, olfactory bulb, PRH, or perforant path (approximately 25 or fewer stimulations; McNamara et al., 1992; Sutula et al., 1995; Experiments 1b, 3b, and 3c). This raises the possibility that the critical variable for determining whether an anterograde spatial effect is produced may be the number of kindling stimulations delivered rather than the site and extent of kindling per se, with approximately 50 representing a critical value. If one makes this assumption, then one would predict that extended kindling of sites that achieve full kindling in less than 50 stimulations would be required for an anterograde spatial deficit to be produced. This prediction is consistent with the results observed. Full kindling of the AM, PP, olfactory bulb, or PRH, which requires 20 or fewer stimulations, does not produce an anterograde spatial deficit (McNamara et al., 1992; Nieminen et al., 1992; Sutula et al., 1995; Experiments 1b, 3a, 3c) whereas extended olfactory bulb kindling (involving as few as approximately 50 stimulations; Sutula et al., 1995) or extended perforant path or AM kindling (200 or more stimulations were the fewest administered; Cammisuli et al., 1997) does. Further investigation of this possibility is warranted and could be readily
achieved by comparing performance after kindling in different sites while holding
constant the number of stimulations (at 20, 50, and 200 stimulations for example).

There is limited evidence regarding the role of extent of kindling in producing
retrograde effects on spatial cognition. However, it appears that retrograde effects can
be induced more easily than anterograde effects, since partial dHPCal kindling
effectively impairs RAM or MWM performance in previously trained subjects (Gilbert
et al., 1996; Leung & Shen, 1991; Leung et al., 1994; Leung et al., 1990; Leung et al.,
1996; Sutherland et al., 1997), which are probably retrograde effects, but not in naive
subjects, which are necessarily anterograde effects (Gilbert et al., 1996; Leung et al.,
1996). In fact, as few as 4 dHPCal ADs have been shown to be capable of disrupting
performance on 2 arm discrimination problems in the RAM learned as much as 32 days
before kindling (Laurent-Demir & Jaffard, 1997). In Experiment 2, I specifically
investigated the effects of extent of kindling on performance of a WM version of the
MWM task. I trained rats and then tested them after 1, 6, 11, and 16 ADs, 1 stage 1
seizure, and 1 stage 5 seizure. This protocol unavoidably confounded both anterograde
and retrograde effects. I found that even 1 AD impaired performance but that
performance subsequently improved such that no deficit was evident after 16 ADs or 1
stage 1 seizure. An impairment reappeared after 1 stage 5 seizure. Based on the above
considerations, the most plausible account for this pattern of results is that the early
phase deficit constitutes a retrograde impairment, consistent with the sensitivity of
retrograde effects to even small amounts of kindling, that the middle phase recovery is
due to successful relearning or the use of effective behavioral compensation strategies,
and that the late phase deficit reflects the appearance of an anterograde effect.
consistent with data previously discussed suggesting that full dHPCal kindling produces an anterograde disruption of spatial cognition.

An additional measure of the extent of kindling that has received some investigation is the use of unilateral versus bilateral kindling. Evidence from studies investigating both spatial and non-spatial cognition suggests that bilateral kindling may be more effective than unilateral kindling in some cases but this may depend upon the site being kindled. In two studies, full bilateral kindling of the AM has been found to produce an impairment in passive avoidance performance relative to the effects of unilateral kindling (Boast & McIntyre, 1977; Peele & Gilbert, 1992). In both studies, non-kindled controls were not tested, leaving it unclear whether bilateral kindling is required to induce the deficit or merely produces a more severe impairment than unilateral kindling. The former contention is supported by the observation that performance by unilaterally kindled groups was near perfect according to the criteria used in these studies, whereas the latter contention is supported by results of a subsequent study (Stone & Gold, 1988) showing that unilateral AM kindling can disrupt passive avoidance performance relative to non-kindled controls. In other studies, bilateral kindling has failed to produce a greater effect than unilateral kindling, although sites other than the AM were kindled. Full unilateral kindling and bilateral kindling of the perforant path do not differ in their failure to affect RAM acquisition (Robinson et al., 1993) or in the mild effect they have on MWM acquisition (McNamara et al., 1992). Similarly, I found that full unilateral PRH kindling and bilateral PRH kindling produce a comparable disruption of 28 day retention of an OD problem (Experiment 1b). A possible explanation for why bilateral kindling may have a greater effect than unilateral
kindling in some cases but not others may relate specifically to the site being kindling. For example, with HPCal or PRH kindling, AD spreads contralaterally early on in kindling and subsequent transfer to the contralateral site following kindling is almost immediate, suggesting that unilateral kindling may induce many of the same effects as bilateral kindling (personal observations). In contrast, AM kindling does not result in the spread of AD to the contralateral AM early into kindling and subsequent transfer is proportionally slower than with the other sites (Cain, 1986; Goddard et al., 1969). Thus, bilateral AM kindling might be expected to produce effects that differ more greatly from those produced by unilateral kindling compared to bilateral perforant path or PRH kindling.

8.4.3. Interval Between Kindling and Testing

Although, the interval between kindling and testing was not specifically investigated in the studies constituting this dissertation, there are several significant observations worth discussing. First, anterograde spatial impairments appear to outlast the acute after-effects of the last seizure. In most studies, behavioral testing has usually been started 1 day to 1 week after the completion of kindling, while, in the present dissertation, spatial testing was always initiated no sooner than 72 hours following the last seizure. Because this period is generally believed to be adequate for the acute after-effects of a seizure to subside, at least some of the deficits observed at these and later time-points must result from other causes. Second, the anterograde effects of kindling on mnemonic function can be quite long lasting. Anterograde effects of kindling on spatial cognition have been observed at periods of 2 weeks or longer following the completion of kindling. Full dHPCal kindling impairs performance on a WM version of
the MWM with testing starting at 2 weeks and continuing over a period up to almost 4 weeks following the completion of kindling (Experiment 3a). Moreover, extended olfactory bulb kindling impairs RAM acquisition with testing starting at 4 weeks following kindling (Sutula et al., 1995). Interestingly, anterograde effects of kindling on non-spatial cognition also appear to be long-lasting. For example, full PRH kindling impairs object recognition in an open field task (Experiment 3b) and full bilateral kindling of the AM impairs passive avoidance performance (Peele & Gilbert, 1992) when tested at 2 weeks following the completion of kindling. In the case of both spatial and non-spatial effects, it cannot be stated whether the observed impairments persist even longer because suitably designed studies testing performance at longer intervals following kindling, and in the absence of the confounding effects of learning opportunities, have not been performed.

Retrograde effects of kindling on spatial cognition are also apparent at relatively lengthy intervals between kindling and subsequent testing. Deficits have been shown in the RAM at 3 to 4 weeks following kindling (Feasey-Truger et al., 1993; Leung & Shen, 1991; Leung et al., 1990; Leung et al., 1996). In some of these studies, continued testing suggested that this impairment abates by 4 weeks post kindling (Feasey-Truger et al., 1993; Leung & Shen, 1991). However, the extensive post-kindling training administered in these studies confounds new learning and recovery, and it is thus unclear whether the impairment is in fact transient or performance eventually reaches control levels due to successful relearning. Recovery and relearning need to be differentiated by examining performance at different post kindling intervals in the absence of the potentially confounding effects of intervening training. This can be done in a within
subjects design by testing subjects on completely novel problems at different time intervals after kindling or in a between subjects design by testing different groups at different intervals between kindling and testing on an identical task. Although these ideal studies have not been performed, Leung and colleagues have completed the most suitable investigation of this issue (Leung & Shen, 1991; Leung et al., 1994). They found that partial dHPCal kindling impairs RAM performance at 3 (Leung & Shen, 1991) but not 6 (Leung et al., 1994) weeks after kindling. Their protocol minimized opportunities for relearning prior to testing at these periods (i.e., 3 intervening trials were administered at 1, 4, and 7 days following kindling). However, a problem with the finding at 6 weeks is that the lack of impairment may relate more to a transient forgetting-induced decline in control levels of performance rather than to any improvement in the performance of the kindled group. In fact, after a few reminder trials, control performance rapidly improved and a marginal deficit (p< 0.10) began to reappear on testing at 7 weeks following kindling. Thus it is not certain that the retrograde effects kindling on spatial cognition are in fact transient over any interval after kindling.

An important point worth restating is that there is presently no strong evidence that any of the mnemonic impairments induced by kindling are transient. Kindled animals are capable of learning and remembering new information, albeit sometimes less efficiently than controls. This is not unexpected since even rats with extensive lesions are often capable of eventually performing tasks that normally depend upon the lesioned structure. For example, rats with large HPCal lesions or transections of the HPC' major subcortical input/output pathway, the fimbria/fornix, can eventually reach excellent
levels of performance on the hippocampally-dependent MWM task (Hannesson & Skelton, 1998; Morris et al., 1990). Thus, performance by kindled animals does improve and can reach control levels on various tasks if sufficient learning opportunities are provided. In this sense, kindling-induced deficits may be described as transient in terms of performance relative to controls. However, such observations do not imply that the underlying impairment produced by kindling is transient, and, as discussed above, appropriate studies to address this question have not yet been undertaken.

8.5. The Characterization of Kindling's Effects on Spatial Cognition: Implications Regarding Underlying Mechanisms

The findings of the present dissertation, consistent with other studies in the field, are that kindling produces a specific anterograde disruption of spatial learning. This deficit is preferentially induced with a stimulation focus in the dHPC, requires at least full kindling, and persists over an interval of at least 2 weeks following kindling, if not longer. In the following section, I will consider the implications of these findings with respect to the types of mechanisms that are likely to mediate the effects of kindling on spatial cognition.

8.5.1. Kindling Produces a Selective Disruption of Spatial Cognition

Given our current knowledge of the neuroanatomy of spatial cognition, the selective disruption of spatial cognition by kindling could most readily be accounted for by changes within the HPC and related structures (Nadel, 1991; O'Keefe & Nadel, 1978). Lesion studies have clearly demonstrated a critical involvement for the HPCal system in performance on a wide range of spatial tasks (Barnes, 1988; Jarrard, 1993;
O'Keefe & Nadel, 1978; Pouget & Benhamou, 1997). Similarly, both electrical recording and imaging studies have demonstrated a critical role for the HPCal system in spatial functions in both rats (O'Keefe & Nadel, 1978; Pouget, Save, & Lenck-Santini, 2000; Wiener, 1996), non-human primates (Rolls, 1999), and humans (Maguire, Burgess, & O'Keefe, 1999). Although a variety of different structures have also been shown to be involved in the two tasks used to investigate spatial cognition following kindling (i.e., the RAM and MWM; Barnes, 1988; Becker, Walker, & Olton, 1980; Brandeis, Brandys, & Yehuda, 1989; Cain & Saucier, 1996), the contributions of these structures are not believed to be as central to spatial processing and mnemonic functions as those of the HPCal system (Nadel, 1990; Nadel, 1991; Pouget & Benhamou, 1997). Thus, these structures are more likely to support functions that are involved in a wider variety of behaviors than those of the HPC. Based on these considerations, the selectivity of the effect observed provides some indication that modifications in the HPCal system are most likely to mediate kindling's effects on spatial cognition. In other words, although the HPC is not involved exclusively in spatial cognition and spatial cognition does not rely exclusively on the HPC, the profound role of the HPC in spatial cognition and the relative selectivity with which spatial effects are observed after kindling makes the HPC the leading anatomical candidate for the site of kindling-induced effects. Further support for this hypothesis comes from the observation that kindling-induced effects on spatial cognition are produced preferentially by HPCal kindling rather than AM or PRH kindling, and that, within the HPCal system, kindling in the dHPC appears to be most capable of disrupting spatial cognition. The dHPC is precisely the area of the HPCal system that appears to be most important for spatial
cognition (Moser et al., 1995; Moser & Moser, 1998).

8.5.2. Kindling Disrupts Spatial Learning but not Short- or Long-Term Spatial Memory

The selective disruption of spatial learning by kindling suggests at least two possible general accounts for the underlying mechanisms. Learning requires that information is suitably transmitted throughout optimal processing and output structures and that enduring changes in neural function are registered therein (i.e., plasticity is induced). Thus, kindling could disrupt spatial learning by altering the capacity for induction of synaptic plasticity within HPCal and/or related circuitries. Alternatively, kindling could disrupt the transmission of signals in circuitry involved in spatial learning such that the flow of information from perceptual centers to the HPCal system, from one region to the next within the HPCal system, and/or from the HPCal system to output systems is compromised. Also, it should be noted that disturbed plasticity and disturbed signal transmission need not be mutually exclusive and, in fact, could operate in a synergistic manner. These possibilities will be discussed in further detail below.

From theoretical considerations alone, learning would seem to require some form of nervous system plasticity and an immediately obvious locale for such changes is the synapse (Eichenbaum, 1996; Jeffery, 1997; Morris, 1989). Despite considerable research aimed at directly linking synaptic plasticity and mnemonic function, definitive empirical evidence linking plasticity and mnemonic function in many neural systems remains elusive and it is theoretical considerations which still provide a major impetus for the belief that synaptic plasticity is an essential part of the processes by which patterns of neural activity generated by normal experience create a memory trace.
Nonetheless, several current hypotheses predict that particular patterns of experience-dependent activity produce modifications in synaptic function, which outlast the experience and lead to altered function in affected circuitries (Elgersma & Silva, 1999; Martin et al., 2000; Wang et al., 1997). These alterations in the activity of the affected circuitries then are manifest in changes in behavioral output, which is considered direct evidence of learning and tacit evidence of neural plasticity.

There are various examples of specific types of synaptic plasticity that might contribute to mnemonic function, but the majority of research investigating the links between mnemonic function and synaptic plasticity has focussed on a one particular class of plasticity termed long-term potentiation (LTP) (Baudry, 1998; Bliss & Collingridge, 1993; Martin et al., 2000; Wang et al., 1997). Much of the interest in LTP can be justified by the fact that LTP possesses many features considered attractive in a hypothetical memory mechanism including associativity, selectivity, and durability (Bliss & Collingridge, 1993; Martin et al., 2000; Morris, 1989). Nonetheless, it should be noted that other forms of plasticity including long-term depression, and some forms of short-term synaptic potentiation, have also been implicated in particular classes of mnemonic function (Baudry, 1998; Silva, Giese, Federov, Frankland, & Kogan, 1998; Wang et al., 1997).

Whatever their precise nature, if changes in synaptic function are essential for the encoding of experience, one means by which kindling could disrupt learning is through the disruption of the mechanisms that underlie the induction of normal synaptic plasticity. One form that this hypothesis may take, which will subsequently be referred to as the “plasticity hypothesis”, is that kindling produces a disruption of the
mechanisms underlying induction of LTP-like plasticity within HPCal or related circuitries and thereby disrupts the encoding of spatial information necessary for optimal spatial task performance. The reader should note that this disruption could involve either an enhancement or an impairment in LTP induction, as long as there was a deviation from the conditions optimal for normal spatial learning (Martin et al., 2000).

An alternative to the plasticity hypothesis is the hypothesis that kindling compromises spatial learning by producing changes in the input, throughput, and/or output of signals within regions involved in normal processing of spatial information. Most present views of cognitive function now recognize that different neural systems support different types of memory functions and these systems compete for control over output systems during the performance of behavioral tasks (Nadel, 1990; Rolls, 2000; Squire, 1992). Thus, the extent to which different neural systems are activated by the stimuli that compose a given task, the extent to which incoming information is transmitted through each memory system, and/or the facility with which a memory system can "command" output structures are important determinants of the types of information that the organism will use to guide behavior in a given task. Thus, shifts in any of these processes would be predicted to produce differences in the behavioral strategy most likely to be used by an organism in any particular testing situation. With respect to spatial cognition, it has been argued that differences in testing environments can determine the extent to which HPCal circuitry is activated by a task and hence the likelihood that a spatial hypothesis will be used to "solve" it (O'Keefe & Nadel, 1978). Thus, spatial learning impairments could result from a shift towards the use of non-spatial strategies in testing situations that might otherwise be optimally solved using
spatial information. Note that this view does not suggest that sensory/perceptual or motor deficits account for impairments. Rather, this view holds that the interface between these systems and those responsible for the processing and encoding of relationships between percepts required for spatial cognition is altered in a way that reduces the probability of stimuli inducing potent spatial processing or potent output from spatial processing systems. Thus, it can be hypothesized that kindling produces a reduction in the efficacy of transmission into, through, and out of HPCal circuitry and thereby reduces the likelihood that spatial hypotheses will be used to guide behavior during the early phases of testing on a novel problem. This, in turn, will be manifest in poorer performance when spatial hypotheses represent the optimal means for solving a task. This view will subsequently be referred to as the “signal transmission hypothesis”.

A third possibility is that there is an interaction between changes in plasticity and changes in signal transmission, which acts synergistically to disrupt spatial learning. For example, plasticity is dependent upon the strength, pattern, and timing of inputs to appropriate processing structures (Larson, Wong, & Lynch, 1986; Pavlides, Greenstein, Grudman, & Winson, 1988; Rose & Dunwiddie, 1986). Thus, an alteration in the strength or pattern of neural inputs to the HPC might exacerbate a reduction in local plastic capacity, thus further reducing the likelihood that plastic changes sufficient for information storage in the HPC are induced. Alternatively, the ability of different processing systems to gain control over motor structures is likely to depend upon the strength and pattern of signals transmitted to these motor structures. Thus, altered plasticity in processing structures could disrupt the magnitude or pattern of outgoing neural signals to output structures, which, in turn, could exacerbate any changes in the
effectiveness of communication between spatial neural systems and motor systems that were already produced by kindling. The above possibilities need not be exclusive and could themselves compound relatively minor modifications in each respective process into a more serious disruption of function that could then become detectable at the behavioral level of analysis used in spatial tasks such as the MWM or RAM.

8.5.3. Kindling's Effects on Spatial Cognition are Site-Specific to the dHPC

With full kindling at least, the effects of kindling on spatial learning are site-specific to the dHPC. This observation has implications both for the types of mechanisms that are likely to underlie this effect and the types of mechanisms that are not. First, this observation suggests that the mechanisms of kindling-induced disruption of spatial cognition must be exclusively, or at least more strongly, induced with a stimulation focus in the dHPC. There are a variety of possible neural changes that might meet this criterion. In general terms, as discussed in the Introduction, two distinct processes can be identified during kindling. The first involves changes in the threshold for eliciting AD and the second involves changes in the propagation of AD. The former is primarily a local phenomenon, while the latter is primarily a circuit phenomenon. Thus, the site-specificity of the behavioral effects of kindling suggests that the underlying mechanisms are more likely to relate to changes in local ADT than to changes in AD propagation. However, it should be acknowledged that changes in local mechanisms controlling propagation from the seizure focus, distinct from those changes specifically involved in changes in ADT, could also be a basis for site-specific behavioral effects. Regardless, the above considerations strongly suggest that changes in characteristic circuits involved in seizure generalization (Applegate, 1998; Burchfiel
et al., 1998; McIntyre & Kelly, 1998), or other circuits characteristically affected by kindling in a variety of sites, could not solely underlie site-specific effects of kindling on spatial cognition or other mnemonic functions. For example, mossy fibre sprouting is observed following kindling in a variety of forebrain sites (Represa & Ben-Ari, 1992; Sutula, Xiao-Xian, Cavasos, & Scott, 1988). The sparing of spatial cognition following full kindling in many of these sites, including the AM, perforant path, medial septum, and olfactory bulbs, strongly argues against the hypothesis that aberrant mossy fibre sprouting is sufficient to cause spatial cognitive impairments.

8.5.4. Anterograde Disruption of Spatial Learning by dHPC Kindling Requires Full Kindling

The anterograde disruptive effect of dHPC kindling on spatial cognition requires that stimulation be continued until full kindling is achieved. This requirement suggests that mechanisms related to seizure generalization may be the same as, or closely related to, those that impair spatial learning. Given the site specificity of kindling’s effects on spatial cognition, this possibility would seem somewhat paradoxical. Full kindling is by definition the stage at which seizures become fully generalized and widespread areas of the brain become activated by the seizure. The requirement that kindling reaches this stage of the process to induce an impairment would seem to suggest that effects involving the characteristic circuitry activated by generalized seizures rather than those directly related to local effects at the kindling site are involved in the impairment. This apparent discrepancy might be accounted for as follows. The process by which AD begins to spread throughout the brain from the seizure focus can be conceptualised as involving multi-stage gating (Burchfiel & Applegate, 1989). Thus, for seizures to
progress in their intensity successive gates must be broken down. The first major gate may involve the transition from stage 0 to stage 1/2 seizures and may involve changes local to the kindling site or in projections that originate in the kindling site. The second gate may involve the transition from stage 1/2 to stage 3/4/5 seizures and is thought to involve changes that are independent of the kindling site. Thus, a kindling-induced deficit may require both the local events associated with the transition through the first gate, hence the requirement for site-specific kindling, and the distal events associated with transition through the second gate, hence the requirement for full kindling.

However, there are also several other means to account for the significance of full kindling in dHPC kindling-induced spatial impairments. With dHPC kindling, AD typically undergoes a notable jump in its duration upon reaching the level of stage 1/2 seizures (personal observations). Thus, local effects associated with multiple bouts of long duration ADs, such as would be experienced during kindling from stage 1 to stage 5 seizures, may differ from the effects of multiple bouts of shorter duration seizures, such as would be experienced during kindling up to stage 1 seizures, and these differing effects may be critical to spatial learning impairments. Alternatively, a disruptive effect on spatial cognition may simply relate to a mechanism that is dependent upon some threshold level of cumulative local seizure activity for its induction, which is reached after approximately the number of seizures required for full dHPC kindling.

8.5.5. Disruption of Spatial Cognition by Kindling is Long Lasting if not Permanent

The anterograde effects of kindling on spatial cognition are observed to be long lasting, if not permanent. Because the kindled state itself reflects a permanent or near
permanent increase in susceptibility to seizure evoking stimuli (Dennison, Teskey, & Cain, 1995; Wada et al., 1974), the durability of the kindling-induced impairment could suggest that mechanisms related to the maintenance of the kindled state could relate to those involved in disrupting spatial learning. While these mechanisms are unknown, this observation does eliminate as candidate mechanisms those effects of kindling which are more transient in nature, such as those with duration of less than two weeks. This also suggests that the mechanisms of kindling-induced mnemonic dysfunction are more likely to relate to enduring plastic effects of kindling per se rather than any of the more acute after-effects of seizures per se.

8.6. Specific Hypotheses of the Mechanisms Mediating Kindling’s Effects on Spatial Cognition

Although the details of any hypothesis of the neural mechanisms of kindling’s effects on spatial cognition will be limited by our current level of understanding of the mechanisms of spatial learning, the spectrum of neural changes associated with kindling, and the relation between the kindling-related variables discussed above and the neural changes associated with kindling, progress on each front is currently sufficient to warrant the formulation of at least a tentative hypothesis. At the minimum, this exercise should highlight the major holes in our relevant knowledge and thus prioritise directions for future experimentation.

Based on the general considerations discussed in the preceding sections, the long-lasting anterograde spatial learning deficit produced by full kindling of the dHPC can be hypothesized to be due to kindling-induced neural changes within HPCal
circuitry, which alter either the capacity for experience-dependent induction of plasticity within HPCα circuitry, the input, throughput, or output of signals in HPCα circuitry, or both. In addition, the mechanisms of these changes may be related to neural effects associated with changes specific to the site being kindled (e.g., reductions in local ADT), the production of generalized seizure activity, and the long-term maintenance of the kindled state.

Specifying details for the above general hypotheses involves determining what consequences of kindling, if any are known, might affect the induction of intra-HPCα plasticity and/or the transmission of signals within HPCα and related circuitry. Then, the extent to which such consequences exhibit a relation to kindling-related parameters that is consistent with the conditions under which spatial impairments are observed should be determined.

What consequences of kindling, if any, might alter mechanisms underlying HPCα plasticity? Because the best-studied candidate mechanism of spatial learning in the HPC is LTP (Bliss & Collingridge, 1993; Martin et al., 2000), the discussion will focus on this form of plasticity. However, it should be noted that kindling may alter the capacity for induction of long-term depression (LTD) in the HPC (Wang & Gean, 1999), and it is becoming increasingly recognized that this form of plasticity may also be involved in hippocampally mediated mnemonic functions (Baudry, 1998; Eichenbaum, 1996; Martin et al., 2000; Wang et al., 1997).

Although a mechanistic relation between LTP and kindling has received theoretical consideration, there has been little investigation of the capacity for LTP induction in the kindled brain. In the only relevant study, metabotropic glutamate
receptor-dependent long-lasting potentiation in basolateral amygdalar neurons in slices taken from AM kindled rats was found to be absent (Neugebauer, Keele, & Shinnick-Gallagher, 1997). These data do not directly speak to the hypothesis that kindling-induced spatial deficits relate to changes in HPCal LTP, since both kindling and LTP were studied outside of the HPC, but they do show that kindling can lead to subsequent alterations in the capacity for LTP induction in the brain.

Other evidence, albeit indirect, suggests that kindling may alter HPCal LTP. This evidence takes the form of studies showing that kindling produces changes in processes and/or molecules involved in LTP. Although all the details of the molecular, physiological, and anatomical mechanisms of LTP have not been worked out, considerable understanding of many aspects of LTP has been achieved over the last 30 years. One relevant observation is that LTP induction in the dentate gyrus and CA1 regions of the HPC is critically dependent upon the function of the NMDA glutamate receptor (Bliss & Collingridge, 1993; Nicoll & Malenka, 1995). Thus, the observation that kindling produces a number of changes related to NMDA receptor function (for reviews see Meldrum, Akbar, & Chapman, 1999; Mody, 1999) suggests that capacity for LTP induction might be altered.

Kindling may alter NMDA receptor function in several ways. Kindling does not appear to have any enduring effects on degree of NMDA binding per se, suggesting that receptor numbers do not change (Meldrum et al., 1999; Mody, 1999). Nor are enduring changes in the expression of mRNAs for the various NMDA receptor subunits seen (Kamphuis, Hendriksen, Diegenbach, & Lopes da Silva, 1995; Kraus, Yeh, Bonhaus, Nadler, & McNamara, 1994). However, an enhancement of NMDA-mediated currents
is observed for up to 60 days after kindling. This is accompanied by a reduced sensitivity to Mg$^{+}$ block (Kohr, De Koninck, & Mody, 1993) and a shift in the relative binding of different pharmacological ligands (Kraus et al., 1994). The bases for changes in NMDA receptor function are not known but could include changes in subunit structure or changes in any one of a variety of regulatory mechanisms (e.g., protein kinases, protein phosphatases, or other proteins such as calmodulin or calcineurin). Regardless of the mechanism, these data are provocative in predicting that NMDA-dependent HPCal LTP might be modified in the kindled brain.

Another potentially relevant change observed in the kindled brain is an increase in kynurenic acid in the dHPC revealed using microdialysis (Wu, Monno, Schwarcz, & Vezzani, 1995). Since kynurenic acid is an endogenous inhibitor of NMDA receptor function via the glycine binding site, this effect could also alter NMDA-mediated plasticity.

Kindling also produces changes in molecules that may be involved in LTP in a manner that may not be directly NMDA receptor related. For example, protein kinase C (PKC) has been shown to be involved in both HPCal LTP (Angenstein & Staak, 1997) and HPCal dependent learning (Van Der Zee, Luiten, & Disterhoft, 1997). After kindling, HPCal PKC activity is persistently increased (Akiyama, 1998), a change that may relate to alterations in metabotropic glutamate receptor function (Angenstein & Staak, 1997; Klapstein, Meldrum, & Mody, 1999). Given PKCs role in LTP, such an effect might be predicted to alter HPCal plasticity in the kindled brain. Interestingly, there is some evidence that PKC activity may directly modulate NMDA receptor function (Chen & Huang, 1991; Chen & Huang, 1992).
Finally, it has recently been shown that kindling can produce a long-lasting increase in the expression of dendritic N-type calcium channels in the CA1 pyramidal cells (Bernstein, Mendonca, Wadia, Burnham, & Jones, 1999). The critical role of calcium in LTP induction suggests that such changes might also alter plastic capacity in the kindled brain.

The above observations represent several possible mechanisms by which kindling might alter subsequent induction of LTP. A variety of other candidate mechanisms that might alter LTP, albeit sometimes in a more indirect manner, also exist but their discussion is beyond the scope of this humble, yet already excessively lengthy, monograph. One final observation worth noting is that early postnatal kainic acid induced seizures lead to subsequent impairments of spatial learning that are associated with disturbed LTP induction (Lynch, Sayin, Bownds, Janumpalli, & Sutula, 2000) suggesting the possibility of an interesting parallel between cognitive dysfunction associated with this model of epilepsy and that seen following kindling.

A second hypothesized mechanism for kindling’s disruption of spatial learning could involve changes in signal transmission within the circuits mediating the processing and translation of spatial information into behavior. This hypothesis, by itself, contends that the mechanisms of plasticity mediating spatial learning are intact but that information either fails to be transmitted into spatial processing centers in a manner suitable for inducing this plasticity or fails to be transmitted out of spatial processing centers in a manner suitable for engaging necessary motor output systems. These possibilities will be considered separately.

In general, there is considerable evidence that kindling induces changes in the
transmission of signals within brain circuits. The potentiation of transmission between a variety of brain regions during and subsequent to kindling, termed kindling-induced potentiation (KIP), has been demonstrated numerous times (Maru & Goddard, 1987b; Racine, Moore, & Evans, 1991; Uno & Ozawa, 1991) and has been shown to resemble LTP, although the two phenomena can be dissociated on several grounds (Cain, 1989). Interestingly, such potentiation of transmission between the AM and specific output pathways mediating visceral responses has been proposed as a mechanism for kindling’s effects on anxiety-related behaviors (Adamec & Young, 2000). At the same time, significant effects on the inhibition of transmission between circuits following kindling have also been noted (Mody, 1999).

If kindling then is capable of altering the interictal transmission of signals within various brain circuitries, what evidence is there that such effects might specifically alter the normal activation of HPCal circuitry by spatial stimuli? There is at least one obvious mechanism by which such an effect might be achieved. As evidenced by the paired-pulse depression procedure, kindling produces an increase in inhibition in the dentate granule cell – perforant path circuitry (Maru & Goddard, 1987a; Tuff, Racine, & Adamec, 1983). The mechanisms of these changes are not known but could relate to changes in GABA_A receptor density or subunit composition (Mody, 1999). An increase in inhibition at dentate granule cells could have profound impact on the ability of spatial stimuli to elicit significant HPCal activation. Hippocampal circuitry can be considered a trisynaptic loop with signals transmitted predominantly, although not exclusively, in sequence from the entorhinal cortex to the dentate gyrus to CA3 cells to CA1 cells and back to the entorhinal cortex. Thus, an increase in inhibition at the dentate level might
be expected to elevate the threshold for signals to pass through the tri-synaptic circuitry in a manner optimal for spatial processing. It is also noteworthy that the induction of HPCal LTP using physiologically veridical stimulation parameters requires high frequency bursts. Thus, increased inhibition in the dentate gyrus may again play a deleterious role in reducing the transmission of strong bursts into the CA regions and thereby reducing the plasticity-inducing capacity of incoming signals.

Some considerations also suggest speculative mechanisms by which kindling may alter the interictal transmission of signals from HPCal circuitry to motor structures. The mechanisms and routes whereby spatial processing structures transmit signals to motor structures that ultimately mediate navigational behavior are not known. However, some evidence suggests that the nucleus accumbens may serve as a relay point for HPCal processing signals to access motor structures mediating spatial behavior (e.g., Floresco, Seamans, & Phillips, 1997). Thus, kindling-induced changes in HPCal interactions with the nucleus accumbens could be speculated to produce changes in the ability of HPCal output to gain command over motor structures. Although no direct evidence exists, several observations are consistent with such a possibility. For example, kindling has been observed to produce a decrease in kynureninic acid concentrations in the accumbens (Loscher, Ebert, & Lehmann, 1996), a decrease in dopamine transporter binding in the striatum (Gordon, Mintz, Rosenne, & Rehavi, 1995), an increase in DA release in the accumbens in response to HPCal AD (Strecker & Moneta, 1994), and potentiation of accumbal responses to AM stimulation (Uno & Ozawa, 1991). While the functional impact of these alterations is not clear, these findings do provide evidence that kindling may alter nucleus accumbens function and
therefore, possibly, hippocampo-accumbens interactions.

8.6.1. Evaluating the Candidate Mechanisms

In the preceding sections, I described two possible hypotheses that might account for kindling's observed disruption of spatial learning (the plasticity hypothesis and the signal transmission hypothesis) and then described specific effects of kindling that might be candidate mechanisms underlying these hypothesized means of disrupting spatial learning. In further evaluating the plausibility of these hypotheses, in the following section, I will consider the extent to which each candidate mechanism exhibits a relation to kindling-related parameters that is consistent with conditions under which a kindling-induced disruption of spatial learning is or is not observed. Before proceeding, I will remind the reader that the kindling-induced spatial impairment is induced by dHPC kindling and not AM or PRH kindling, by full and not partial kindling, and lasts for at least 16 days following the last kindling stimulation. Also, I must acknowledge that the specific mechanisms considered as possible mediators of the various hypothetical means by which kindling disrupts spatial learning are not exhaustive and therefore, even if a particular mechanism does not meet the above criteria, alternative means, more consistent with the criteria, may exist. Regardless, this exercise should still be fruitful in narrowing down the possibilities and generating testable predictions that can be prioritised for subsequent experimental assessment.

Several possible mechanisms that could mediate a kindling-induced disruption of intra-HPCal plasticity were previously discussed including changes in NMDA receptor function, PKC levels, kynurenic acid levels, and CA++ channel function. These show varying degrees of consistency in their relation to the "kindling parameter restrictions"
associated with the deficit (see Table 10). With respect to interval between kindling and testing, NMDA, PKC, and Ca++ channel changes are all sufficiently long-lasting, having been demonstrated at periods of up to 28, 112, and 28 days following the last kindling stimulation respectively (Akiyama, 1998; Bernstein et al., 1999; Mody, 1999). It is unclear whether changes in kynurenic acid meet this criterion since effects have been observed at 7 but not 49 days following kindling (Loscher et al., 1996; Wu et al., 1995). With respect to kindling site specificity, HPCal PKC changes are indeed preferentially induced by dHPC kindling, although smaller effects are also seen after AM kindling (Akiyama, 1998). Conversely, kynurenic acid effects are observed equally after either dHPC or AM kindling (Wu et al., 1995), while the site specificity of effects on NMDA receptors and Ca++ channels is unknown. Lastly, with respect to kindling extent, changes in kynurenic acid meet this criterion by being observed after full but not partial kindling (Wu et al., 1995). Changes in NMDA receptors, PKC levels, and Ca++ channels are all observed after full kindling but it is not known whether these effects are observed to the same degree after partial kindling (Akiyama, 1998; Bernstein et al., 1999; Mody, 1999). Thus, the above considerations suggest that each of these mechanisms, except perhaps kynurenic acid changes, are plausible means by which a plasticity-related specific learning deficit might be selectively induced by full dHPC kindling. However, there are considerable holes in the data required to evaluate the completeness of the extent to which these effects consistently vary with all of the kindling-related parameter restrictions associated with the deficit. These holes represent obvious points for subsequent experimentation.
Table 10. Consistency of plasticity-related kindling-induced neural changes in their relation to parameters associated with the production of a spatial learning deficit. ✓ indicates that the neural change is observed under the condition indicated whereas X indicates that it is not. ? indicates that the relation of the neural change to the particular condition is unknown. Durability refers to whether the change is still observed at least two weeks following the last kindling stimulation. The optimal pattern for a candidate mechanism would be ✓ ✓ X ✓, which parallels the circumstances under which the spatial learning deficit was observed.

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Several possible mechanisms that could mediate a kindling-induced disruption of signal transmission into, through, and out of HPCal circuitry were also discussed in the preceding section including changes in intra-HPCal inhibition and hippocampo-accumbal communication. These show a limited degree of consistency in their relation to "kindling parameter restrictions" associated with the deficit (see Table 11). With respect to interval between kindling and testing, changes in recurrent inhibition in the dentate gyrus and CA1, changes in HPCal GABA, receptor characteristics, and changes in hippocampo-accumbal responses have all been observed at sufficiently long intervals after kindling (i.e., 28 days or more) (de Jonge & Racine, 1987; Gordon et al., 1995; Loscher et al., 1996; Titulaer, Ghijsen, Kamphuis, De Rijk, & Lopes da Silva, 1995a; Titulaer, Kamphuis, & Lopes da Silva, 1995b; Titulaer, Kamphuis, & Lopes da Silva, 1995c; Titulaer, Kamphuis, Pool, van Heerikhuize, & Lopes da Silva, 1994). With respect to kindling-site specificity, changes in GABA, receptors and inhibition have been observed not only after dHPC kindling but also in many cases with comparable magnitude after AM or PP kindling (de Jonge & Racine, 1987; Maru & Goddard, 1987a; Mody, 1999; Oliver & Miller, 1985; Tuff et al., 1983), while changes in hippocampo-accumbal interactions are unknown after dHPC kindling, having only been observed following AM kindling (Gordon et al., 1995; Loscher et al., 1996). Finally, with respect to kindling extent, changes in GABA, receptors and inhibition, in at least some cases, do not appear to relate to kindling extent because similar effects are observed following partial and full kindling (Kamphuis, De Rijk, & Lopes da Silva, 1994). Conversely, at least one study suggests that changes in hippocampo-accumbal interactions may relate to extent of kindling with an effect being observed after...
Table 11. Consistency of signal transmission-related kindling-induced neural changes in their relation to parameters associated with the production of a spatial learning deficit. √ indicates that the neural change is observed under the condition indicated whereas X indicates that it is not. ? indicates that the relation of the neural change to the particular condition is unknown. Durability refers to whether the change is still observed at least two weeks following the last kindling stimulation. The optimal pattern for a candidate mechanism would be √ √ X √ X, which parallels the circumstances under which the spatial learning deficit was observed.

<table>
<thead>
<tr>
<th></th>
<th>Durability</th>
<th>Kindling site specificity</th>
<th>Kindling Extent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>dHPC</td>
<td>Other sites</td>
</tr>
<tr>
<td>Inhibition</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>GABAₐ</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>HPC-NAcc</td>
<td>√</td>
<td>?</td>
<td>√</td>
</tr>
</tbody>
</table>
stage 3 but not stage 1 seizures (Strecker & Moneta, 1994). Thus, overall, the above considerations suggest that the mechanisms considered as candidates to underlie kindling-induced changes in HPCal-related signal transmission largely fail to meet the restrictions imposed by the relation of these parameters to the observed learning effect. Thus, they are unlikely to be the sole bases for kindling's effects on spatial cognition.

Having considered the above specific mechanisms as possible bases for several hypothetical means by which kindling disrupts spatial cognition, it is worth noting briefly that there are many other kindling-induced effects that show a specific relation to some of the kindling-related parameters deemed critical for the induction of a spatial learning deficit. For example, kindling produces kindling-site specific differences in immediate early gene expression during kindling in the dHPC, PRH, or AM (Sato et al., 1998). In this study, expression in the dentate gyrus during the early phases of kindling was only observed with a dHPC focus. There are also a variety of other effects that have been observed which show a relation to kindling site (Baimbridge, Mody, & Miller, 1985; Friedl, Clusmann, Kral, Dietrich, & Schramm, 1999; Hosford et al., 1995; Klapstein et al., 1999; Schenk & Snow, 1994), extent of kindling (Bendotti, A., Tarizzo, & Samanin, 1993; Greenwood, Abdou, Meeker, & Hayward, 1994; Meyerhoff, Bates, & Kubek, 1990; Vezzani et al., 1992), and the interval following kindling (Bragdon, Taylor, McNamara, & Wilson, 1988; Vreugdenhil, Faas, & Wadman, 1998). These effects represent possible points for further investigation. However, the significance of many of these findings is difficult to ascertain because observations relating them to more than one kindling-related parameter tied to the learning impairment are lacking, as is a theoretical framework for hypothesizing the implications of these effects for learning.
and memory function. Nonetheless, these observations do further support the idea that kindling-related parameters can have a significant impact on the type and extent of neural changes observed following kindling and underscore the possible significance of these types of considerations for determining the likely mechanisms of kindling-associated learning dysfunction.

8.6.2. Testing a Working Hypothesis

The preceding section has shown that several of the candidate mechanisms for the plasticity hypothesis exhibit a more consistent relation to the kindling-related parameters associated with the kindling-induced spatial learning deficit than do the candidate mechanisms for the signal transmission hypothesis. Thus, the currently most tenable hypothesis of the basis of full dHPC kindling’s disruption of spatial learning is that kindling disturbs mechanisms involved in the induction of plasticity within HPCal circuitry in a manner that relates to changes in NMDA receptor characteristics, levels of PKC, and possibly increases in density of Ca++ channels. It should be acknowledged, however, that changes in signal transmission, though unlikely to be solely responsible for kindling-induced spatial impairments, could also be a factor in exacerbating the effects related to disturbed plasticity.

There are several means of testing the plasticity hypothesis as a basis for kindling’s disruption of spatial learning. The most obvious and direct test of a requisite condition of this hypothesis is to determine whether HPCal plasticity, and LTP in particular, is affected by full dHPC kindling. It should be noted that a complete blockade of LTP is not necessarily expected. Rather, a change in the input/output relations of LTP or a shift in the optimal conditions for LTP induction could well serve
as the bases, at least in part, for the subtle reduction in spatial learning efficiency seen in kindled rats. Moreover, such subtle changes are more likely to be associated with kindling, as seen in other examples of metaplasticity (Abraham & Tate, 1997). Other experiments could then determine whether HPCal LTP is preferentially affected by dHPC kindling versus kindling in other sites and by full kindling rather than partial kindling. Finally, the longevity of effects on LTP could be examined along with corresponding studies to further establish the post-kindling duration of kindling-induced spatial learning impairments.

Other tests of the plasticity hypothesis could involve further investigations of the specific candidate mechanisms, such as NMDA receptor, PKC, and CA++ channel changes and their relation to kindling site, kindling extent, and the interval following the completion of kindling. Such studies would be useful in completing the determination of the extent to which these effects show a similar relation to these parameters as does the kindling-induced spatial learning impairment. Ultimately, more ambitious experiments might then aim to determine whether manipulations that reverse or attenuate any of these effects would have a corresponding impact on spatial learning impairments.

8.7. Concluding Remarks

In a general sense, the studies in the present dissertation were designed to address questions regarding the effects of brain changes associated with the kindled state on subsequent mnemonic function. They clearly show that the kindled state can indeed be associated with a disruption of the mechanisms mediating normal mnemonic
function. In a more specific sense, these studies addressed questions regarding the
effects of kindling on spatial cognition. In this case, they provide strong evidence that
full kindling of the dHPC produces a selective and enduring disruption of spatial
cognition, which is manifest primarily in an impairment of learning. Moreover, they
show that this impairment exhibits a distinct relation to kindling-related parameters such
as the site of kindling, the extent of kindling, and the interval between kindling and
testing such that it is preferentially induced by kindling of the dHPC but not the AM or
PRH, by full and not partial kindling, and is seen at intervals of at least 16 days
following kindling. Based on the nature of the deficit and its relation to these kindling-
related parameters a speculative hypothesis of the mechanisms of kindling’s effects on
spatial cognition was derived as follows. Kindling impairs spatial learning through a
disruption of the induction of intra-HPCa1l plasticity that may be mediated by changes in
NMDA receptor function, PKC activity, and/or Ca** channel activity. Thus, the present
dissertation suggests that not only does kindling produce plastic changes that are
reflected in enduring changes in susceptibility to seizure evoking-stimuli and changes in
environmentally-evoked behavioral responses (e.g., anxiety-related behaviors), but it
also may produce changes in plasticity itself (i.e., metaplasticity). Kindling-induced
metaplastic effects, in addition to having deleterious effects on functions requiring
plasticity, such as learning and memory, may also have implications for the continued
evolution of the epileptic condition itself. Determining the causes of metaplastic
changes in epilepsy and designing suitable interventions to reverse them may then have
significant implications both for the treatment of mnemonic dysfunction in epilepsy and
the treatment and control of seizure progression as well. The hypotheses deduced from
the characteristics of the kindling-induced disruption of spatial cognition observed here provide a reasonable starting point for this endeavor.
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10. APPENDIX

10.1. Curriculum Vitae

Darren Keith Hannesson

Home Address
1401- 125 5th Ave. N.
Saskatoon, SK
S7K 6A5
(306) 956-3915

Work Address
Room A011
Medical Research Bld.
University of Sask.
Saskatoon, SK
(306) 966-6587

Academic Preparation

1996 – Present
Doctorate of Philosophy
Department of Psychology
University of Saskatchewan

1995
Master of Arts
Department of Psychology
University of Victoria

1992
Bachelor of Arts (Advanced)
Department of Psychology
University of Regina

Dissertation

Title: Characterization of the effects of kindling on spatial cognition: Implications for the mechanisms of kindling-induced mnemonic dysfunction

Awards and Distinctions

2001 NSERC PDF

1998 – 1999 University of Saskatchewan Scholarship

1993 – 1994 Howard E. Petch Research Scholarship
1992 – 1994  NSERC PGS A
1992 – 1993  President's Research Scholarship
1991 – 1992  Scribner Prize in Biology
1991 – 1992  Jesuit Father's Prize
1986 – 1992  General Proficiency Scholarships
1985 – 1986  Liefeld Scholarship in Science
1985 – 1986  Scribner Prize in Biology

Teaching Experience

Instructor
May to July, 2001
Courses: Evolutionary Psychology; Introduction to Biopsychology
Sept to Dec, 1997
Course: Introduction to Biopsychology

Teacher's Assistant
Sept, 1995 to April 1999
Courses: Introduction to Biopsychology, Intermediate Biopsychology,
Advanced Biopsychology, Introductory Statistics, Advanced
Undergraduate Statistics – Univariate, Advanced Undergraduate Statistics
– Multivariate, Graduate Statistics – Univariate, Industrial Psychology,
Cognition and Perception, Human Neuropsychology, Evolutionary
Psychology

Publications

Peer-Reviewed Journal Articles:

Hannesson, D. K., Wallace, A. E., Pollock, M., Corley, S., & Corcoran, M. E.
(submitted). The relation between extent of dorsal hippocampal kindling and DMTP

Anti-cannabinoid withdrawal action of lithium: involvement of oxytocinergic neuronal
activation. The Journal of Neuroscience.


Book Chapters:


Abstracts and Conference Presentations:


