AN EXAMINATION OF KINDLING'S EFFECT ON SPATIAL COGNITION

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Abstract

Kindling involves the progressive development of epileptiform activity that culminates in generalized seizures in response to repeated electrical stimulation of the brain. Kindling induces widespread changes in synaptic sensitivity and neuronal reactivity. These neuroplastic changes are evident in altered memory and behavior.

This research was designed to further our understanding of kindling-induced deficits in spatial cognition. Two questions were examined: 1)does entorhinal cortex kindling disrupt spatial cognition; and 2)can bilateral bifocal kindling, of two brain regions known to participate in spatial cognition, produce larger cognitive deficits than unifocal kindling? This research attempted to confirm the spatial cognitive effects produced by unifocal dorsal hippocampal (dHPC) kindling, as a positive control. In contrast, the spatial cognitive effects produce by unifocal entorhinal cortex (EC) and bifocal kindling (i.e., EC kindling with subsequent contralateral dHPC kindling) are unknown and were examined here. Rats were subjected to unifocal EC kindling, unifocal dHPC kindling, or bifocal kindling. Rats exhibited fully generalized seizures prior to Morris water maze training from days 2 to 31. Visible platform trials were used to examine escape motivation and gross motor coordination, and all groups performed adequately.

Consistent with previous research, dHPC kindling disrupted performance during acquisition trials; however, EC and bifocal kindling failed

to disrupt acquisition. During retention trials, the bifocal kindling group displayed a disruption in performance; however, dHPC and lateral EC kindling failed to affect retention. The bifocal kindled group failed to display larger deficits than the unifocal kindled groups.

These data suggest that the number of kindling stimulations given to a particular site may play a critical role in site-dependent disruption of memory.

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Dedication

I dedicate this dissertation to my parents, Rudolph and Celeste Wolfe. They provided me with an interest in invention, the thirst for knowledge and the believe that in life, anything is possible. Throughout my life they have supported both my ideas and dreams. I will always grateful to them for the life I have. Thank you, Mom and Dad.

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List of Abbreviations

Afterdischarge	AD
Afterdischarge threshold	ADT
Dorsal hippocampus	dHPC
Entorhinal cortex	EC
Hidden Platform	HP
Lateral entorhinal cortex	LEC
Medial entorhinal cortex	MEC
Morris water maze	MWM
Probe	P
Radial arm maze	RAM
Temporal lobe epilepsy	TLE
Visible Platform	VP

1. Introduction

Over thirty-five years ago, the phenomenon of kindling was considered to be a nuisance observed in studies of long-term potentiation (Bliss & Lomo, 1970). Kindling was discovered and named by Graham Goddard (Goddard, McIntyre, & Leech, 1969) who documented the development of seizures through focal electrical stimulation of the brain. While the literature has grown extensively, the mechanisms of kindling remain largely unknown. Simultaneously, kindling has become an useful method for examining characteristics of epilepsy and neural plasticity.

In my thesis I examined kindling's effect on spatial cognition. I intend on furthering our understanding of kindling-induced behavioral aberrations, by utilizing the Morris water maze paradigm to examine kindling's effects on spatially dependent mnemonic processes. These results may be related to temporal lobe epilepsy's effect on mnemonic processes.

1.1 Rationale for studying kindling

In North America epilepsy afflicts 1 in 100 people (Kurland, 1977). The most prevalent form of epilepsy is temporal lobe epilepsy (TLE) (Devinsky & Luciano, 1991). TLE is classified as a complex partial seizure. This type of epilepsy originates as a focal seizure in the temporal lobe and then secondarily generalizes with accompanying tonic/clonic behavioral

convulsions.

The most common rationale for studying kindling is that it exhibits characteristics that are congruent with TLE. At a fundamental level, kindling is documented by the progression of convulsive and electrographic activity. Electrical kindling requires a focal stimulus of a sufficient intensity to evoke electrographic epileptiform activity that is documented in terms of afterdischarge (AD) intensity and duration and the accompanying convulsive response.

Similarly, correlative evidence supports the assumption that kindling models TLE. "Model" refers to an induced phenomenon that displays properties similar to a phenomenon observed in nature, rather than a scaled replica of an object. There are several lines of evidence validating kindling as a model of TLE. First, drugs produce similar effects in kindled convulsions and complex partial seizures in humans (Adamec, 1990; Loscher, Jackel, & Czuczwar, 1986). Second, similar patterns of hippocampal sclerosis and mossy fiber sprouting can be observed in extended kindling and TLE (Cavazos, Das, & Sutula, 1994; Cavazos, Golarai, & Sutula, 1991; Kotloski, Lynch, Lauersdorf, & Sutula, 2002; Sutula, Lauersdorf, Lynch, Jurgella, & Woodard, 1995; Swanson, 1995). Third, recurrent spontaneous motor seizures can be produced by extensive kindling, a defining feature of the clinical epilepsies (Pinel & Rovner, 1978; Wada, Sato, & Corcoran, 1974). These similarities to epilepsy provide the foundation for the use of kindling for studying focal epilepsies

(Engel, 1998; Sato, Racine, & McIntyre, 1990).

An additional reason for studying kindling is that the procedure induces a multitude of neuroplastic changes, which are evident in the propagation of AD activity, localized reduction of the AD threshold (ADT), and kindling transfer. These changes in neuronal plasticity can be induced in the adult mammalian brain without evidence of any overt neuronal damage (Kotloski et al., 2002), providing support for using kindling as a general technique for studying neural plasticity (Goddard et al., 1969).

1.2 Kindling and kindling characteristics

1.2.1 Definition

Kindling is a procedure by which the repeated administration of a weak sub-convulsive electrical stimulus to focal brain sites via chronically indwelling electrode induces a progressive enhancement in the responsiveness of the brain to the stimulation. The induction of AD produces a gradual decline in ADT that is exclusive to site the of stimulation (Racine, 1972a). As kindling progresses, the evoked AD reliably grows in intensity, duration, complexity and propagation (Racine, 1972a). The propagation of AD to other neural regions is correlated with the progression in seizure severity of behavioral seizures. Racine developed a 6 point scale to characterize development of the behavioral seizures. Stage 0 is characterized by little or no behavioral change but may consist of increased exploratory behavior; Stage 1 is indicated by immobility and rhythmatic mastication; Stage 2 is indicated by rhythmatic

head clonus; Stage 3 is indicated by unilateral forelimb clonus contralateral to the site of stimulation; Stage 4 is indicated by bilateral forelimb clonus; Stage 5 is indicated by hindlimb clonus associated with rearing and falling. Extended kindling will progressively enhance the severity of seizures; behavioral correlates have been classified up to a 9 point scale (Pinel & Rovner, 1978). These scales were developed for electrical kindling and are representative for the kindling of limbic regions.

1.2.2 Stimulation parameters, intensity and rate

Kindling is effective with a variety of stimulation patterns and durations. Typically, the stimulation used is a 1 sec train of balanced biphasic square wave pulses at 60 pulses/sec. However, biphasic sine wave stimulation, trains of 60 seconds and frequencies ranging from 25 to 150 pulses/sec can utilized for kindling (Goddard et al., 1969). In some cases a stimulation frequency of 3 pulses/sec has been effective in kindling (Corcoran & Cain, 1980).

The intensity required to induce kindling is site-dependent, ranging from 10 **:**A to 3000 **:**A (personal observation). For example, the ADT range for the dorsal hippocampus (dHPC) is 20-80 **:**A, whereas the ADT range for the piriform cortex is 300-600 **:**A (personal observations). However, a suprathreshold stimulation is required for kindling of convulsive seizures. Subthreshold stimulation intensity is insufficient to kindle or to decrease the number of stimulations required to kindle a generalized seizure. However,

similar to suprathreshold stimulations, subthreshold stimulations will reduce the ADT at the site of stimulation (Racine, 1972a).

Another site-dependent characteristic of kindling is the kindling rate. The kindling rate (or the number of stimulations required to induce a generalized seizure) is quite variable between regions. For example, the dHPC requires 45-60 stimulations to elicit a generalized or stage 5 seizure, whereas the entorhinal cortex (EC) requires 15-30 stimulations and the piriform cortex requires 5-10 stimulations (Racine, 1972b).

1.2.3 Cortical versus limbic kindling

A general dichotomy exists in the kindling pattern of cortical and limbic regions. The limbic kindling pattern is evident in stimulation of the amygdala, dHPC, and olfactory bulb. These sites exhibit relatively low ADTs with progressive AD durations. Initially no behavioral manifestations occur with kindling; however, as limbic kindling continues the appearance and degree of accompanying convulsions progress in a predictable sequence culminating in a generalized seizure. The pattern of cortical kindling, evident in stimulation of the claustrum, motor cortex, and anterior neocortex, is quite different from kindling of limbic structures. Cortical kindling is associated with high ADTs with short AD durations that remain relatively constant throughout kindling. Behavioral convulsions occur early in kindling, often coinciding with the stimulation train. Despite these contrasts, if cortical kindling is continued the seizure will progress into a state that resembles limbic-type seizures (i.e.,

extended AD durations, and limbic clonic-tonic convulsions) (Racine, 1975; Seidel & Corcoran, 1986).

1.2.4 Kindling transfer

Kindling involves extensive reorganization of neural circuits, proximal and distal to the site of stimulation. Thus, kindling facilitates neuronal excitability and synaptic transmission throughout most regions of the brain. The "transfer effect" provides evidence of propagated neuronal hyperexcitability, in which the kindling at one site (primary) enhances the seizure susceptibility of other sites (secondary). Compared to primary site kindling, the secondary site requires significantly fewer kindling sessions to elicit a motor seizure. For example, primary amygdaloid kindling requires a mean of 10.6 stimulations; however, subsequent to dHPC kindling, only 1.8 stimulations of the amygdala are required to induce a fully generalized seizure (Burnham, 1975). This represents an 84% savings. In addition, lesion studies indicate that the "transfer effect" does not depend on the integrity of the primary structure. Racine (Racine, 1972b) lesioned the primary site prior to secondary site kindling and observed transfer rates similar to secondary site kindling with an intact primary site. These results suggest that kindling alters neuronal excitability outside the area stimulated.

1.3 Rationale for studying the consequences of kindling

The most compelling reason for studying the effects of kindling on memory function is their potential resemblance to the memory dysfunction

frequently observed in and self-reported by patients with epilepsy. Research suggests that epilepsy and memory are intimately associated. The frequency of memory deficits is significantly greater in epileptic patients than in other comparable populations (Pedersen & Dam, 1986), and aberrations in memory are most commonly observed in patients with complex partial seizures (Delaney, Rosen, Mattson , & Novelly, 1980; Milner, 1975). Disturbances in verbal memory are most frequently observed (Dupont et al., 2000; Giovagnoli & Giuliano, 1999); however, aberrations in visuospatial memory and motor coordination often accompany verbal memory deficits (Breier, Plenger, Castillo et al., 1996; Delaney et al., 1980; Prevey, Delaney, Cramer, Mattson, & Group, 1998).

Clinical studies suggest that patients with epilepsy are conscious of memory problems. Epileptic patients more frequently complain of memory deficits and demonstrate increased scores on subjective rating of memory versus a comparable population (Hendriks, Aldenkamp, Van dre Vlught, Alpherts, & Vermeulen, 2002; Thompson & Corcoran, 1992). In addition, the perception of memory loss can precede or occur simultaneously with the onset of complex partial seizures or cryptogenic seizures (Gallassi, Morreale, Lorusso, Pazzaglia, & Lugaresi, 1988; Kalviainen, Aikia, Helkala, Mervaala, & Riekkinen, 1992). However, these self-rated memory deficits are positively correlated with the interval since seizure onset (Hendriks et al., 2002) and often underestimated the frequency, severity, and extent of the memory

disruptions (Giovagnoli, Mascheroni, & Avanzini, 1997; Thompson & Corcoran, 1992). Furthermore, measures concerning the quality of life are inversely correlated with epilepsy (Baker, Jacoby, Buck, Stalgis, & Monnet; Thompson & Corcoran, 1992).

The mechanisms underlying the memory dysfunction observed in epilepsy remain unknown. It is likely that damage to structures involved in memory provide some contribution to the deficit. Commonly, epilepsy is accompanied by hippocampal sclerosis, which is defined as neuronal loss and gliosis of the hippocampus. The damage may extend to other regions including the EC, lateral temporal cortex, and regions beyond the hippocampal formation (DeCarli, Hatta, Fazilat, Gaillard, & Theodore, 1998; Lee et al., 1998). However, inconsistent results have been reported when mnemonic function is exmained in TLE patients with hippocampal sclerosis as compared to TLE patients without hippocampal sclerosis. Hippocampal sclerosis has been observed to increase mnemonic dysfunction (Breier, Plenger, Wheless et al., 1996; Hermann, Seidenberg, Schoenfeld, & Davis, 1997) or to fail to alter the magnitude of mnemonic dysfunction (Giovagnoli et al., 1997). However, the brain activation patterns of TLE patients with hippocampal sclerosis differ from controls when performing verbal memory encoding and retrieval (Dupont et al., 2000). This study was limited by the exclusion of TLE patients without hippocampal sclerosis. Thus, the results are ambiguous and either the neuronal damage or the TLE could be responsible

for the altered activation patterns.

In clinical epilepsy research, controlling lesion placement, size, and cause is difficult. However, lesions can be avoided using animal models. Seizures in animals can be fully kindled without inducing overt brain damage (Kotloski et al., 2002) and yet fully kindled animals exhibit mnemonic and behavioral aberrations (Boast & McIntyre, 1977; Hannesson & Corcoran, 2000; Peele & Gilbert, 1992). Thus, brain damage is probably not responsible for the disruption in behavior; rather, evidence suggests that the altered neuronal plasticity is the critical factor.

Mnemonic consequences of epilepsy are difficult to study in a clinical setting due to individual patient confounds. Two forms of confound are present in clinical studies of epilepsy: seizure characteristics and individual's characteristics. Seizure characteristics consist of: seizure severity, frequency, intensity, duration, focus, age of onset, and the interval between seizure and testing. Confounds of the individual's characteristics consist of: drug history, presence of a precipitating event, underlying or associated pathology, baseline levels of functioning, and psychosocial impact of epilepsy. Kindling largely eliminates the confounds, while enabling the manipulation of the seizure characteristics including: seizure focus, severity, duration, age of onset, drug history, and the interval between seizure and testing. This degree of control validates kindling as an excellent preparation for the study of epilepsy and in particular epileptogensis (Engel, 1998; Sato et al., 1990).

1.4 Behavioral consequences of kindling

Mnemonic dysfunction is highly associated with epilepsy. Similarly, kindling has consistently induce aberrations in mnemonic function. To facilitate the discussion of kindling-induced mnemonic deficits, I will separately consider tasks that require allocentric cues (spatial tasks) and tasks that do not require allocentric cues (non-spatial tasks). When investigating the effects of kindling, parameters of the extent of kindling, site of stimulation, and behavioral task are critical considerations (Hannesson & Corcoran 2000). The extent of kindling can be generalized into three degrees: "partial kindling" including the first AD, non-convulsive seizures, and hemiconvulsions; "full kindling" including 1 fully generalized convulsion to 30 fully generalized convulsions; and "extended kindling" including a minimum of 30 fully generalized.

1.4.1 Non-spatial tasks

A number of studies have documented that amygdaloid kindling impairs performance on aversively motivated tasks. This research has utilized multiple versions of conditioning tasks that require the formation of an association between sensory stimuli and an aversive event, and thus emotional cognition is require for optimal performance.

Amygdaloid kindling has been consistently shown to disrupt aversive conditioning (Boast & McIntyre, 1977; McIntyre & Molino, 1972; Peele & Gilbert, 1992; Stone & Gold, 1988). The initial studies investigated the

effects on aversive conditioning when preceded by full amygdaloid kindling. McIntyre and Molino (1972) first observed that unilateral amygdaloid kindling, in conjunction with a contralateral amygdaloid lesion, impaired the acquisition of a conditioned response. Subsequently, Boast and McIntyre (1977) observed that bilateral amygdaloid kindling was effective in disrupting passive avoidance. Peele and Gilbert (1992) replicated these results, while Stone and Gold (1988) extended the results documenting that unilateral amygdaloid kindling was effective in disrupting passive avoidance. Similarly, full amygdaloid kindling impairs the retention of a shock-motivated brightness discrimination in the Y-maze (Becker & Grecksch, 1992; Becker et al., 1992). Amygdaloid kindling disrupts the retention but not the acquisition of a shock-motivated active avoidance task, suggesting that general emotional memory is intact (Hannesson & Corcoran, 2000).

Amygdaloid kindling has been observed to affect measures of anxiety-like behaviors. Amygdaloid kindled rats display reduced exploration time and episodes of entry into the open arms of the elevated-plus maze, suggesting an anxiogenic effect (Adamec & Shallow, 2000; Helfer, Deransart, Marescaux, & Depaulis, 1996; Nieminen et al., 1992). Adamec and Shallow (2000) suggested that the anxiogenic effects are dependent on the site kindled within the amygdala. They observed an anxiogenic effect after full kindling the posterior central nucleus of the amygdala, no effect after kindling of the medial central nucleus, and an anxiolytic effect after kindling the anterior central nucleus.

However, these results have yet to be replicated. Conversely, dHPC kindling and perirhinal cortex kindling failed to alter anxiety-like behavior (Darren Keith Hannesson, 2001). These data suggest that the nature of kindling-induced impairments in emotionality and emotional memory may relate specifically to the kindling site and the neuronal processing demands of the region.

1.4.2 Spatial tasks

Primarily two tasks have been utilized to assess performance of spatial cognition: the radial arm maze (RAM) and the Morris water maze (MWM). In a typical RAM, appetitive rewards are positioned distal to the center region in each of the eight radiating arms. Optimal performance requires that an animal visit each arm once, retrieving bait from all eight arms without revisiting arms. The arms of a RAM are undifferentiated by intra-maze clues, causing performance to be dependent on the differentiation of extra-maze spatial clues.

The typical protocol of the RAM task examines a short term memory store termed working memory. Working memory contains information concerning which arms have been visited during each trial. Typically, every arm possesses bait, thus minimizing the demands on reference memory, information that remains constant during and between trials. However, the RAM can be utilized to examine the performance of reference memory by baiting a fraction of the arms. This protocol requires the animal to

differentiate between baited arms and visited arms.

The MWM is a circular pool with undifferentiated walls filled with cool opaque water obscuring the location of a submerged escape platform. Rats must locate the submerged platform utilizing extra-maze spatial clues due the absence of reliable intra-maze. Contrary to the RAM, the typical MWM protocol is dependent on reference memory since the platform location remains in a constant location both during and between trials. However, the MWM protocol can be manipulated to examine working memory by relocating the escape platform in different sessions.

1.4.2.1 Radial-arm maze

Initial research concentrated on the effect of dHPC kindling, specifically the CA1 field, on spatial cognitive performance in the RAM. Lopes da Silva and colleagues (Lopes da Silva, Gorter, & Wadman, 1986) first investigated the effects of full dHPC kindling on the performance of working/reference memory in the RAM. They demonstrated an impairment in working memory and reference memory while testing concurrently with kindling but an impairment only in reference memory after the completion of kindling. Leung and colleagues extended these results, demonstrating that either full or partial dHPC kindling impaired working memory performance in the standard RAM task (Leung, Boon, Kaibara, & Innis, 1990; Leung, Brzozowski, & Shen, 1996; Leung & Shen, 1991; Leung, Zhao, & Shen, 1994). Feasey-Truger and colleges (Feasey-Truger, Kargl , & ten Bruggencate, 1993)

examined the effects of full kindling of the dentate gyrus, demonstrating a disruption in reference memory but not working memory in the RAM. These data suggest that reference memory may be more susceptible to kindling-induced disruptions than working memory in the RAM task (Hannesson &Corcoran, 2000).

It is unclear in the research discussed above whether kindling produces retrograde or anterograde disruption of performance. Because RAM training precedes kindling, kindling may have disrupted memory for previously learned locations (retrograde amnesia) or acquired memory during testing trials (anterograde amnesia). However, to investigate anterograde mnemonic disruptions produced by kindling, several studies have reversed the protocol by establishing kindling prior to RAM training. Sutula and colleagues (1995), provided evidence of anterograde amnesia induced by extended olfactory bulb kindling, by kindling 1 month prior to RAM training. Although performance by extended kindled rats was disrupted, there were no impairments after either partial or full kindling. Utilizing similar paradigms, full amygdaloid kindling and unilateral or bilateral perforant path kindling failed to disrupt RAM acquisition (Letty, Lerner-Natoli, & Rondouin, 1995; Robinson, McNeill, & Reed, 1993). These results suggest that the extent of kindling and the site of stimulation are critical for the anterograde disruption of RAM performance. Considering results obtained in the MWM (see below), it is possible that full dHPC kindling would disrupt subsequent acquisition of the RAM task.

1.4.2.2 Morris water maze

When kindling is established before MWM training, deficits induced by full kindling appear to be regionally specific, but extended kindling can reduce the regional specificity of the deficits. Gilbert and colleagues (Gilbert, McNamara, & Corcoran, 1996) demonstrated that full but not partial kindling of the dHPC impaired acquisition in the MWM. These results were replicated and extended by Hannesson and colleagues (2001), who observed an impairment in MWM acquisition following full dHPC kindling. Conversely, full amygdaloid, lateral septal, ventral hippocampal, or perforant path kindling failed to disrupt subsequent acquisition in the MWM (G. Holmes et al., 1993; McNamara, Kirkby, dePape, & Corcoran, 1992; McNamara, Kirkby, dePape, Skelton, & Corcoran, 1993; Nieminen et al., 1992). However, extended amygdaloid or perforant path kindling disrupted acquisition in the MWM (Cammisuli et al., 1997). These results suggest that the extent of kindling and the kindling site are critical in producing deficits in spatial cognition.

A reduction of the site-specific mnemonic deficits has been described in studies where training precedes kindling or is performed concurrently. When MWM training precedes kindling, performance is disrupted in both partial and fully dHPC kindled rats (Gilbert, Hannesson, & Corcoran, 2000; Gilbert et al., 1996). Similarly the induction of kindled seizures in the perforant path, amygdala, and septum either prior to or immediately following daily training

sessions was sufficient to disrupt performance (McNamara et al., 1992).

Hannesson (2001) suggested that these findings demonstrate three features of kindling's effect on spatial cognition. First, the disruption in cognition produced by kindling is regionally specific, with the dHPC being especially sensitive. Second, the extent of kindling is critical in producing anterograde deficits, with extended and full kindling being effective but partial kindling being ineffective. Finally, these data demonstrate a difference in the sensitivities of anterograde and retrograde effects to the disruptive effects of kindling, with retrograde effects being more sensitive to less extensive degrees of kindling.

1.5 Unanswered questions concerning kindling-induced behavioral deficits

The anterograde deficits produced by full kindling appear to be regionally specific. The deficits closely correspond to the function of the structure that have been suggested by lesion and activation studies. Hence, similar effects on inhibitory avoidance behavior are produced by amygdaloid lesions and amygdaloid kindling (Boast & McIntyre, 1977). Similarly, both full dHPC kindling and dHPC lesions impair spatial memory (Compton, Griffith, McDaniel, Foster, & Davis, 1997; Good & Honey, 1997; Steffenach, Sloviter, Moser, & Moser, 2002).

To date, the behavioral and mnemonic effects of EC kindling have not been studied. The EC has reciprocal monosynaptic connections with the dentate gyrus, CA1 region of the hippocampus, and cortical regions (for

review of EC synaptic connections and lesion studies, see Schwarcz & Witter, 2002). The EC has been implicated in the retention of spatial information in activation and lesion studies (Glasier, Janis, Roof, & Stein, 1999; Good & Honey, 1997; Oswald & Good, 2000), yet lesions of the EC spare acquisition in both rats (Bannerman et al., 2001), and monkeys (Suzuki, Miller, & Desimone, 1997; Sybirska, Davachi, & Goldman-Rakic, 2000). Thus, full EC kindling should theoretically induce deficits in retention of spatial tasks.

It is possible that cognitive and behavioral impairments would be greater following multifocal kindling (i.e., kindling of multiple sites), especially if the two regions play similar roles in cognition and behavior. Even though memory and behavior have not been examined in patients with multifocal epilepsy, several measures indicate that multifocal epilepsy is more severe than unifocal epilepsy including: mortality rates, psychomotor retardation, surgical outcome, and association with other cerebral diseases (M. D. Holmes et al., 2000; Ishii et al., 2002; Lawn, Westmoreland, & Sharbrough, 2000; Sahin, Menache, Holmes, & Riviello, 2001; Van Lierde, Van Paesschen, Dupont, Maes, & Sciot, 2003).

1.6 Research direction

My research has three main goals: 1)to investigate the mnemonic effects of full EC kindling on spatial cognition; 2)to replicate the deficit in spatial cognition induced by full dHPC kindling; and 3)examine the mnemonic effects of bifocal kindling on spatial cognition.

1.6.1 Experiment 1

In experiment 1, I investigated the effects of full EC kindling on subsequent performance on a control task, and acquisition and retention in the MWM. Due the EC's function in the retention of spatial information, I predicted that EC kindling would disrupt retention but not acquisition of spatial information.

1.6.2 Experiment 2

In experiment 2, I investigated the effects of full dHPC and full bifocal (EC and dHPC) kindling on subsequent performance on a control task, and acquisition and retention in the MWM. This study replicates previous demonstrations of dHPC kindling's effect on spatial cognition, as a positive control, and extends it by examining the mnemonic effects of bifocal kindling. Included in the study were comparisons of bifocal and unifocal kindled groups. The two sites chosen were the dHPC and the EC due to their involvement in spatial cognition.

2. Methods

Due to the similar procedure used in experiments 1 and 2, the methods will be combined for description. Male Long-Evans hooded rats weighing 225-250 g were housed in groups and handled daily for one week prior to any manipulation. All experimental manipulations occurred during the light phase of the 12:12 hour light/dark cycle. After surgery rats were housed individually with food and water available ad libitum.

2.1 Surgery

Rats were anaesthetized with Isoflurane™ and given a subcutaneous injection of Anafen™ (1 cc/kg) as a postsurgical analgesic. The rats were placed in a stereotaxic apparatus, with skull level. Bipolar electrodes with a tip separation of 0.4 to 0.5 mm were constructed of enamel-insulated nichrome wire (127 m dia) and female amphenol pins. Electrodes were implanted in the left EC and contralateral dHPC using the following coordinates relative to bregma: EC, -7.8 mm (AP), 5 mm (ML), -6.5 mm (DV); and dHPC, -3.5 mm (AP), 2.6 mm (ML), -3.1 mm (DV). The amphenol pins were inserted into a plastic 9-pin pedestal, and the electrode assembly was secured to the skull by four jewelers screws and dental acrylic. One jeweler screw served as the ground reference electrode and was positioned over the anterior cortex. The surgery was completed by the application of a topical

antibiotic/steroid (Topagen™) to the wound.

2.2 Kindling

Kindling was initiated after a postsurgical recovery period of 7 to 10 days. Rats were randomly assigned to one of four groups: EC kindled, dHPC kindled, bifocal kindled (EC and dHPC), and control. During the initial kindling session the minimum intensity of stimulation sufficient to evoke an AD was determined. Electrical stimulation consisting of a 1 sec train of balanced biphasic square pulses at 60 pps was supplied by a Grass S8800 stimulator with an initial threshold stimulation intensity of 100:A for the EC and 10:A for the dHPC. If the intensity was insufficient to induce five sec of AD, stimulation was applied successively at a 1-min interval. The intensity was increased in increments of 100:A for EC and 10:A for the dHPC until AD was evoked. The minimum effective intensity was arbitrarily defined as the ADT. Seizures were kindled with stimulation applied at ADT. Control rats were age-matched, received implantation of electrodes, and were connected to the kindling lead and placed in the kindling box daily, but did not receive electrical stimulation.

2.2.1 Unifocal kindling

Unifocal kindling groups received stimulation applied to a single site, either the EC or the dHPC. The criterion for completion of kindling was 5 fully generalized stage 5 seizures (Racine, 1972b).

2.2.2 Bifocal Kindling

The bifocal kindling group received stimulation applied first to the EC until the criterion for kindling was met, and then to the dHPC. At the beginning of kindling, the ADT was determined for the EC; on the subsequent day the ADT for the dHPC was determined. Kindling stimulation was applied once daily to the EC until the criterion was met, stimulation was suspended for 7 days, and then stimulation was applied once daily to the dHPC to criterion. During the suspension of stimulation, rats were connected to the kindling lead and placed in the kindling box daily without electrical stimulation. Because the criterion for full kindling was induction of 5 fully generalized seizures at each site, the bifocal kindled rats experienced 10 stage 5 seizures prior to behavioral testing.

2.3 Water maze

A rectangular room housed the water maze pool distal to the door, and a computer was located proximal to the door. A video camera was secured to the ceiling above the center of the pool. The walls was adorned with multiple posters as external spatial cues. The room was illuminated by four halogen lamps orientated towards the ceiling creating a square around the pool, and constant background noise was provided by soft music.

The water maze was constructed of a white industrial plastic with a diameter of 200 cm and walls 45cm in height. The pool was filled with water 26 cm deep, $26^{oC} \pm 1^{oC}$ in temperature, and rendered opaque with polyethylene pellets measuring 1 x 2 x 2 mm. During hidden platform (HP)

trials a clear plexiglass platform measuring 23 cm in height supporting a white grid platform measuring 10 cm by 12 cm was secured in the NE quadrant of the pool. It was submerged 3 cm below the surface of the water. For visible platform (VP) trials, a black wooden block covered with a metal grid was secured to the platform and extruded 3 cm above the water.

Rats' swim paths were monitored by a computerized video-tracking system supplemented by a VCR as a backup. Ethovision™ was employed to analyze distance swum, number of quadrant entries, and dwell time in individual trials.

2.4 Water maze procedures

On each trial the rat was gently placed into the water facing the exterior pool wall at one of three pseudorandomly chosen locations: Southwest, Northwest, or Southeast. Each rat was allowed to swim until finding the platform or until 60 sec elapsed, at which point it was gently guided to the platform. The rat was allowed to perch on the platform for 10 sec and then was removed to a holding pen situated proximal to a 250W red heating lamp. Inter-trial interval was maintained between 2 and 4 min.

VP training commenced two days after the final kindling stimulation. The training consisted of 6 trials in which the platform was pseudorandomly placed in four locations: North, South, West, or East. Each location was equidistant from the edge of the pool and unique to the platform placement during HP trials. The VP trials were intended to reduce stress and thigmotaxic

behaviors, while providing a measure of motivation and motor coordination. VP performance is known to be independent of spatial cognition, and it is insensitive to hippocampal damage (Hannesson & Corcoran, 2000).

On post-kindling day 3, rats were subjected to 19 trials, 18 HP and 1 probe. During the 19 trials, rats were released from three psuedorandom start locations. HP trials involved the rats searching for a submerged escape platform located in the northeast quadrant. After completion of 15 consecutive HP trials, a probe trial was introduced, in which the platform was removed and the rat was allowed to swim for 60 seconds. The probe trial provides a measure of the rats' memory for the location of the HP uncontaminated by escape on to the HP. After the probe trial, the rat was subjected to three additional HP trials to ensure full training.

Retention of memory was assessed on days 10 and 31 after kindling. Each day the rat was subjected to 4 trials, 3 HP trials followed by 1 probe trial. Starting locations for each trial were pseudorandomly organized, with a differing sequence each day. The behavioral testing paradigm is summarized in Table 2.1.

2.5 Histology

Following the completion of behavioral testing animals were sacrificed by CO_2 gas inhalation. Brains were fixed in 4% paraformaldehyde and transferred to a 30% sucrose solution prior to sectioning. Frozen 40:A sections were taken through the dHPC and the EC. Every section through the

electrode track was mounted and stained with cresyl violet. Electrode placements were determined by comparing sections to plates from Paxinos and Watson (1997).

Post-Kindling	Day 2	Day 3	Day 10	Day 31
	Pre-training	Acquisition	7 Day Retention	28 Day Retention
Trials	6 VP	15HP, 1P, 3HP	3HP, 1P	3HP, 1P

Table 2.1 Behavioral testing procedure schedule.

2.6 Data Analysis

SPSS for Windows was used to analyze the data. Distance, latency and direct swim data for acquisition and retention trials were subjected to analyses with the Kruskal-Wallis non-parametric test for main effects, supplemented by the Mann-Whitney non-parametric test for simple effects. Non-parametric analyses were performed due to violations in the assumption of homogeneity of variance. However, for examination of chance performance during probe trials t-tests were used.

3. Results

3.1 Experiment 1

3.1.1 Histology

The placements of EC electrodes in the left hemisphere of the EC kindled group were bimodal, between the lateral EC (LEC) and medial EC (MEC) (see Figure 3.1). A significant difference in ADT was observed between LEC and MEC groups; thus two groups were formed, LEC and MEC kindled rats. The contralateral electrodes for the LEC and MEC groups were located in or proximal to the CA1 region of the dHPC. The number of rats in each group was as follows: LEC = 6, MEC = 5, and control = 11.

3.1.2 Kindling

The MEC possessed a significantly higher ADT than the LEC ($U = 0.5$, $z = -1.66$, $p<0.01$). The mean ADT in the MEC was of 1760 ± 413 :A, and 23.2 ± 3.9 stimulations were required to kindle to 5 generalized seizures; whereas the mean ADT in the LEC was 450 ± 67 :A and 28.8 ± 5.1 stimulations were required to kindled to 5 generalized seizures. The difference in rate kindling was not significant. Lesions were not observed in either area.

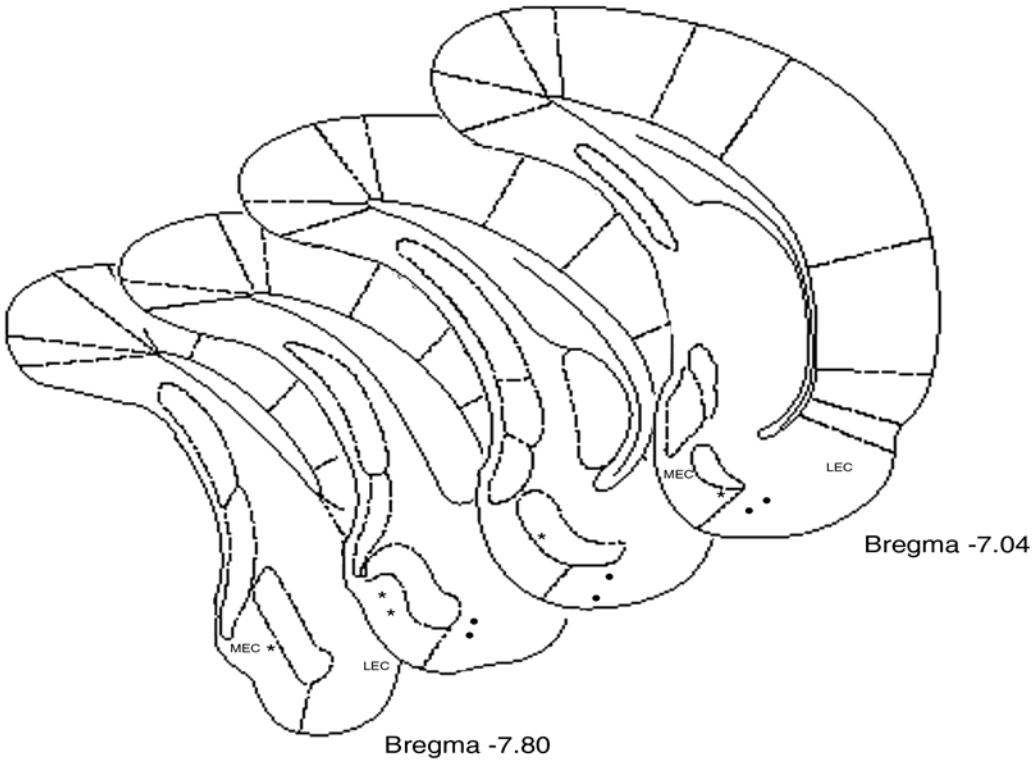

Fig. 3.1 Location of the lower tip of the stimulation electrode in EC kindled rats. Plates are posterior to bregma and were adapted from Paxinos and Watson (1997). *=MEC electrode placement , •=LEC electrode placement (dHPC electrode placements not shown)

3.1.3 MWM

3.1.3.1 Visible Platform trials

All groups performed equally well on VP trials (H^2=5.71, p>0.05) (see Figure 3.2). This measure provides evidence that deficits on HP trials are not due to disruption in motor coordination or motivation.

3.1.3.2 Acquisition trials

For acquisition trials with HP, a significant main effect on the sixth grouping (T_{18}) of trials (H^2=6.50, p<0.05)(see Figure 3.3). Analysis of the simple effects revealed a significantly impaired performance by the MEC kindled group, which exhibited greater swim distances to the HP as compared to the control group (T_{18} U=21.00, z=-2.59, p<0.01). Inspection of data from individual rats indicated that 2 of the 5 rats in the MEC group completely failed to learn the location of the HP. Figure 3.4 shows the data from the 2 rats that failed to learn, compared to data from the other 3 rats in the MEC group and the control group.

Analysis of direct swims failed to reveal any impairments (H^2=3.46, p>0.05). Direct swims are defined as a swim path that is within an alleyway 12 inches wide and extending from the point of release to the platform.

3.1.3.3 Retention trials

A significant main effect was observed on the retention trials on day 10 but not day 31 (D_{10} H^2=8.56, p<0.02; D_{31} H^2=3.48, p>0.05)(see Figure 3.3).

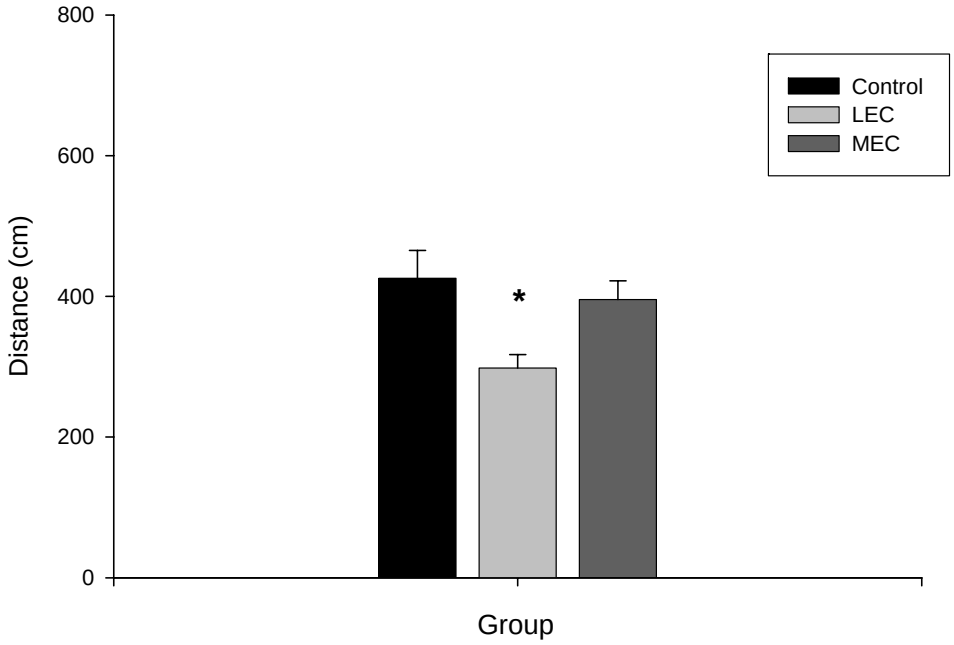

Fig. 3.2. Mean escape distances on 6 VP trials.

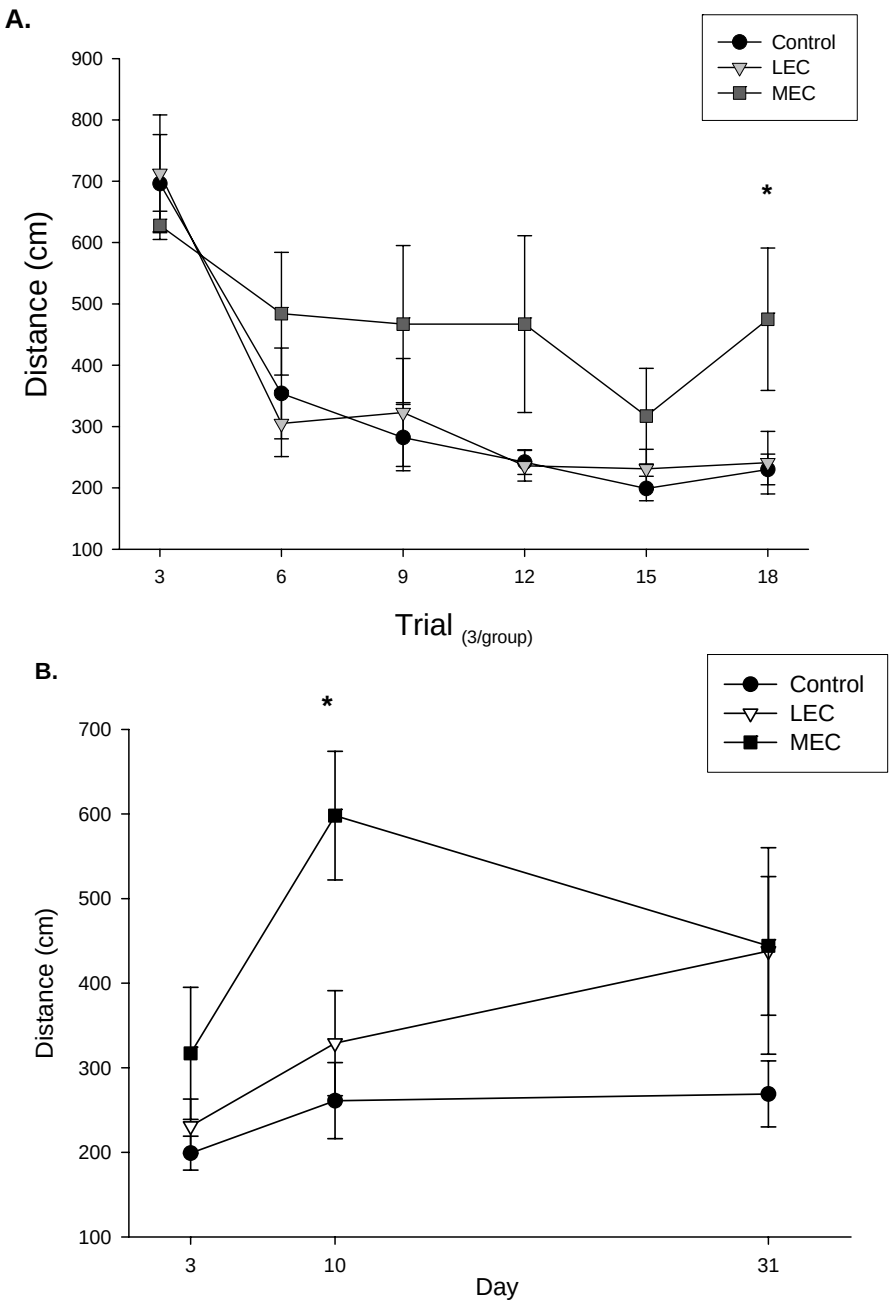

Fig. 3.3 MWM performance on HP trials. **A.** Mean escape distances on acquisition trails. **B.** Mean escape distances on retention trials.
***** Denotes MEC kindled group is significantly different from LEC kindled and Control groups, $p<0.05$. (3 trials/block)

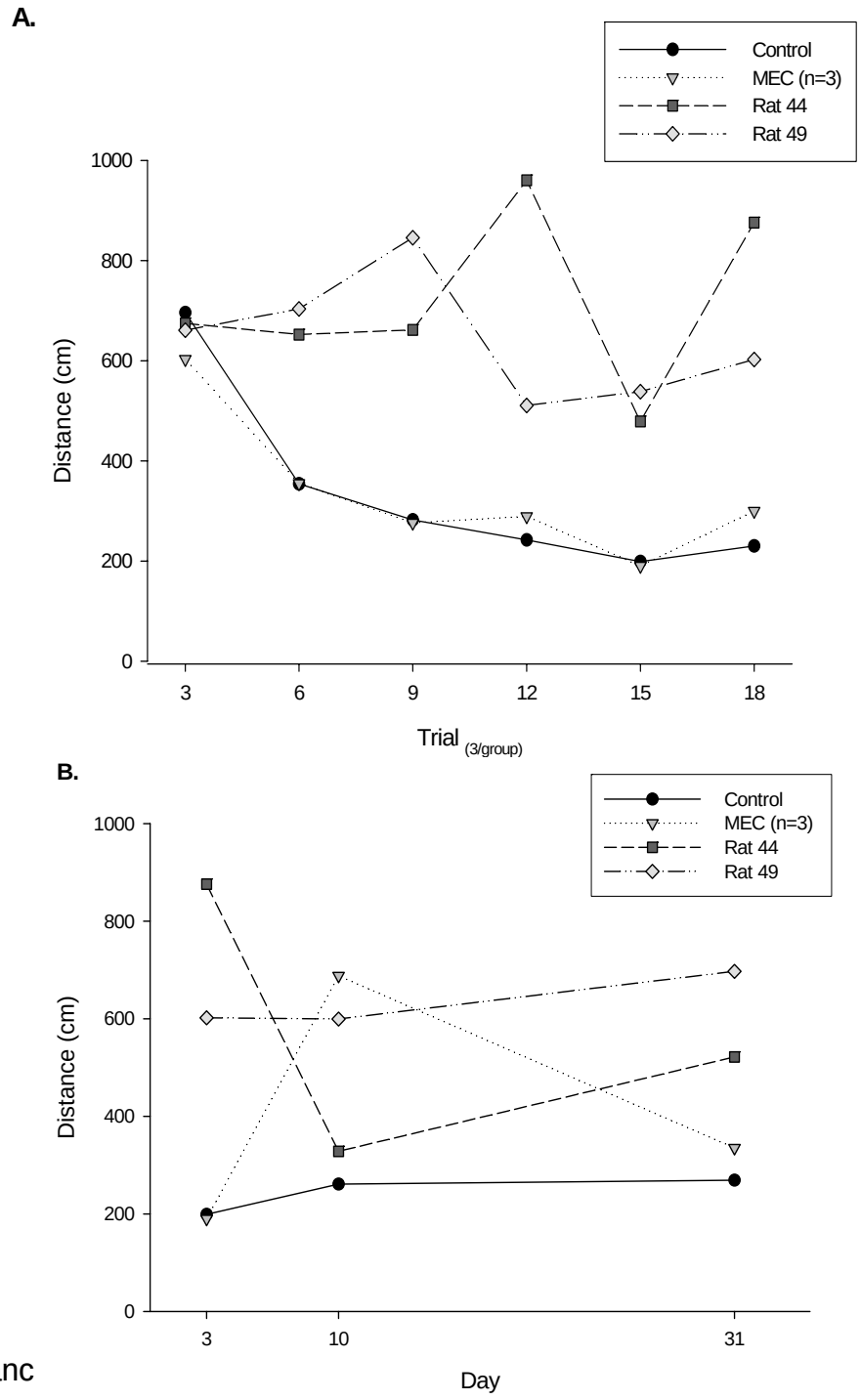

Fig 3.4 Performance of MEC kindled rats on HP trials. **A.** Mean escape distances on acquisition trails. **B.** Mean escape distances on retention trials. MEC (n=3) group acquired the location of the HP, whereas Rat 44 and Rat 49 failed to acquire the location of the HP.

Analysis of the simple main effects revealed that the MEC kindled group required a significantly greater distance to locate the HP during retention trials on day 10 as compared to the control group (D_{10} U=4.00, z=-2.66, p<0.01). Similarly, the MEC kindled group displayed a performance deficit on day 10 retention trials as compared to the LEC kindled group (D_{10} U=2.00, z=-2.37, p<0.02), but the difference was non-significant on day 31(D_{31} U=13.00, z=-0.365, p>0.05). Inspection of data from the 2 rats from the MEC group that failed to learn showed that these rats displayed deficits in retention on both days 10 and 31 (Figure 3.4). It is noteworthy that the other 3 rats that performed at control levels in acquisition also showed a deficit in retention on day 10, but not on day 31.

3.1.3.4 Probe trials

On all probe trials LEC kindled and control rats performed better than at chance levels (t(5)$4.9, p#0.004; t(9)$4.41, p#0.001, respectively). However, MEC kindled rats performed better than at chance levels only on day 31 (t(4)=8.2, p=0.01)(see Figure 3.5). A main effect was observed on day 10 probe (H^2=12.12, p=0.016). Analysis of the simple effects revealed that the control group spent a significantly greater time spent in the platform quadrant as compared to the MEC group on the day 10 probe trial (U=6.00, z=-2.44, p=0.013). Thus the MEC group was exhibiting poorer memory than controls on day 10.

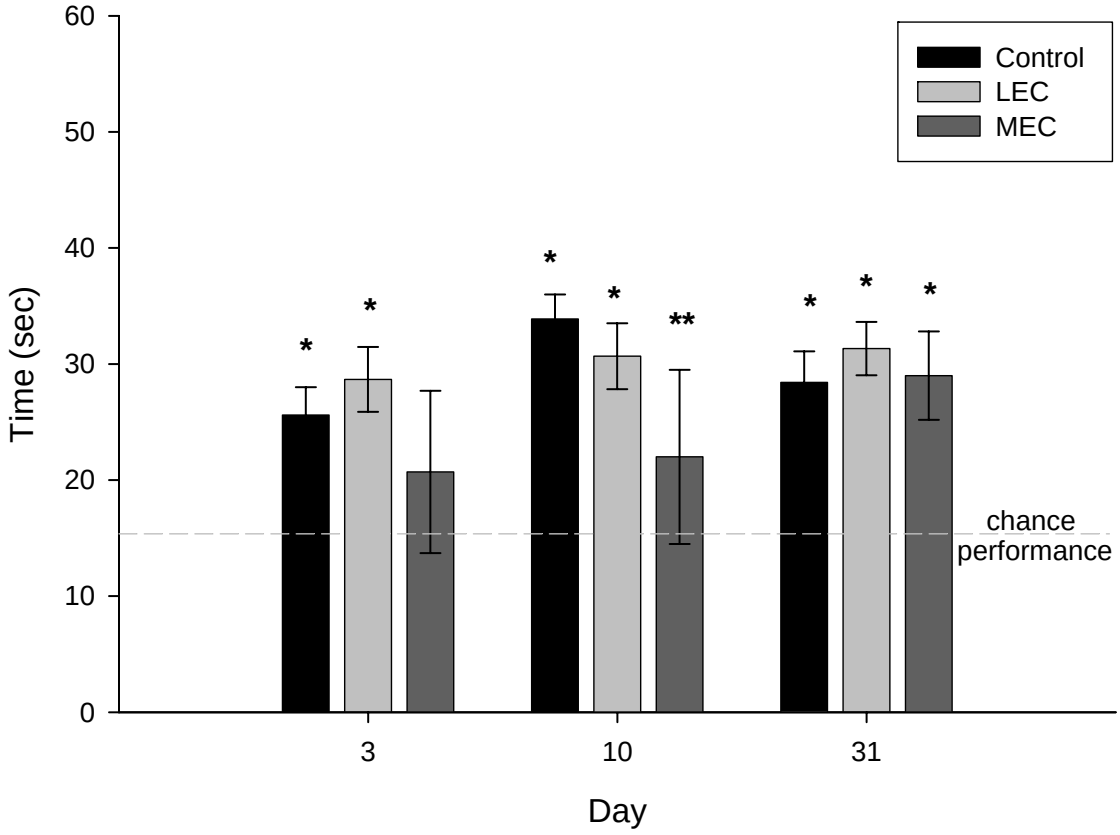

Fig. 3.5 Dwell time in platform quadrant on probe trials. * Denotes significantly different from chance performance, $p<0.05$. ** Denotes significantly different from control, $p<0.02$.

3.2 Experiment 2

3.2.1 Histology

For electrode placements for LEC and control groups, refer to Experiment 1. The right hemisphere electrodes of the dHPC kindled group were located in the CA1 region of the dHPC; contralateral electrodes were in or proximal to the EC (see Figure 3.6). In the bifocal kindled animals, electrodes were placed exclusively in the LEC of the left hemisphere, and contralateral electrodes were located in the CA1 field of the dHPC (see Figure 3.7). The LEC group was used for comparison due to the placement of EC electrodes in the bifocal group exclusively in the LEC. The number of rats in each group was as follows: LEC = 6, dHPC = 11, bifocal = 11, and control = 11.

3.2.2 Kindling

The bifocal kindled group possessed a mean ADT of 490 ± 56 **:**A in the LEC and 22 ± 5 **:**A in the dHPC. The mean kindling rate of the EC in the bifocal kindled group was 20.8 ± 3.2 ADs and of the dHPC was 10.8 ± 4.4 ADs. In contrast, the unifocal dHPC kindling group displayed a mean ADT of 32 ± 5 **:**A and required 41.3 ± 12.6 ADs to fully kindle. Comparing the unifocal to the bifocal dHPC kindling, the bifocal dHPC kindling group showed a significantly faster kindling rate($U=0.00$, $z=-3.98$, $p<0.001$), representing a 76% savings in the number of ADs required to induce 5 generalized seizures.

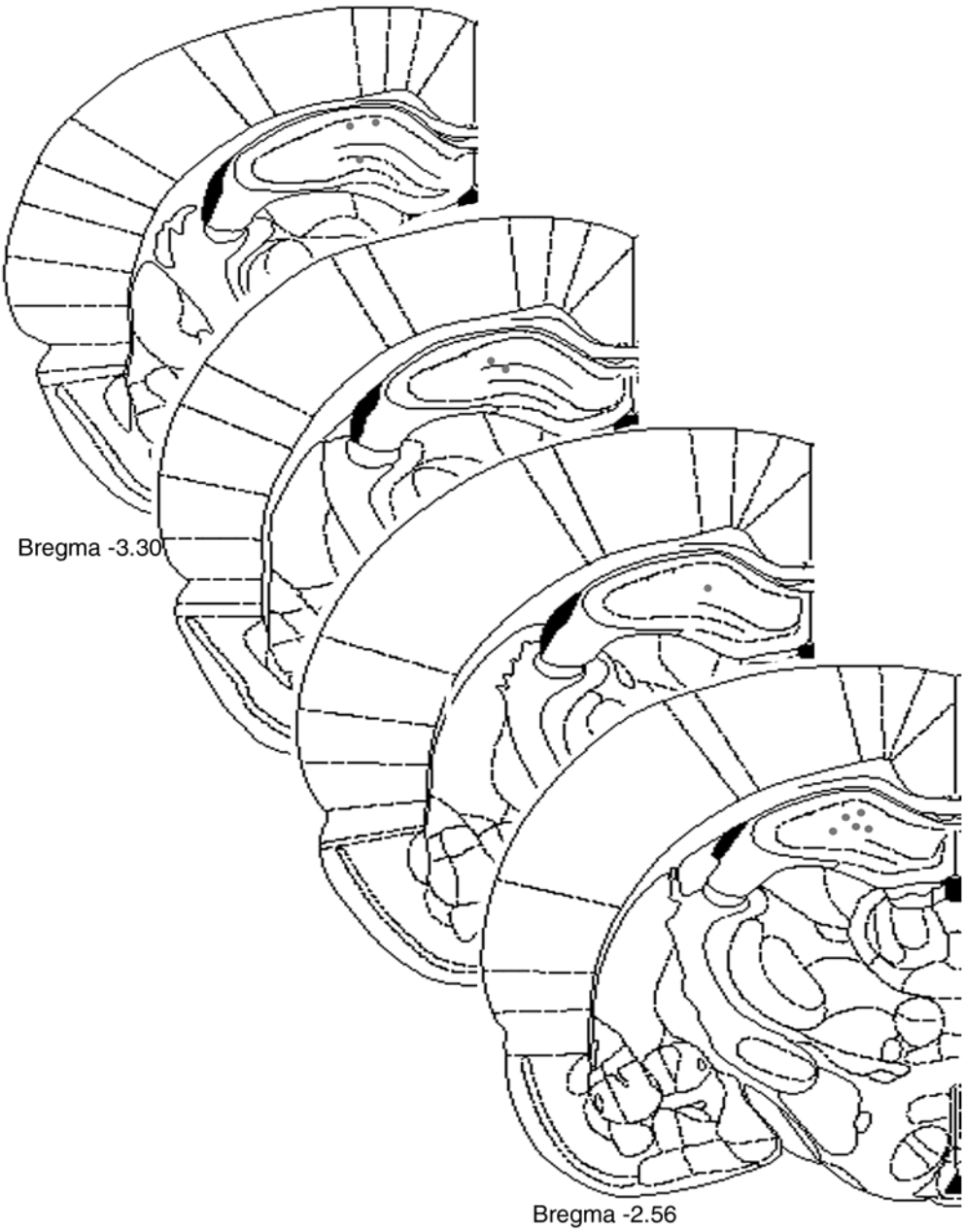

Fig. 3.6 Location of the lower tip of the stimulation electrode in dHPC kindled rats. Plates are posterior to bregma and were adapted from Paxinos and Watson (1997). (EC placements not shown)

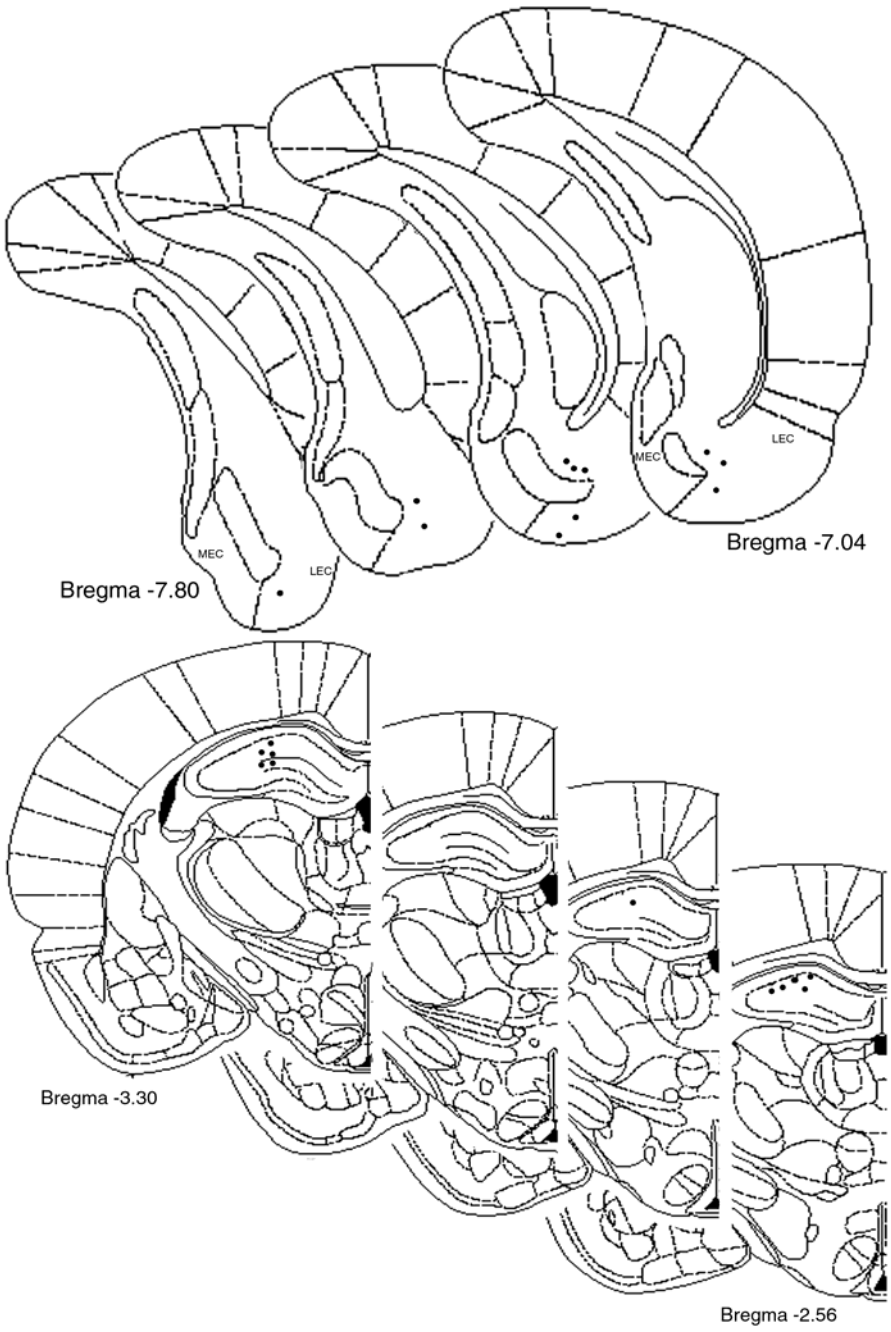

Fig. 3.7 Location of the lower tip of the stimulation electrode in bifocal kindled rats. Plates are posterior to bregma and were adapted from Paxinos and Watson (1997).

3.2.3 MWM

3.2.3.1 Visible platform trials

A significant main effect was observed in the distance swum during the VP trials (H^2=8.50, p<0.05) (see Figure 3.8). Further analysis revealed enhanced performance by LEC kindled rats. These rats required less distance to swim to the VP than control rats (U=13.00, z=-2.01, p<0.05).

3.2.3.2 Acquisition trials

In the HP acquisition trials, the Kruskal-Wallis test revealed a significant main effect on the trial six grouping (T_6) of trials (H^2=9.95, p< 0.05) (see Figure 3.9). Analysis of the simple effects revealed impaired performance by the dHPC group, which required a greater distance to locate the HP as compared to the control group (T_6 U=21.00, z-2.59, p<0.01).

Despite the induction of 10 fully generalized seizures, 5 of which were generated by dHPC kindling, bifocal kindling failed to impair acquisition performance in the MWM as compared to control rats, (p>0.05). Performance during acquisition did not differ significantly between the bifocal group and the dHPC group (p>0.05), and analysis of direct swims failed to reveal any impairments (H^2=3.19, p>0.05).

3.2.3.3 Retention trials

Analysis revealed a main effect for day 10 and day 31 retention trials (D_{10} H2=8.22, p<0.05, D_{31} H^2=9.15, p<0.03) (see Figure 3.9). Further analysis

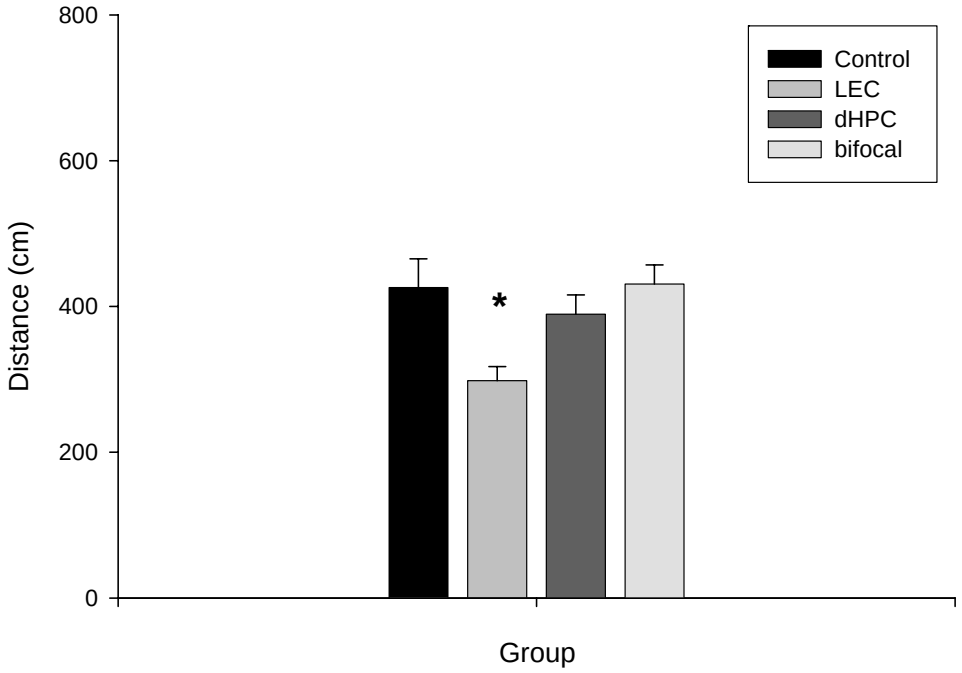

Fig. 3.8 Mean escape distances on 6 VP trials. ***** Denotes that the LEC kindled group is significantly different from the control group, $p<0.05$.

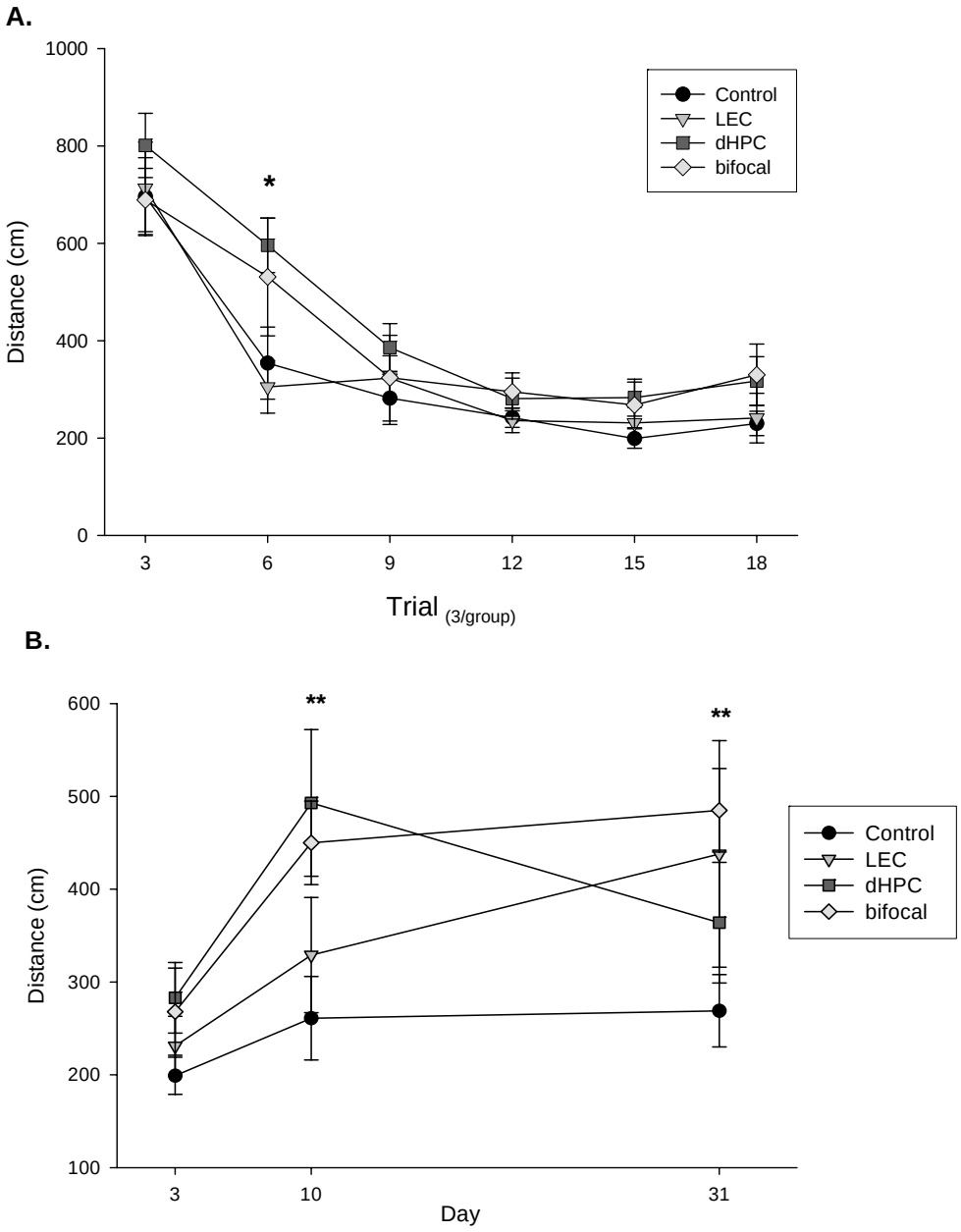

Fig. 3.9 Performance on HP trials. **A.** Mean escape distances on acquisition trails. **B.** Mean escape distances on retention trials. *****Denotes that the dHPC kindled group is significantly different from control, $p<0.05$. ****** Denotes that the bifocal kindled group is significantly different from the control group, $p<0.003$. (3 trials/block)

indicated that the bifocal kindled group displayed an impairment in performance during both sets of retention trials as compared to the control group (D_{10} U=15.00, z=-2.99, p<0.01; D_{31} U =17.00, z=-2.87, p=<0.01). However, no significant differences were observed comparing bifocal kindled and LEC kindled rats (D_{10} U=20.00, z=-1.3, p>0.05, D_{31} U=23.00, z=-1.005, p>0.05).

3.2.3.4 Probe trials

On all probe trials rats performed better than at chance levels (t(10)s $4.1, p#0.02). However, a main effect was observed on the day 10 probe (H^2=9.29, p<0.03) (see Figure 3.10). Analysis of the simple effects reveal a significantly greater time spent in the platform quadrant by the control rats as compared to the dHPC kindled and bifocal kindled groups on the day 10 probe trials (D_{10} U=19.00, z=-2.73, p=0.005; D_{10} U=30.50, z=-2.00, p=0.047, respectively). These data suggest that even though the rats were performing at better than chance levels, there was a significant impairment in the performance of both kindled groups.

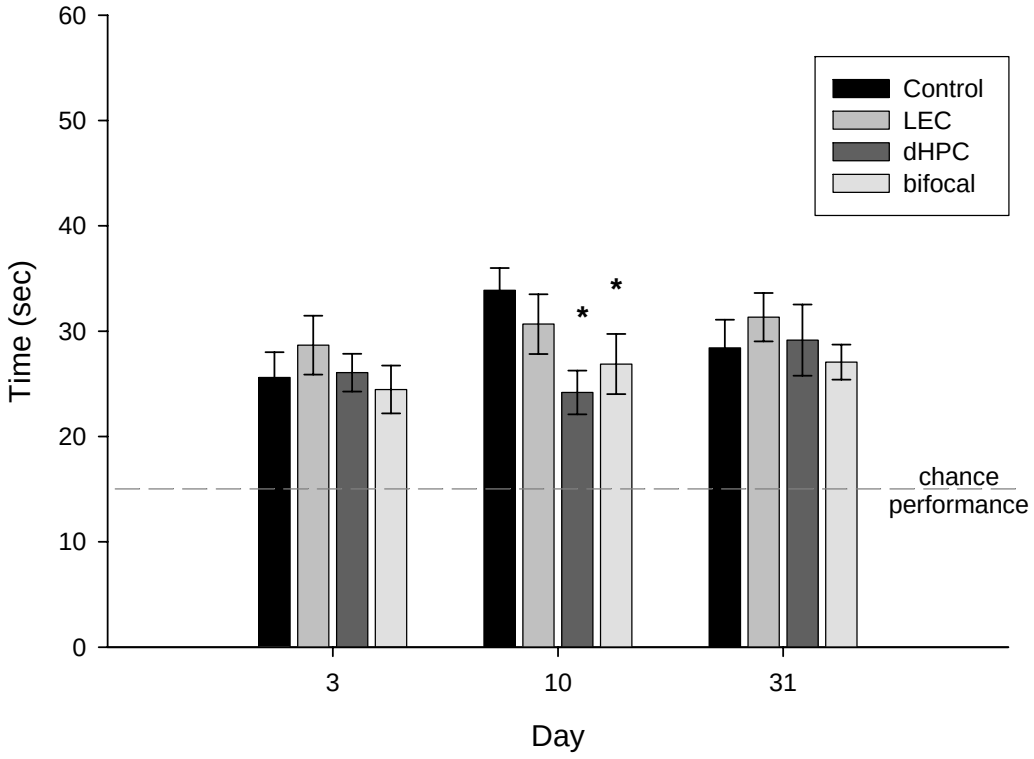

Fig. 3.10 Dwell time in platform quadrant on probe trials. All groups performed significantly better than chance performance, $p<0.05$.
* Denotes significantly different from control, $p<0.05$.

4. Discussion

After kindling there was no disruption in performance in the non-spatial pretraining performed with VP trials. In fact, LEC kindled performed significantly better than control rats, for reasons that are not immediately apparent. However, as a measure of escape motivation, motor coordination, and general mnemonic deficits, the performance of the LEC kindled subjects was unimpaired. These results suggest that the observed deficits in spatial cognition are due to effects on memory rather than to gross behavioral disruption (Hannesson, Mohapel et al., 2001).

My research suggests that LEC kindling failed to produce spatial cognitive deficits, whereas MEC kindling produced deficits in acquisition and retention of spatial information. However, caution must exercised when interpreting the results from the MEC kindling group, because 2 of 5 MEC kindled rats failed to acquire the HP location and, not surprisingly showed a deficit in retention on days 10. The MEC kindled group also performed at chance levels during 2 of 3 probe trials. These 2 rats showed exceedingly high ADT intensities with no histological evidence of lesions. Additional MEC kindled rats should be tested, to determine whether this a reliable effect. However, the behavioral results are consistent with the findings of Ferinteanu and colleagues (Ferbinteanu, Holsinger, & McDonald, 1999), who observed

impaired place learning in the MWM with lesions of the medial perforant path but not with lateral perforant path lesions. The perforant path is a monosynaptic reciprocal pathway between the EC and the hippocampus, with the lateral perforant path originating in the LEC and the medial perforant path originating in the MEC. In addition, McNaughton and Barnes (McNaughton & Barnes, 1977) observed differing field potential responses in the dentate gyrus to stimulation of the MEC and LEC. The population spikes observed after stimulation of the MEC displayed shorter latencies and higher amplitudes than those evoked by stimulation of the LEC, indicating a higher efficiency of the medial perforant path in the activation of the dentate gyrus (McNaughton & Barnes, 1977). This evidence suggests that the MEC may play a greater role in spatial cognition than the LEC.

Perhaps the more surprising result is that the 3 rats from the MEC group that performed at control levels in acquisition also showed a deficit in retention on day 10, but not day 31. No explanation for this finding is immediately apparent. A replication with a larger number of rats is required, to ensure that the result is reliable.

When LEC kindling is subsequently followed with dHPC (bifocal) kindling, retention deficits are observed. Examination of swim paths of the bifocal kindled rats revealed no thigmotaxic patterns during the retention trials; generally, the swim paths were located in the central region of the pool. This suggests that retention deficits observed in bifocal kindled rats

were due to inappropriate search patterns rather than an inappropriate behavioral response such as thigmotaxis.

Even though bifocal kindling disrupted performance during retention trials, as compared to control, no significant difference was observed between the effects of bifocal kindling and unifocal LEC kindling. I can suggest three possible explanations for these results. First, an additional 5 stage 5 seizures were evoked in the bifocal kindled rats as compared to the LEC kindled rats. The additional five stage 5 seizures may produce the disruption in spatial memory. Extended kindling has been observed to reduce the site-specificity of mnemonic dysfunctions observed in full kindling. Full amygdaloid and full olfactory bulb kindling fail to impair performance in spatial cognitive tasks, yet performance deficits are observed after extended kindling of either site (Cammisuli et al., 1997; Sutula et al., 1995).

Second, assuming that the disruption is mediated by the LEC, the bifocal kindled rats received an average of 11 additional stimulations. These additional stimulations may have generated the disruption in spatial cognition. There is no definitive evidence indicating whether the kindling stimulations or the generation of a generalized seizures are responsible for the production of kindling-induced cognitive deficits. Finally, LEC and dHPC kindling may have a potentiating effect on the deficits in retention of spatial information, and thus both sites must be kindled to produce a retention deficit.

My study with bifocal kindling is the first to examine possible kindling-induced site-specific disruptions in learning and memory. Previously little attention has been paid to the effects of bilateral kindling on subsequent learning. Both unilateral and bilateral perforant path kindling fails to alter subsequent spatial learning in the WMW (McNamara et al., 1992). Similarly, both unilateral and bilateral perforant path kindling fails to alter subsequent learning in the RAM (Robinson et al., 1993). However, Peele and Gilbert (1992) observed that bilateral amygdaloid kindling produces a greater impairment on a passive avoidance task than unilateral amygdaloid kindling. Unilateral amygdaloid kindling disrupts performance in passive avoidance tasks (Stone & Gold, 1988), whereas unilateral perforant path kindling fails to alter spatial cognition in both the MWM and RAM tasks (McNamara et al., 1992; Robinson et al., 1993). These results suggest that bilateral kindling can increase the magnitude of mnemonic deficits produced by unilateral kindling. Theoretically, if the cognitive disruptions I observed after MEC kindling are replicable, bifocal kindling of the MEC and dHPC may produce even larger cognitive disruptions.

One limitation of this study is the definition of "retention". The impairment in performance during retention trials demonstrates either disruption of the retention of memory or of the retrieval of memory. In this experiment, one cannot differentiate between retention and retrieval deficits.

Surprising results were observed in comparing the acquisition performance of the bifocal kindled and the dHPC kindled groups. Consistent with previous research, dHPC kindling disrupted the acquisition of the HP location as compared to controls (Hannesson, Howland et al., 2001). Conversely, bifocal kindling before acquisition trials failed to produce a significant difference from controls and dPHC kindling. Two potential confounds may account for these results. First, kindling transfer was evident in secondary site dHPC kindling in the bifocal kindling group. Primary kindling of the dHPC required a mean of 41 stimulations, whereas secondary dHPC kindling required a mean of only 10 stimulations. This represents a savings of 76%. Thus, even though primary and secondary dHPC kindling produce equivalent stage 5 seizures, the dHPC was stimulated fewer times during secondary site kindling.

Second, nonspatial pre-training during VP trials may have minimized the effect seen during acquisition trials. Saucier and colleagues (Saucier, Hargreaves, Boon, Vanderwolf, & Cain, 1996) observed that nonspatial pretraining eliminates spatial learning deficits produced by the *N*-methyl-D-aspartate antagonist NPC17742. Their study subjected rats to 12 nonspatial pretraining trials, whereas I subjected rats to 6 trials. Extensive pretraining in Saucier's study completely eliminated the deficit, whereas the more limited pretraining in my study may have only reduced the size of the deficit. In future work it will be interesting to see whether more extensive exposure

to VP trials (i.e., pretraining) can eliminate the kindling-induced deficit altogether.

Collectively, these results suggest that in the induction of mnemonic deficits the number of stimulations given to a site may be more critical than the induction of generalized seizures. The bifocal kindling group experienced 5 additional generalized seizures and received 76% fewer dHPC stimulations than the unifocal dHPC kindling group, yet failed to show a disruption in acquisition, which is characteristic of dHPC kindling. However, it is premature to conclude that number of stimulations is critical in the induction of mnemonic deficits, due to the previously mentioned confounds.

4.1 Further Experiments

In order to complete a more thorough investigation of bifocal kindling, I have developed additional procedures to be incorporated in further research. The first procedure is the addition of a group consisting of LEC kindled rats, kindled to a criterion of 10 stage 5 seizures. This will clarify whether the retention deficits observed after bifocal kindling are due to the combination of LEC and dHPC kindling or primarily due to LEC kindling and enhanced with the subsequent generalized seizures.

The second procedure will involve the elimination of the VP trials. The VP trials may have minimized differences during acquisition of the HP location. By repeating the bifocal kindling (LEC kindling prior to dHPC) and eliminating the VP trials during WMW testing, a deficit in acquisition may be

observed. If this procedure fails to result in a disruption in the acquisition of spatial cognition, then a third procedure may be of interest:

The third procedure will be the addition of a bifocal group with a reversed order of kindling (i.e., dHPC prior to LEC kindling). This result would determine whether the order of kindling the two sites is critical in development of spatial cognitive deficits. For example, unifocal dHPC kindling disrupted performance during acquisition; however, when dHPC kindling was subsequent to LEC kindling, no deficit in acquisition was observed. Theoretically, full dHPC kindling prior to LEC kindling may result in a deficit in acquisition.

4.2 Conclusions

First, consistent with previous research, full dHPC kindling disrupts acquisition but not retention in subsequent MWM testing. Second, full LEC kindling is ineffective in producing any spatial cognitive deficits. Third, bifocal kindling does not increase the magnitude of observed deficits. The performance of bifocal kindled animals on both acquisition and retention trials did not differ significantly from dHPC and LEC kindled animals.

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