Hippocampal Kindling and Kindled Seizures Impair
Performance in the Morris Water Maze

by

Trevor Howard Gilbert
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We accept this thesis as conforming
to the required standard

Dr. M.E. Corcoran, Supervisor (Department of Psychology)

Dr. C. Mateer, Departmental Member (Department of Psychology)

Dr. C. Hawryshyn, Outside Member (Department of Biology)

Dr. D. Paul, External Examiner (Department of Biology)

University of Victoria

Supervisor: Dr. Michael E. Corcoran

ABSTRACT

The hippocampus is thought to play an important role in the processing of spatial information, in that interference in normal hippocampal functioning can produce deficits in performance of a wide variety of tasks that use spatial navigation for optimal performance. The effects of hippocampal kindling on subsequent learning and memory of a spatial task have been assessed in several studies. Although the studies vary in procedure and site of stimulation, their results fairly consistently suggest that epileptiform activity within the hippocampal formation impairs performance. I have attempted to assess the spatial performance of rats in the Morris water maze task during and subsequent to kindling of hippocampal field CA1.

I used two procedures: (1) seizures were kindled with stimulation of CA1 preceding training, or just prior to daily training in the water maze (acquisition); and (2) maze training was imposed until performance stabilized, and then seizures were kindled with stimulation of CA1 preceding testing, or just prior to daily testing in the maze (retention).

In Experiment 1, kindling of field CA1 to a convulsive stage (kindling), but not a preconvulsive stage (partial kindling), produced performance deficits in acquisition in the water maze. In Experiment 2, CA1 kindling to convulsive and preconvulsive stages produced performance deficits in retention in the water maze. In Experiment 3, nonconvulsive afterdischarge (AD) and kindled seizures (generalized convulsions),

evoked just prior to daily testing in the water maze, produced performance deficits in acquisition and retention, respectively.

These results strongly suggest that the processing of spatial information is vulnerable to both transient epileptiform activity and the long-lasting increase in neural excitability (the kindled state). Collectively, these findings are consistent with previous results suggesting that kindled epileptiform activity in the hippocampus impairs performance in tasks sensitive to spatial learning and memory.

Examiners:

Dr. M.E. Corcoran, Supervisor (Department of Psychology)

Dr. C. Mateer, Departmental Member (Department of Psychology)

Dr. C. Hawryshyn, Outside Member (Department of Biology)

Dr. D. Paul, External Examiner (Department of Biology)

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Introduction

Epileptiform activity can be induced by electrical stimulation in many structures in the forebrain and some sites in the brainstem. The intermittent application of low-intensity electrical pulses to specific regions of the brain leads to the progressive development of generalized motor seizures (Goddard, 1967; Goddard, McIntyre, and Leech, 1969). This phenomenon, known as kindling, is associated with a permanent state of enhanced susceptibility to seizures and various biochemical and electrophysiological alterations in a number of forebrain sites (Corcoran, 1988; Cain, 1989).

Kindling of behavioural seizures by stimulation of limbic sites in rats has been characterized according to a series of stages (Racine, 1972a, 1972b) that are associated with the gradual evolution and propagation of afterdischarge (AD). Initial AD elicits only nonconvulsive indicators such as automatisms (e.g., grooming) and freezing. The first convulsive behaviours to emerge typically involve myoclonus (e.g., facial twitches) and chewing, referred to as stage 1 seizures. During stage 2, the neck muscles become involved, resulting in bobbing of the head in addition to more intense mouth movements. Stage 3 seizures consist of unilateral clonus of the forelimb, usually on the contralateral side. In stage 4, clonus of the forelimbs becomes bilateral, and stage 5 generalized seizures are associated with rearing and falling, presumably as a consequence of development of clonus in the hindlimbs. At each stage of kindling, signs of the earlier stages are typically seen, but are incorporated into the more advanced stage.

In my thesis I shall concentrate on kindling of the hippocampal formation, which has been intensively investigated in the study of the neurobiological mechanisms of learning and memory (Alvarez and Squire, 1994; Morris, Kandel, and Squire, 1988; Squire, 1986; Squire, 1992a; Squire, 1992b; Squire and Cave, 1991; Vanderwolf and Cain, 1994). The hippocampus is thought to play a significant role in the processing of spatial information, in that interference in normal hippocampal functioning can produce deficits in performance of a wide variety of tasks that use spatial navigation for optimal performance (Barnes, 1988; Jarrard, 1993; Morris, Garrud, Rawlins, and O'Keefe, 1982). For example, lesions of the hippocampus have been found to impair place learning in the Morris water maze (Morris et al., 1982). Although the hippocampus is typically associated with spatial memory (Nadel, 1991; Jarrard, Okaichi, Steward, and Goldschmidt, 1984; Jaffard, Beracochea, and Cho, 1991), several other limbic sites have been implicated as well (Sutherland and McDonald, 1990). Lesions of the medial septum also impair place learning in the water maze (Kelsey and Landry, 1988). Also, it has been found that amygdaloid lesions impair avoidance conditioning even though they do not impair place learning (Morris, 1984).

Epileptiform activity and memory. Because the hippocampus, septum, and amygdala are highly susceptible to kindling, it might be expected that the development of epileptiform activity within them would have effects on at least some aspects of memory (Becker, Grecksch, Ruthrich, Pohle, Marx, and Matthies, 1992; Holmes, Chronopoulos, Stafstrom, Mikati, Thurber, Hyde, and Thompson, 1993; Peinado-

Manzano, 1994). In the clinical setting, for example, it is not uncommon for patients with temporal lobe epilepsy to display memory impairments before, during, and after epileptic seizures (Gallassi, Morreale, Lorusso, Pazzaglia, and Lugaresi, 1988; Halgren and Wilson, 1985). Kesner (1982) has found that limbic stimulation produced amnesic syndromes in a variety of epilepsy models in rats. In addition, seizure propagation in hippocampal CA1 field produces a retrograde but not anterograde impairment of working memory but no impairment in reference memory (Knowlton, McGowan, and Olton, 1985; Knowlton, Shapiro, and Olton, 1989; Olton and Wolf, 1981). As commonly used, working memory refers to a form of short-term memory that makes use of information that can change from trial to trial (i.e., trial-dependent memory), and reference memory refers to a form of long-term memory that makes use of information that is constant from trial to trial (i.e., trial-independent memory). Furthermore, as will be discussed in detail below, kindling with stimulation of CA1 produced deficits in both working memory (Leung, Boon, Kaibara, and Innis, 1990; Leung and Shen, 1991; Leung, Zhao, and Shen, 1994) and reference memory (Lopes da Silva, Gorter, and Wadman, 1986), both using the radial arm maze to assess spatial performance. Leung et al. (1990) reported that kindling of hippocampal field CA1 to the point of either non-convulsive or convulsive seizures disrupts performance in the 8-arm radial maze for a minimum of 3 weeks after the last afterdischarge (AD). They suggested that this disruption was caused primarily by hippocampal ADs and was not necessarily associated with the behavioural seizures.

Morris water maze. There is evidence that kindled seizures can affect spatial

learning in the Morris water maze, which I used to measure spatial learning. Briefly, the Morris water maze consists of a large pool filled with water, which is clouded by adding milk powder. The rat is trained to locate a submerged escape platform, relying on spatial cues for navigation (see Methods for detail). The evidence indicates that there is a dissociation between the effects of kindling (i.e., the state of increased epileptogenicity) and the effects of seizures. Most deficits in water maze performance are detected only in the postictal state, shortly after seizures, and not after kindling itself. For example, the subseizure stimulation of the dentate gyrus (DG) was found to impair place learning in the Morris water maze (Parker and Wally, 1988). Similarly, the triggering of seizures by stimulation of the perforant path (PP), septum, or amygdala prior to daily training impaired place learning, but had little effect on visible platform training or swim speed (McNamara, Kirkby, dePape, and Corcoran, 1992; McNamara, Kirkby, dePape, Skelton, and Corcoran, 1993). The visible platform task is a cue task in which rats are required to navigate to a visible platform (extended above the water). This task was designed to assess whether any general sensorimotor or motivational factors interfere with the rats' ability to escape, in a simpler situation in which escape could be guided by proximal rather than distal spatial cues. Similarly, the analysis of swim speeds helps to determine whether there is a deficit in the motivation to escape. Seizures induced by stimulation of the PP after daily training also impaired place learning. This was indicated by the increased latencies, both during acquisition and reversal phases, and by the lack of a quadrant bias during the probe trials. That is, on the final trial of the last day of acquisition, a

probe trial was given to assess the strength and accuracy of initial learning, in which the rats were required to swim in the pool for 60 s without an escape platform. Analysis of behaviour during the probe trial permits the investigator to determine the amount of time spent by the rat searching for the escape platform. The rationale is as follows: if a rat tends to swim in one quadrant substantially more than in others, this may indicate that the rat has learned that the platform is in that particular region.

In the study of McNamara et al. (1992), all seizure groups failed to show a target quadrant bias on the postacquisition probe, and only the amygdala seizure group showed a bias on the postreversal probe. Conversely, place learning was unimpaired by kindling itself, defined operationally as performance in rats tested 24 hrs after the last generalized seizure induced by kindling of the PP, septum, or amygdala. Furthermore, the probe trials indicated that controls and the kindled groups at all stimulation sites showed a significant bias for the target quadrant. The results indicate that seizures, but not kindling itself, impede place learning in the Morris water maze.

McNamara et al. (1992) maintained that the performance deficit produced by seizures was not a consequence of behavioural or motivational impairments, as neither swim speed nor visible platform training was affected by the induction of seizures. In an alternative task that made use of proximal spatial cues, the rats were required to swim to a visible platform (as mentioned above) located in the center of a different quadrant on each trial. The rats tested in the postictal state exhibited control levels of performance on the cue task. This result is important, because it has been found that sustained stimulation to the PP produces profound deficits in performance and loss of

pyramidal cells in the CA1 and CA3 regions of the hippocampus (Rogers, Barnes, Mitchell, and Tilson, 1989). Normal performance on the visible platform task in the McNamara et al. study suggests that any neuronal death associated with kindling was not extensive enough to result in deficits in all forms of learning in the water maze.

The findings of McNamara et al. (1992) are not entirely in agreement with the results of Knowlton et al. (1989), who found that hippocampal seizures impaired working memory but spared reference memory. McNamara et al. suggested that because the Morris water maze task is viewed as requiring mostly reference memory (e.g., Morris, 1983), reference memory is also susceptible to disruption by hippocampal seizures. In addition, McNamara found that induction of seizures prior to daily training produced an anterograde impairment; however, Olton and Wolf (1981) found that hippocampal seizures failed to produce anterograde impairments in working memory. It is possible that these differences may have been due to the severity and duration of seizures in the two studies. Rats in the Olton and Wolf study were referred to as having electrophysiological seizures, whereas the rats in the McNamara study had consistent generalized motor seizures. However, recall that rats in the Olton and Wolf study displayed profound retrograde amnesia.

Knowlton et al. (1985) detected deficits in the radial arm maze following seizures even up to 8 hours after training. As mentioned previously, the induction of seizures immediately following daily training in the water maze also produced significant impairment in place learning (McNamara et al., 1992). Although McNamara and colleagues did not find an overall disruption in place learning in the

kindled group, they did find a significant impairment on the first day. This could be indicative of a proactive effect of seizures that can last for 24 hrs. McNamara et al. suggested that the deficit produced by post-trial seizures might not be due to simply a retrograde influence on memory.

Radial-arm maze. Most studies have examined the effects of kindling on spatial memory in the 8-arm radial maze or closely related tasks. In variants of the task, certain arms are consistently baited with food on all trials. Error in reference memory are measured by the frequency with which well-trained rats enter the non-baited arms, and errors in working memory are measured by the frequency with which rats enter the baited arms more than once in a testing session. Robinson et al. (1993) compared the transient and persistent effects of kindling the PP on the acquisition of two radial maze tasks. Seizures were triggered in one group 30-45 minutes prior to each learning trial. In the other group, training commenced 24 hr after the last generalized seizure. They found learning impairments only in the group trained shortly after seizures, and suggested that the deficit is related to the transient aftereffects of an AD rather than to the persistent changes in neuronal firing caused by the kindled state.

Feasey-Truger, Kargl, and ten Bruggencate (1993) also used the 8-arm radial maze to assess the effects of hippocampal kindling on working and reference memory. They examined the effects of kindling of the DG. During the kindling phase, there was a marked increase in the number of reference memory errors. The difference in error scores between the experimental and control rats was significant for the entire

kindling period. An interesting note was that Feasey-Truger et al. found no significant differences between the pre-seizure scores and the period in which the rats displayed stage 5 seizures. The reference memory error scores were still significantly greater than those of controls during the first three weeks of a layoff period without stimulation; however, there was a gradual improvement in performance over the next 4 weeks. Feasey-Truger et al. found that DG kindling failed to produce a working memory impairment in any phase of their experiment. They suggested that the reference memory impairment was due primarily to the hippocampal ADs, since the deficit in performance was apparent in the preconvulsive stages of kindling. Also, the degree of reference memory impairment did not differ between rats that were displaying stage 5 seizures and those that were displaying only nonconvulsive seizures. Thus generalized seizures were not required for impairment of reference memory.

Although it appears that hippocampal kindling results in impaired performance of a spatial task sensitive to reference memory, other factors must be considered. For example, it is possible that nonmemory deficits in motor or sensorimotor/perceptual components were responsible for the impaired performance. Ehlers and Koob (1985) detected locomotor deficits for as long as 2 hr after hippocampal seizures. Although this finding raises the possibility that the deficits in maze performance were due to motor problems and not deficits in memory, it is unlikely that motor deficits can provide the entire explanation. If in fact motor deficits were responsible for the observed impairment in reference memory in the study of Feasey-Truger et al. (1993), for example, one might expect that there would also be an impairment in working

memory, which would be equally vulnerable to motor problems. Feasey-Truger and colleagues did not find any significant differences between experimental and control rats in the number of errors in working memory. Furthermore, they found that errors in reference memory persisted for 3 weeks after the discontinuation of kindling stimulation, seemingly well beyond the point at which any simple motor deficit would occur. With regard to the seizure-induced deficit observed in the Morris water maze (McNamara et al., 1992), recall that no differences were found between experimental and control rats in the ability to find a visible platform in the water maze, a task that is presumed to use non-spatial capabilities but that should be equally sensitive to motor or other general behavioural deficits.

The results of Feasey-Truger et al. (1993) are in partial agreement with the study of Lopes da Silva et al. (1986), who reported that kindling of field CA1 of the hippocampus produces deficits in reference memory in the radial maze. However, Lopes da Silva et al. also reported transient deficits in working memory during the kindling phase, as have been reported in other studies involving CA1 kindling (Knowlton et al., 1989; Leung et al., 1990; Olton and Wolf, 1981; Olton, Walker, and Wolf, 1982). Recall that Robinson et al. (1993) found both reference and working memory deficits in the radial maze after PP kindled seizures, and McNamara et al. (1992) found reference memory deficits in the water maze, also after PP kindled seizures. The fact that disturbances in working memory were found in some but not all studies suggests that deficits in certain components of spatial memory (and possibly learning and memory in general) may differ as a function of site and task. In other

words, results may vary as a function both of the structure within the hippocampal formation that is kindled (DG, CA1, or PP) and of the task that is used to assess the memory impairment (water maze or radial maze).

The above discussion suggests that hippocampal kindling and hippocampal lesions produce similar patterns of disruption in learning and memory. Thus it appears that kindling may act as a functional lesion, altering neural activity throughout the hippocampal formation and any anatomically connected structures that may play a significant role in the processing of spatial information.

Evidence reviewed above indicates that spatial memory in the radial arm maze is disrupted by CA1 kindling (e.g., Leung et al., 1990) whereas spatial memory in the Morris water maze is unaffected by PP kindling (McNamara et al., 1992). To identify the role of the particular task chosen to measure spatial learning (radial arm maze vs. Morris water maze) and the role of the site of kindling (PP vs. CA1), I examined spatial learning and memory in the Morris water maze during and subsequent to kindling of field CA1 of the hippocampus. I also compared the effects of kindling to the effects of kindled seizures or AD. Based upon the research discussed above, I hypothesized that both kindling and kindled seizures would disrupt spatial learning (acquisition) and spatial memory (retention) in the water maze.

General Methods

Subjects. The subjects were 85 male Long-Evans hooded rats (Charles River, Quebec) weighing 250-400 g at the time of surgery. They were housed individually in shoebox cages with food and water available ad libitum. The colony room was maintained on a 12:12 h light-dark cycle. All testing took place during the light cycle.

Surgery. The rats were anesthetized with sodium pentobarbital (65 mg/kg) administered intraperitoneally and received stereotaxic implantation of bipolar stimulating/recording electrodes bilaterally into field CA1 of the hippocampus. Relative to bregma, the coordinates were -3.6 AP, +2.6 ML, and -3.2 DV from skull, with the incisor bar set at -3.9. The electrodes consisted of twisted strands of Nichrome wire, insulated except at the tips, with a total diameter of 127 μm. One of the electrodes of the bipolar pair was cut 0.5 mm shorter than the other. The deep electrode was targeted at the CA1 stratum radiatum, and the surface electrode was targeted at the alveus. A surgical screw connected to a strand of insulated stainless steel wire was secured to the right frontal pole and served as the ground/reference electrode. Gold-plated Amphenol pins were soldered to the ends of all wires and were inserted into a plastic pedestal that was affixed to the skull using dental acrylic and 3 additional surgical screws. The rats were given a period of at least one week for recovery.

Kindling. The kindling procedure commenced following the postsurgical recovery period or 24 hrs after the last day of an initial training period (see below for specific Experiment for detailed procedures). The rats were connected to the

recording/stimulation lead and placed in a shielded plexiglass chamber, and baseline referential recordings of EEG from one wire of each electrode were taken for approximately 30 s. Electrical stimulation consisted of a 1-s train of constant current balanced biphasic square-wave pulses 1.0 ms in duration and delivered at 60/s. Stimulation was delivered via the electrode in one hemisphere at a rate of 1/min at an initial intensity of 20 μA and increasing in 10 μA steps until AD was elicited. AD was defined as a train of spikey epileptiform transients, each of which had an amplitude of at least double the baseline EEG and a duration of 80 msec or less. The lowest intensity of stimulation that induced AD was arbitrarily defined as threshold (ADT). ADT in the contralateral hippocampus was measured on the next day. The side chosen for daily stimulation was the one that displayed the typical electrographic manifestations expected from field CA1. These included hippocampal theta rhythm at the stratum radiatum with an amplitude greater than 0.5 mV, a period of secondary AD after triggering of primary AD in field CA1, and an initially low ADT (Leung et al., 1990). The rats were then stimulated unilaterally once daily at an intensity 50 μA above threshold, and EEG was recorded for at least 1 min after stimulation. Behavioural seizures were classified according to Racine's scale (1972a).

Rats in all *kindled* groups received stimulation until 3 consecutive stage 5 seizures were elicited (see specific Experiments below for group description and assignment). At this point, stimulation was discontinued and water maze testing was instituted beginning 24 h after the last seizure. Rats in all *partial-kindled* groups received stimulation comparable to the number of ADs in the kindled group, but in the

absence of consistent generalized behavioural seizures. Rats in these groups received stimulation to parallel the kindled group, or until a predetermined number of ADs was elicited (see below). In other words, in an attempt to roughly match the variability in the number of stimulations required to evoke generalized seizures in the kindled group, stimulation in some rats in the partial-kindled group was terminated after they had exhibited a similar (variable) number of ADs as rats in the kindled group. Stimulation in other rats in the partial-kindled group was terminated after they had experienced an arbitrary number of ADs. Testing in the maze for partial-kindled rats also commenced 24 h after the last AD or seizure. Rats in the *AD* or *seizure* groups received stimulation evoking AD or a generalized seizure 25-45 min prior to daily testing in the water maze. All controls carried implanted electrodes and received comparable handling but no electrical stimulation.

Apparatus. The Morris water maze consisted of a circular pool 150 cm in diameter and 45 cm in height with a white inner surface. The pool was filled to a height of 25 cm of water at approximately 22 ° C. The water was made opaque by addition of powdered skim milk. The hidden escape platform (13 x 13 cm) consisted of a plastic grate affixed to a metal hydraulic stand (*Atlantis* platform) that could be easily raised or lowered. It was submerged approximately 2 cm below the water surface so that it was invisible at water level. The visible platform (described below) was a black stand (13 x 13 cm) that extended 5 cm above the surface of the water.

Acquisition and *Retention Phases*. During acquisition and retention (as defined below), the submerged escape platform was located in the center of the northwest

quadrant. All groups were given 4 trials daily for 7 consecutive days. On each trial the rat was placed in the water facing the pool wall at one of 4 randomly determined starting locations (north, west, east, or south pole). The rat's swim path, distance (cm), and escape latency were measured with a video tracking system (Chromotrack 4.02; SD Instruments). Once the rat found the platform, it was permitted to remain on it for 10 s; if it did not find the platform within 60 s, it was guided to it and permitted to remain on it for 10 s. After each trial the rat was placed in a holding cage under a brooding lamp for warmth, with an intertrial interval of approximately 3 min.

After the last trial on the final day of acquisition or retention (day 7), a probe trial was given to assess the strength and accuracy of initial learning or relearning. This procedure consisted of releasing the rats from the southern pole and allowing them to swim for 30 s without an escape platform (which had been lowered to the bottom of the pool). At the end of the 30 s the platform was raised to normal depth, and the rat was permitted to swim until it located the platform. Once the rat found the platform, it was allowed to remain on it for 10 s.

Reversal Phase. On the day following the last day of acquisition or retention, rats in Experiments 1 and 2 were trained to locate the submerged platform in a different quadrant; this was to assess their proficiency at learning a new location. The platform was relocated to the center of the quadrant diagonally opposite (southeast) to the previous location (northwest). As in the acquisition and retention phases, all rats were given 4 trials daily for 7 consecutive days, followed by a 30 s probe trial after

the final trial on the last day.

Seizure Phase. On the day following the completion of reversal, experimental rats in Experiments 1 and 2 received kindling stimulation 25-45 min prior to daily testing. This was to assess the transient effects of epileptiform activity on maze performance of *experienced* rats. The platform remained in the same quadrant as in the Reversal Phase (southeast). As in the previous phases, all rats were given 4 trials daily for 7 consecutive days followed by a 30 s probe trial after the final trial on the last day.

Histology. At the completion of behavioural testing, all rats were deeply anesthetized with sodium pentobarbital and transcardially perfused with physiological saline. The brains were removed and fixed in 10% formalin. Frozen coronal sections 80 μm thick were taken and stained with cresyl violet to verify electrode placements.

Data Analysis. The swim path lengths and escape latencies were analyzed using analysis of variance (ANOVA) with repeated measures (Wilkinson, 1990). Comparisons were made post-hoc using Tukey's test. The acceptable level for statistical significance was $P < 0.05$.

EXPERIMENT 1

According to Leung et al. (1990), kindling of hippocampal field CA1 disrupts learned responses in the radial-arm maze, a task sensitive to spatial learning and memory. Leung reported that performance in the radial-arm maze was disrupted regardless of whether nonconvulsive or convulsive seizures had been kindled. The purpose of Experiment 1 was to assess the effects of CA1 kindling on subsequent learning (*acquisition*) in the Morris water maze.

Methods

Thirty-seven rats were randomly assigned to 1 of 4 groups. As mentioned above, rats in the kindled group (n = 13) received daily stimulation until 3 consecutive stage 5 seizures were evoked. Rats in the partial-kindled group (n = 7) received daily stimulation to parallel the kindled group, or until 30 ADs were elicited. The third and fourth groups served as yoked controls (n = 11 and 6, respectively); these rats carried implanted electrodes and were handled during the kindling phase, but did not receive any stimulation. For all groups, training in the water maze began 24 hr after the last seizure or AD. There was no further application of kindling stimulation over the initial course of training (i.e., during acquisition and reversal). On the day following the last day of acquisition, rats in all groups were trained to locate the submerged platform in a new location (Reversal Phase) [see General Methods]. On the day following the last day of reversal, experimental rats received kindling stimulation 25-45 min prior to daily testing (Seizure Phase). Controls received similar handling but no kindling stimulation.

Results

Kindling. Rats in the *kindled* group required 36.54 $\pm$ 6.98 ADs (mean $\pm$ S.E.M.) before the first stage 5 seizure was evoked and 46.77 $\pm$ 6.38 ADs before three consecutive stage 5 seizures were evoked. The initial ADT for this group was 28.46 $\pm$ 4.21 μA. Rats in the *partial-kindled* group were kindled to 27.14 $\pm$ 3.99 ADs and displayed an initial ADT of 25.71 $\pm$ 3.14 μA. Four rats in the partial-kindled group displayed nonconvulsive seizures, two rats displayed partial (stage 1 and 2) convulsive seizures, and one rat displayed one stage 5 seizure. The AD of each rat typically consisted of a primary AD followed by a period of flat EEG and then a secondary AD within approximately 60 s.

Acquisition. Rats in the kindled group displayed an impairment in learning the location of the submerged escape platform, as indicated by their longer swim distances as compared to controls (Fig. 1). An overall ANOVA of the kindled group's swim distance revealed a significant groups effect [F (1, 94) = 9.31, $P < 0.003$] and days effect [F (6, 564) = 39.71, $P < 0.0001$] but a nonsignificant interaction between groups and days [F (12, 564) = 1.53, $P = 0.167$]. Post-hoc analysis revealed that the kindled group swam significantly longer distances than controls on Days 3, 4, 5, and 7 ($P < 0.033$). A representative swim path of a typical rat on the first day of acquisition is shown in Figure 2. It is clear that the rat has not yet learned of an escape platform. Representative swim paths of a kindled and a control rat on Day 4 of acquisition are shown in Figure 3. It is evident that the kindled rat did find the escape platform despite the longer swim path.

Figure 1. Effects of CA1 kindling on Morris water maze performance during acquisition. Rats in the kindled group were impaired in learning the location of the submerged escape platform during acquisition, as indicated by their longer swim distances (means $\pm$ S.E.M.) as compared to controls. Note that the kindled group also showed longer swim distances during both reversal and the seizure phase. $*P < 0.05$.

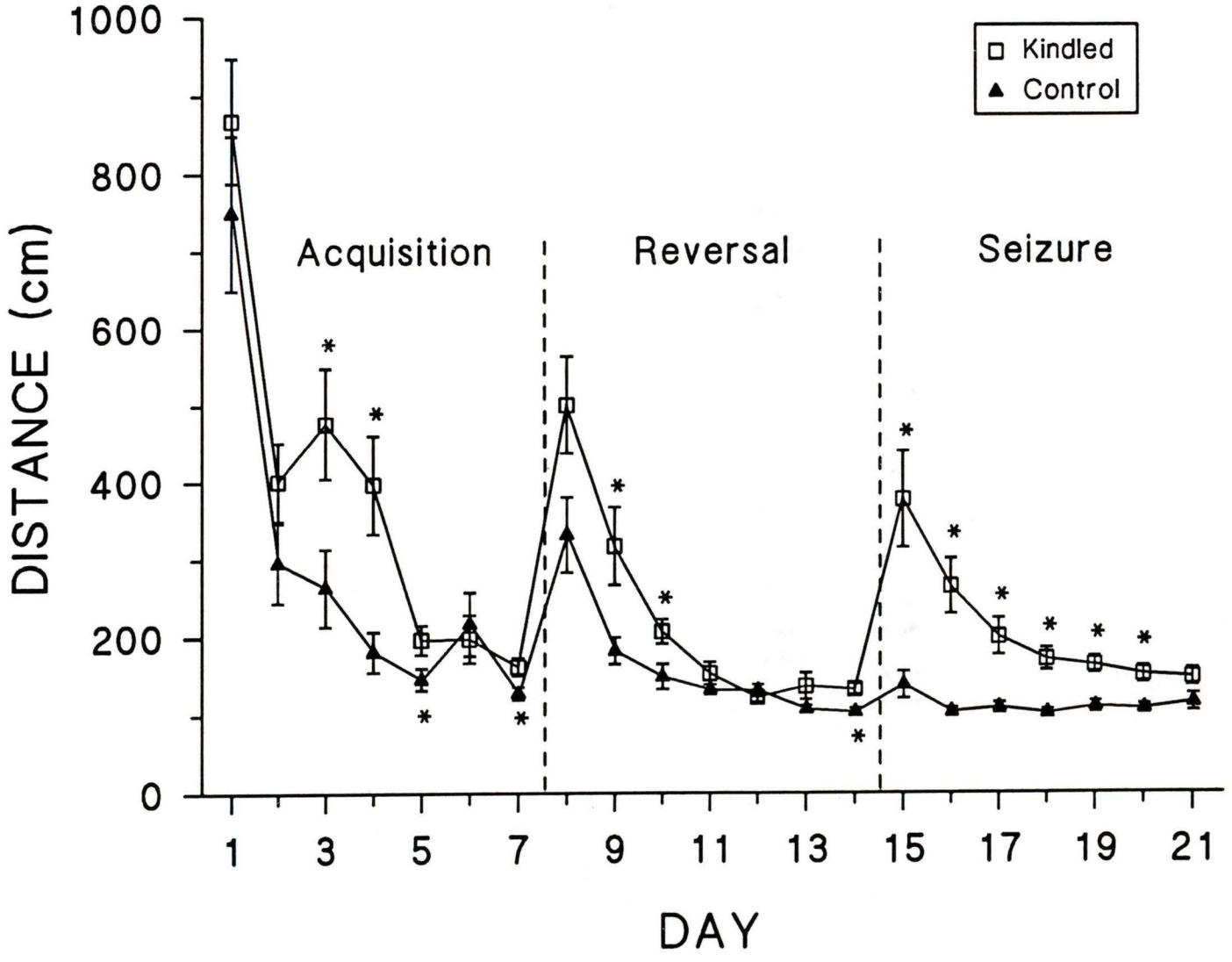

1000
800
600
400
200
0
DISTANCE (cm)
Kindled
Control
Acquisition
Reversal
Seizure
1
3
5
7
9
11
13
15
17
19
21
DAY

Figure 2. Representative swim path of a typical rat on the first day of acquisition. Note the circuitous swim path. It is clear that the rat was not aware of any escape platform and therefore did not show a bias for any quadrant.

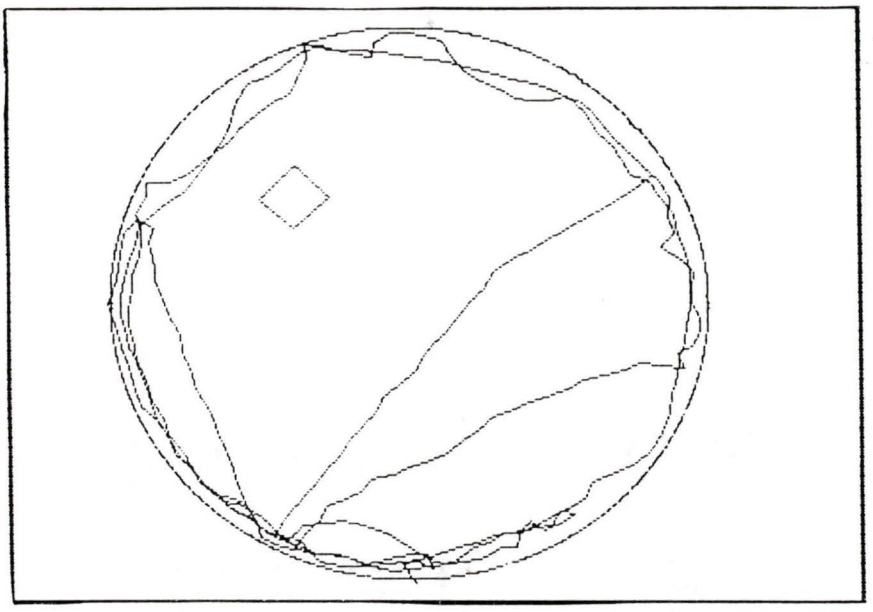
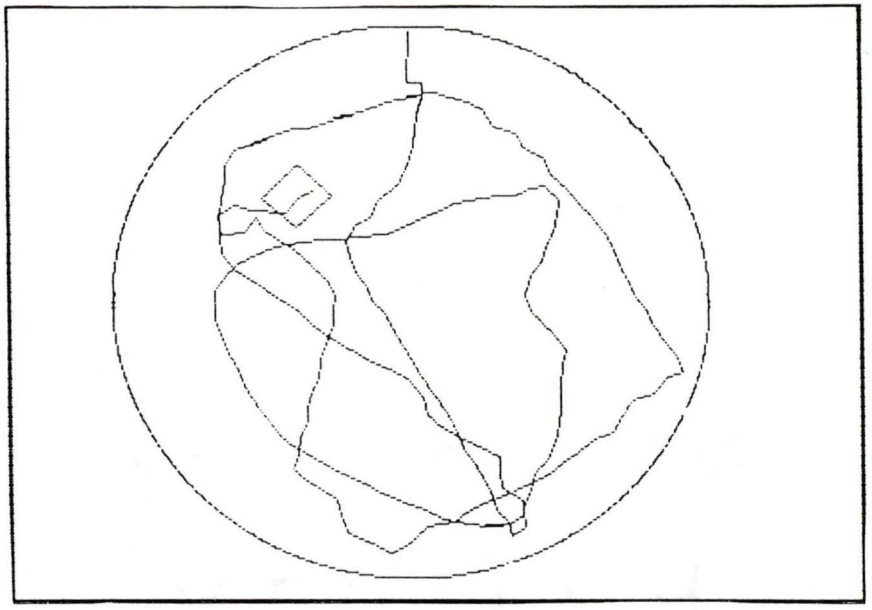
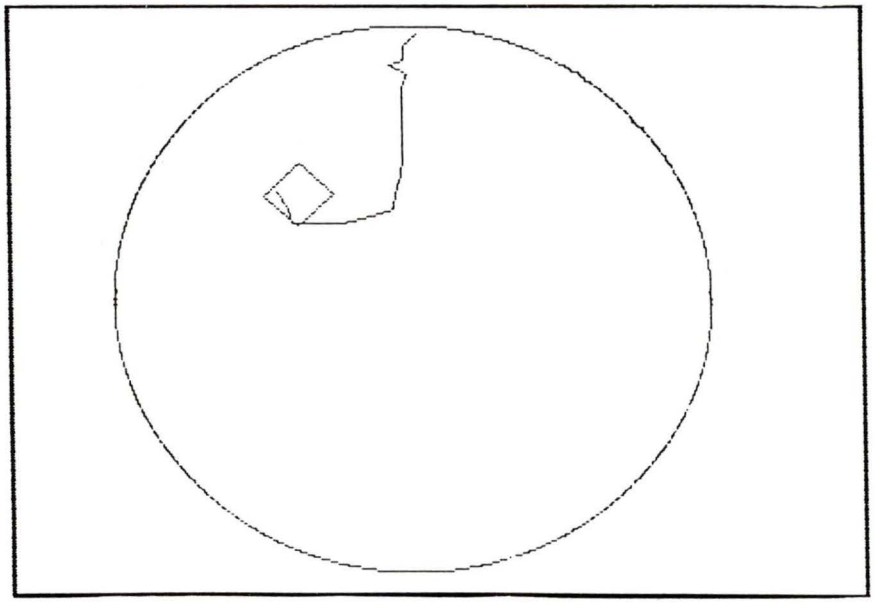


Figure 3. Representative swim paths of a kindled rat (upper trace) compared to its control (lower trace) on Day 4 of acquisition. Note that the kindled rat eventually found the escape platform despite an elongated swim path.





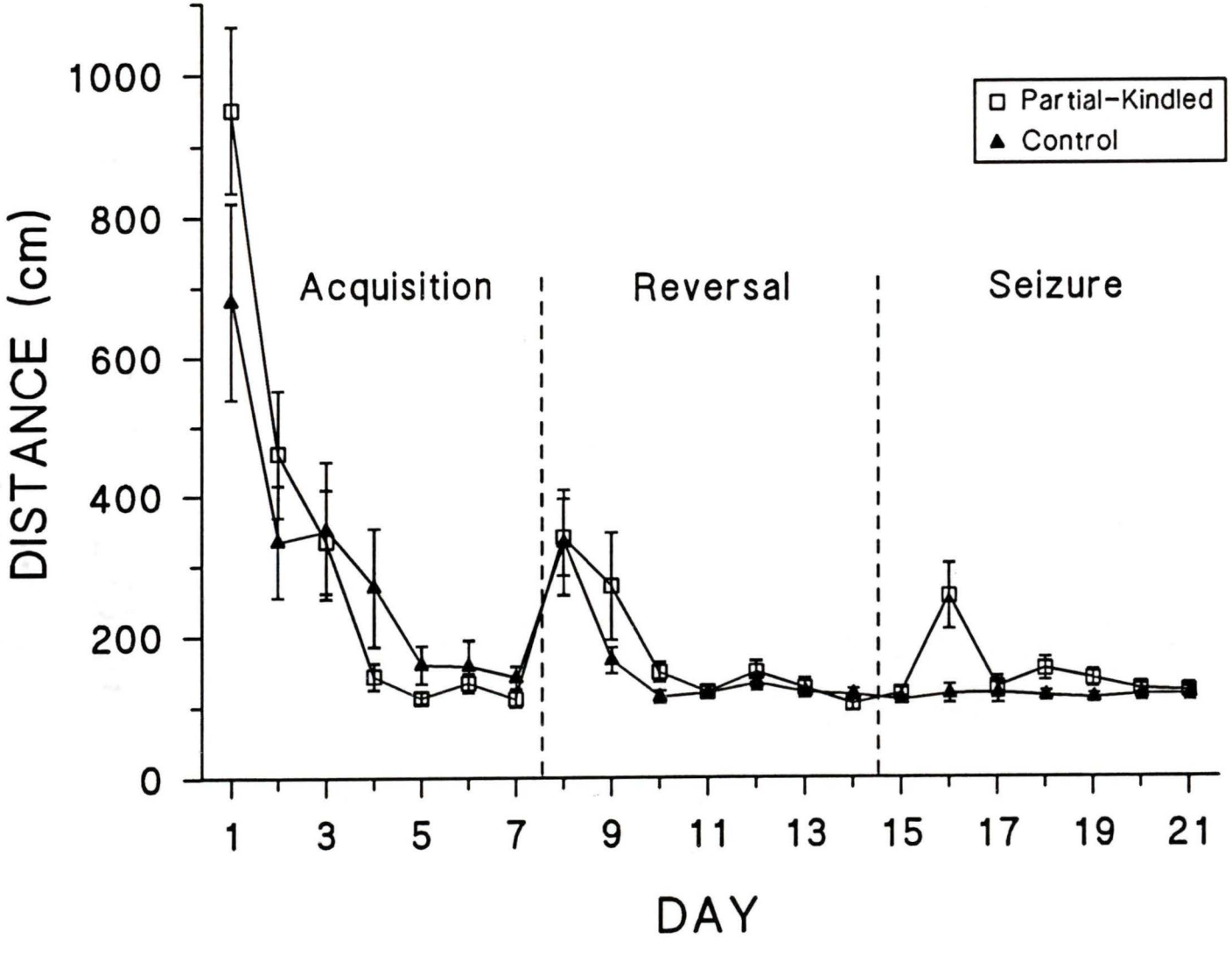
Rats in the partial-kindled group were unimpaired in learning the location of the escape platform as compared to controls (Fig. 4). An overall ANOVA of the partial-kindled group's swim distance revealed a nonsignificant groups effect but a significant days effect [F (6, 276) = 20.63, $P < 0.0001$]. The interaction between groups and days was not significant.

Reversal. Rats in the kindled group showed an impairment in learning the new location of the submerged platform when it was moved directly opposite to the previous quadrant (Fig. 1). An overall ANOVA on swim distance during reversal revealed a significant groups effect [F (1, 58) = 6.08, $P < 0.017$] and days effect [F (6, 348) = 23.01, $P < 0.0001$] but a nonsignificant interaction between groups and days [F (12, 348) = 1.74, $P = 0.110$]. Post-hoc analysis revealed that the kindled group swam significantly longer distances than controls on Days 9, 10, and 14 ($P < 0.040$).

Rats in the partial-kindled group were unimpaired in learning the new location of the submerged platform as compared to controls (Fig. 4). An overall ANOVA on swim distance of the partial-kindled group revealed a nonsignificant groups effect but a significant days effect [F (6, 108) = 4.78, $P < 0.0001$]. The interaction between groups and days was not significant.

Post-Seizure. Triggering a generalized seizure in the kindled group with stimulation of field CA1 25-45 min before daily testing produced a profound deficit in performance, as indicated by the longer swim distances required by the kindled group to locate the submerged platform (Fig. 1). An overall ANOVA on swim distances of

Figure 4. Effects of CA1 partial kindling on maze performance during acquisition. Rats in the partial-kindled group were unimpaired in learning the location of the submerged platform during the acquisition, reversal, and seizure phases.


Partial-Kindled
Control
DISTANCE (cm)
1000
800
600
400
200
0
Acquisition
Reversal
Seizure
1
3
5
7
9
11
13
15
17
19
21
DAY

the kindled group revealed a significant groups effect [F (1, 38) = 29.34, $P <$ 0.0001], days effect [F (6, 228) = 5.71, $P < 0.0001$], and a significant interaction between groups and days [F (12, 228) = 4.72, $P < 0.0001$]. Post-hoc analysis revealed that the kindled group swam significantly longer distances than controls on Days 15-20 ($P < 0.041$) but not on Day 21 ($P = 0.206$).

Triggering an AD in the partial-kindled group 25-45 min before daily testing did not produce a significant deficit in performance (Fig. 4). Analysis of swim distances of the partial-kindled group revealed a nonsignificant groups effect, days effect, and a nonsignificant interaction between groups and days.

Probe Trials. Experimental rats in both the kindled and partial-kindled groups demonstrated a bias for the target quadrant similar to that of controls during the postacquisition, postreversal, and postseizure probe trials. Analysis of the probe trials revealed nonsignificant differences between control and experimental rats in the amount of time spent in the target quadrant searching for the submerged platform (Fig. 5 & 6).

Discussion

The kindling of hippocampal field CA1 until 3 consecutive stage 5 seizures were evoked produced deficits in acquisition in the Morris water maze, as compared to unstimulated controls. This finding partially agrees with the results of Leung et al. (1990), who reported that kindling of convulsive or nonconvulsive seizures with stimulation of field CA1 impaired performance in the radial-arm maze. In disagreement with Leung's results, however, I found in the present experiment that

Figure 5. Probe trial performance following CA1 kindling (Experiment 1). Rats in the kindled group demonstrated dwell times in the target quadrant similar to that of controls during all 3 probe trials (postacquisition, postreversal, and postseizure). Error bars represent S.E.M.

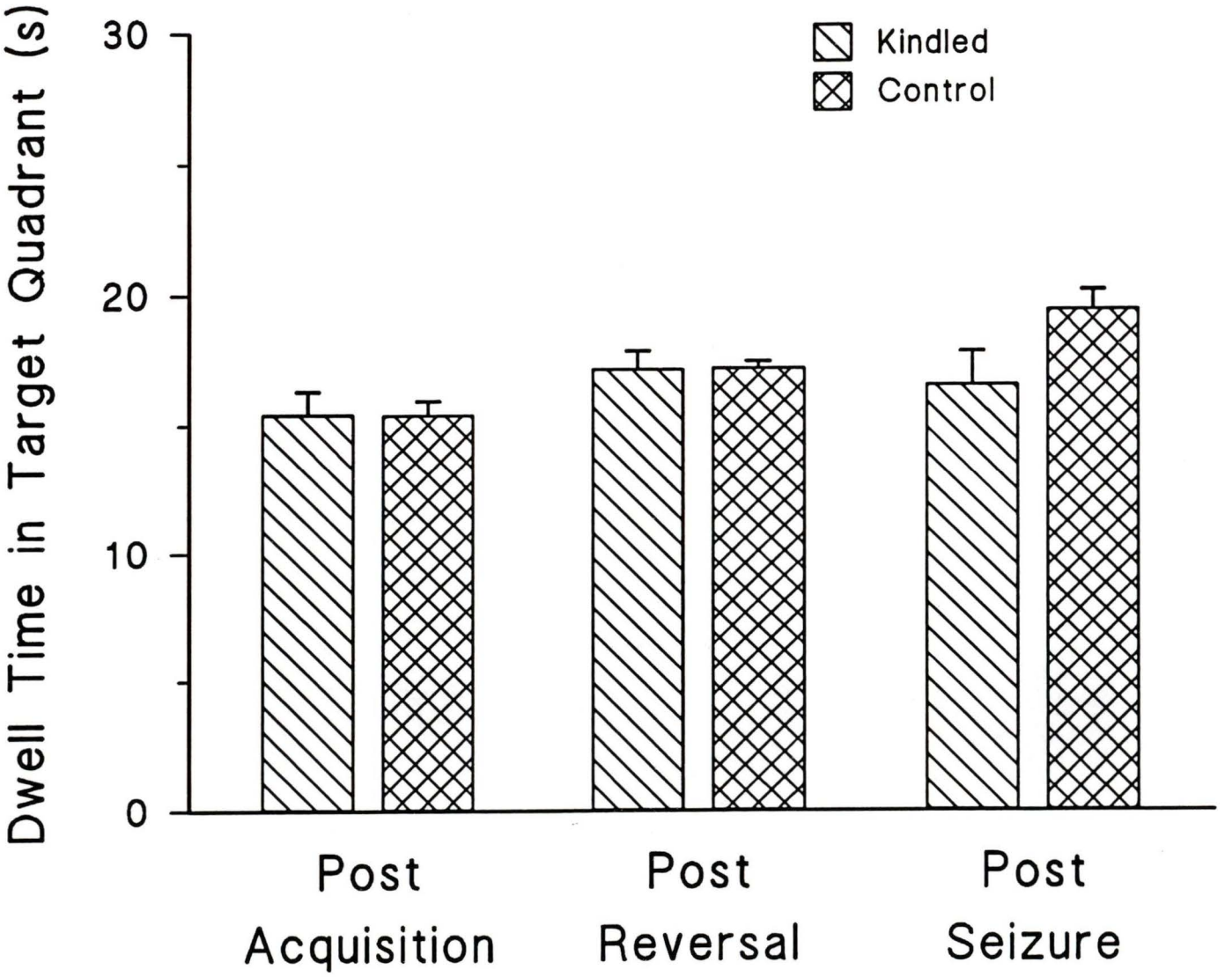
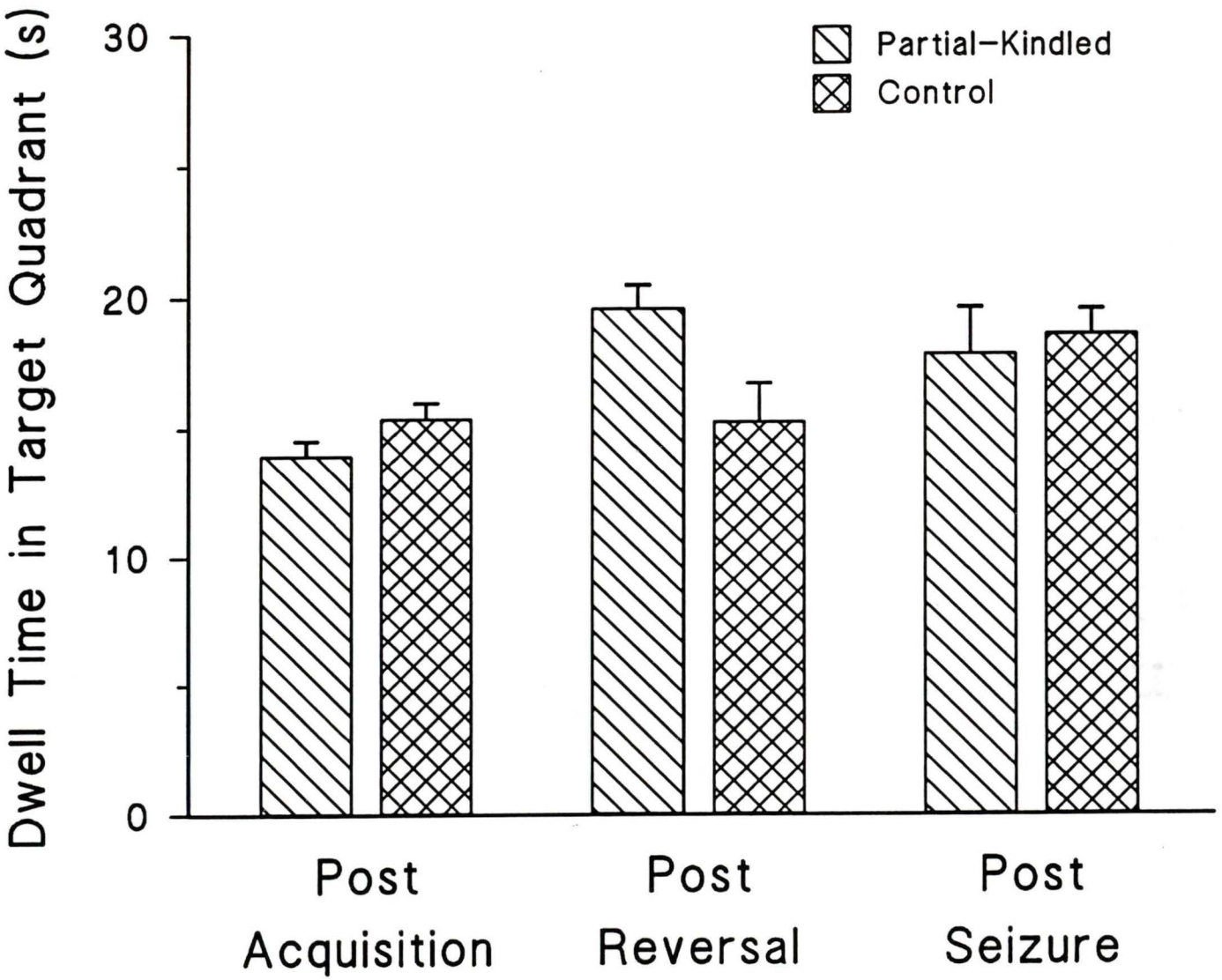

Dwell Time in Target Quadrant (s)
30
20
10
0
Kindled
Control
Post Acquisition
Post Reversal
Post Seizure

Figure 6. Probe trial performance following CA1 partial kindling (Experiment 1). Rats in the partial-kindled group demonstrated dwell times in the target quadrant similar to that of controls during all 3 probe trials (postacquisition, postreversal, and postseizure).


Dwell Time in Target Quadrant (s)
30
20
10
0
Partial–Kindled
Control
Post Acquisition
Post Reversal
Post Seizure

rats in the partial-kindled group were unimpaired in the water maze compared to controls. Leung reported that the deficits persisted at least 3 weeks and concluded that the disruption in performance was due primarily to the ADs and was not necessarily associated with the generalized seizures.

It is possible that the discrepancy in results regarding partial kindling is due to a potential confound in the procedures I used to constitute the partial-kindled group. The partial-kindled group essentially comprised two subgroups, a loosely *matched* group (i.e., matched to the number of ADs leading to generalized seizures in the kindled group) and a *cutoff* group, in which stimulation was suspended after 30 ADs. This procedure may have imposed a selection bias, as it is possible that rats that were matched to the kindled group to equate for total number of ADs were inherently less susceptible to kindling and possibly also to the effects of kindling on maze learning (whereas, it would have to be argued, Leung's group showed a deficit because he suspended kindling stimulation after an arbitrary number of ADs and thereby avoided a selection bias). If the argument about selection is accurate, then it would be predicted that the speed of kindling should be negatively correlated with maze performance. In other words, the prediction would be that rats showing more rapid kindling would also show a greater deficit in maze performance after kindling. I tested this hypothesis by calculating the correlation between the number of ADs to the first stage 5 seizure in the kindled group and the total distance swum in the first 4 days. The correlation was nonsignificant [r = -0.3996, P = 0.1762]. Although this does not conclusively rule out a relation between susceptibility to kindling and

susceptibility to kindling-induced disruption of performance in the water maze, it does suggest that any such relation must be very weak.

Another potential confounding is the relation between the total number of stimulations and the effects on maze performance in the partial-kindled group. A correlation between the total number of ADs and total distance swum in the first 4 days was also nonsignificant [r = -0.125, P = 0.79]. Thus it appears that the effects on maze performance are not necessarily related to either the speed at which generalized seizures are kindled or the total number of ADs evoked. Rather, deficits in performance seem most clearly related to the *state* of kindling (either nonconvulsive or convulsive).

Rats in the kindled group showed impaired performance during both the reversal and seizure phases. The impairment in reversal is not surprising, as the rats' performance was still impaired on 2 of the last 3 days of acquisition. This suggests that the kindled group was slower to acquire reversal simply because they were not performing at control levels during the last few days of acquisition. Perhaps if the number of days in acquisition were extended, the kindled group would perform at control levels and thus might acquire reversal more quickly. The deficit in performance during the seizure phase is not consistent with the results of Leung et al. (1990), and will be examined further in the discussion at the end of Experiment 2.

The performance of the partial-kindled group during the reversal and seizure phases was not impaired. The lack of impairment during reversal is consistent with the lack of impairment during acquisition and simply indicates that the rats were able

to use the appropriate search strategies to locate the submerged platform. The lack of impairment in the seizure phase will be discussed in Experiment 2.

EXPERIMENT 2

Most studies relating CA1 kindling and spatial memory have examined *retention* in the radial-arm maze. A common procedure has been to train the rats to a criterion number of errors (i.e., incorrect arm entries) prior to kindling and then to retest them during kindling or after the cessation of kindling (Leung et al., 1990; Lopes da Silva et al., 1986; Olton and Wolf, 1981). The purpose of Experiment 2 therefore was to examine the effects of CA1 kindling on subsequent relearning (*retention*) in the water maze.

Methods

Twenty-six rats were trained in the water maze (4 trials per day for 7 consecutive days) prior to the commencement of kindling. The rats were then randomly assigned to 1 of 4 groups. The kindled group ($n = 7$), partial-kindled group ($n = 8$), and controls ($n = 6$ and 5, respectively) were subjected to the same postkindling procedures and phases as rats in Experiment 1.

Results

Kindling. Rats in the *kindled* group required 27.57 $\pm$ 3.73 ADs before the first stage 5 seizure was evoked and 35.71 $\pm$ 3.47 ADs before three consecutive stage 5 seizures were evoked. The initial ADT for this group was 20 $\pm$ 0.0 μA. Rats in the *partial-kindled* group were kindled to 42.13 $\pm$ 5.89 ADs and displayed nonconvulsive seizures. The initial ADT for the partial-kindled group was 22.5 $\pm$ 1.96 μA. The mean interval between the end of initial training and the beginning of retention testing for the kindled and partial-kindled groups was 50.9 days and 52 days,

respectively.

Retention. Rats in the kindled group displayed an impairment in relearning the location of the submerged escape platform, as indicated by their longer swim distances as compared to controls (Fig. 7). The controls were able to quickly locate the escape platform when they were retested in the water maze. This suggests that significant "forgetting" did not occur in the controls. An overall ANOVA on swim distance of the kindled group during retention revealed a significant groups effect [F (1, 49) = 12.09, $P < 0.001$] and days effect [F (6, 294) = 4.95, $P < 0.0001$] but a nonsignificant interaction between groups and days [F (12, 294) = 1.92, $P = 0.077$]. Post-hoc analysis revealed that the kindled group swam significantly longer distances than controls on Days 2, 3, and 4 ($P < 0.0383$).

Rats in the partial-kindled group also displayed a significant impairment in relearning the location of the escape platform as compared to controls (Fig. 8). An overall ANOVA on swim distance of the partial-kindled group during acquisition revealed a significant groups effect [F (1, 49) = 5.41, $P < 0.024$] and days effect [F (6, 294) = 6.18, $P < 0.0001$], but a nonsignificant interaction between groups and days [F (12, 294) = 1.55, $P = 0.163$]. Post-hoc analysis revealed that the partial-kindled group swam significantly longer distances than controls on Day 2 only ($P < 0.0382$); the difference on Day 3 was marginally nonsignificant ($P = 0.053$). Representative swim paths of a partial-kindled and control rat on Day 2 of retention are shown in Figure 9.

Reversal. Rats in the kindled group were unimpaired in learning the new

Figure 7. Effects of CA1 kindling on maze performance during retention. Rats in the kindled group were impaired in relearning the location of the submerged escape platform during retention, as indicated by their longer swim distances as compared to controls. Note that maze performance was unimpaired during reversal, only to be hindered once daily kindling stimulation was instituted. $*P < 0.05$.

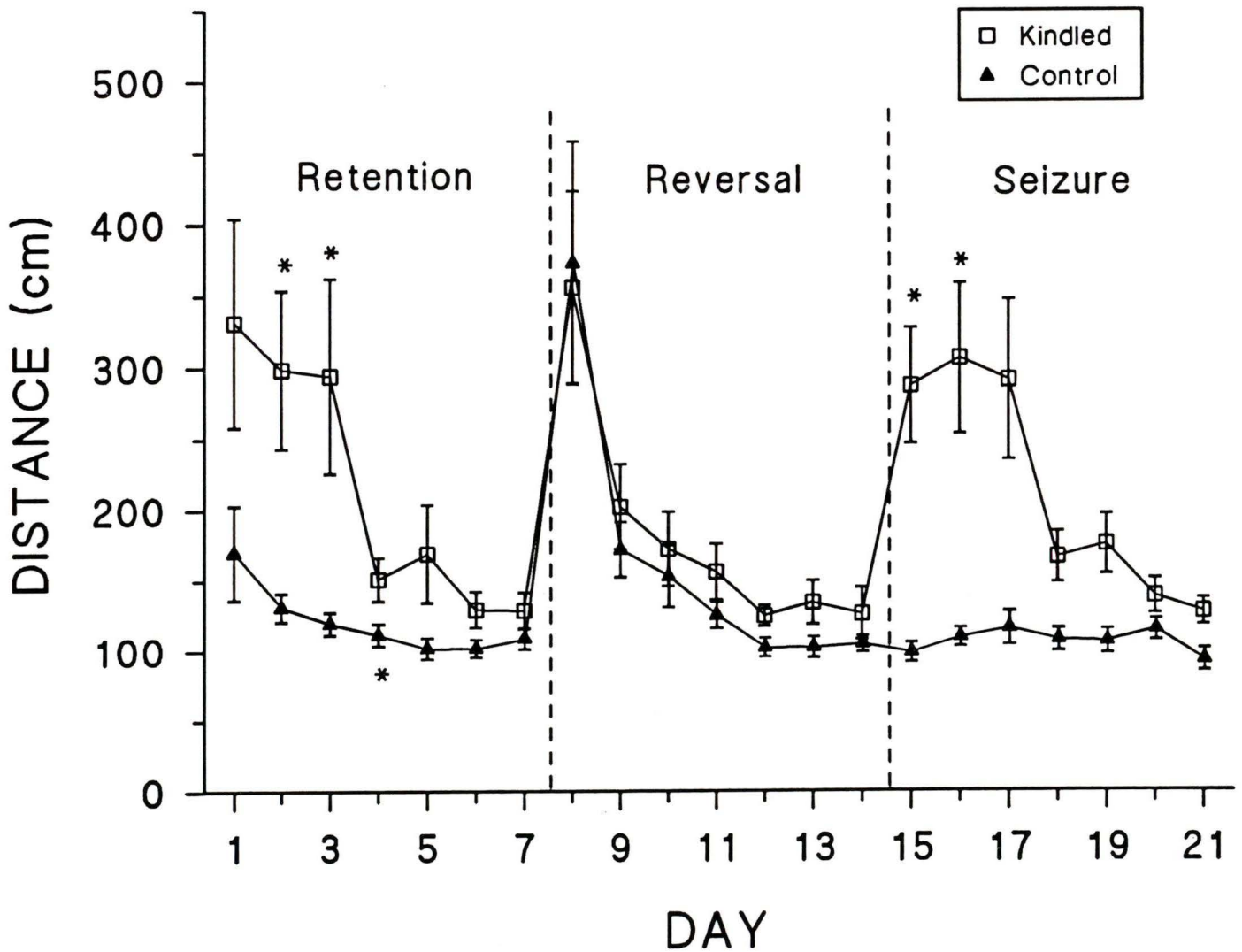

Kindled
Control
Retention
Reversal
Seizure
DISTANCE (cm)
500
400
300
200
100
0
1
3
5
7
9
11
13
15
17
19
21
DAY

Figure 8. Effects of CA1 partial kindling on maze performance during retention. Rats in the partial-kindled group were impaired in relearning the location of the submerged escape platform during retention, as indicated by their longer swim distances as compared to controls. Note that maze performance was unimpaired during reversal. Note also that despite a pronounced tendency for longer swim distances during the seizure phase, the partial-kindled group did not differ significantly from controls. $*P < 0.05$.

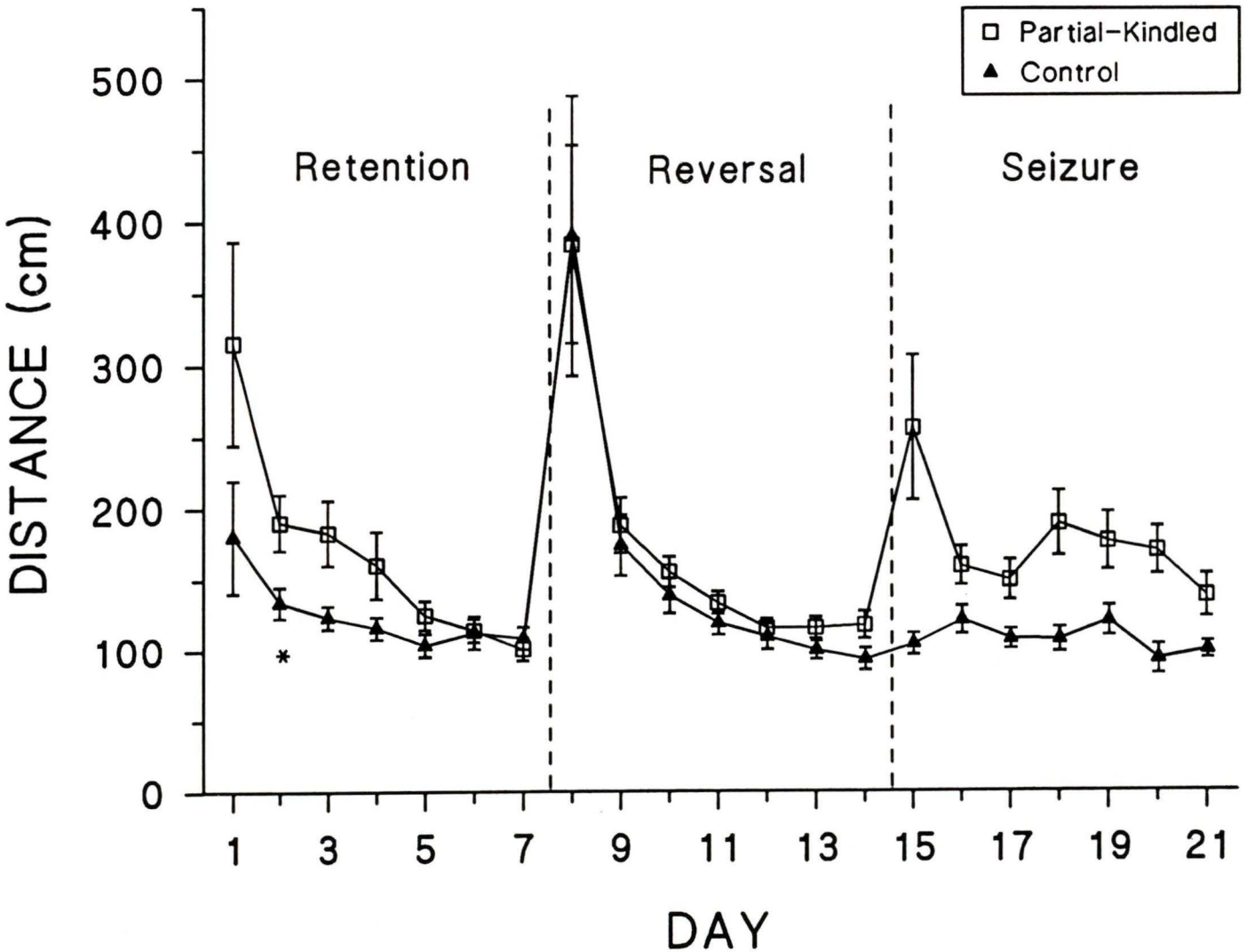
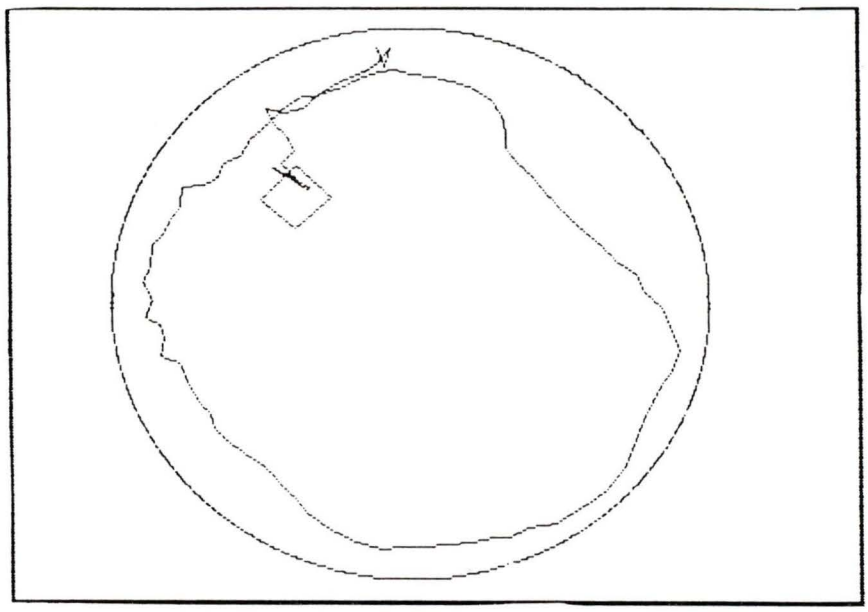
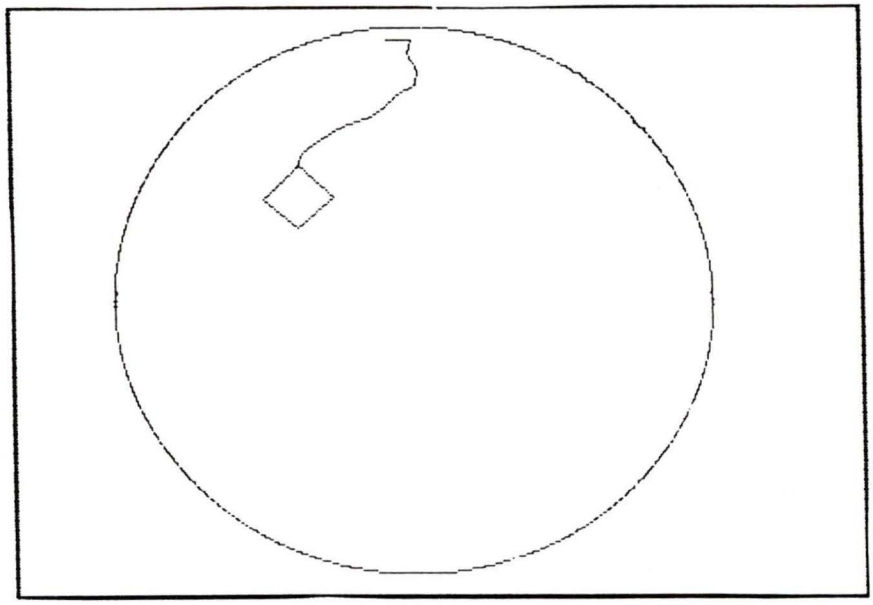

Partial-Kindled
Control
Retention
Reversal
Seizure
DISTANCE (cm)
500
400
300
200
100
0
*
1
3
5
7
9
11
13
15
17
19
21
DAY

Figure 9. Representative swim paths of a partial-kindled rat (upper trace) compared to its control (lower trace) on Day 2 of retention. Note the longer swim path in the partial-kindled rat.





location of the submerged platform when it was moved directly opposite to the previous quadrant (Fig. 7). An overall ANOVA on swim distance during reversal revealed a nonsignificant groups effect, but a significant days effect [F (6, 252) = 12.19, $P < 0.0001$]. The interaction between groups and days was not significant.

Rats in the partial-kindled group were also unimpaired in learning the new location of the submerged platform (Fig. 8). An overall ANOVA on swim distance of the partial-kindled group revealed a nonsignificant groups effect, but a significant days effect [F (6, 174) = 10.9, $P < 0.0001$]. The interaction between groups and days was also not significant.

Post-Seizure. Triggering a generalized seizure in the kindled group with stimulation of field CA1 25-45 min before daily testing produced a deficit in performance, as indicated by the longer swim distances required by the kindled group to locate the submerged platform (Fig. 7). An overall ANOVA on swim distances of the kindled group during the post-seizure phase revealed a significant groups effect [F (1, 17) = 11.08, $P < 0.004$] but a nonsignificant days effect and interaction between groups and days. Post-hoc analysis revealed that the kindled group swam significantly longer distances than controls on Days 15 and 16 ($P < 0.0239$). The differences on Days 17-19 were marginally nonsignificant ($P > 0.0558$).

Triggering an AD in the partial-kindled group 25-45 min before daily testing did not significantly affect performance (Fig. 8). Analysis of swim distances of the partial-kindled group revealed a nonsignificant groups and days effects and a nonsignificant interaction between groups and days.

Probe Trials. Experimental rats in both the kindled and partial-kindled groups demonstrated a bias for the target quadrant similar to that of controls during the postretention, postreversal, and postseizure probe trials. Analysis of the probe trials revealed nonsignificant differences between control and experimental rats in the amount of time spent in the target quadrant searching for the submerged platform (Figs. 10 & 11).

Discussion

The kindling of nonconvulsive or convulsive seizures from stimulation of field CA1 produced deficits in retention in the Morris water maze, as compared to controls. This finding supports the results of Leung et al. (1990), who reported that kindling of nonconvulsive or generalized seizures disrupts performance in the radial-arm maze. The procedure of Experiment 2 is closer to that of Leung et al. than was Experiment 1, in that Leung et al. trained their rats in the maze prior to the commencement of kindling.

Rats in the kindled and partial-kindled groups were not impaired during the reversal phase, and only the kindled group was impaired during the seizure phase. The lack of impairment in reversal is not surprising, as the rats were performing near control levels during the last 3 days of retention. This suggests that the rats learned that there was an escape platform and were able to incorporate the necessary search strategies to escape, regardless of the location of the platform. The deficit in performance I observed in the kindled groups during the seizure phase (Experiments 1 and 2) is inconsistent with the results of Leung et al. (1990), who reported recovery of

Figure 10. Probe trial performance following CA1 kindling (Experiment 2). Rats in the kindled group demonstrated dwell times in the target quadrant similar to that of controls during all 3 probe trials (postretention, postreversal, and postseizure).

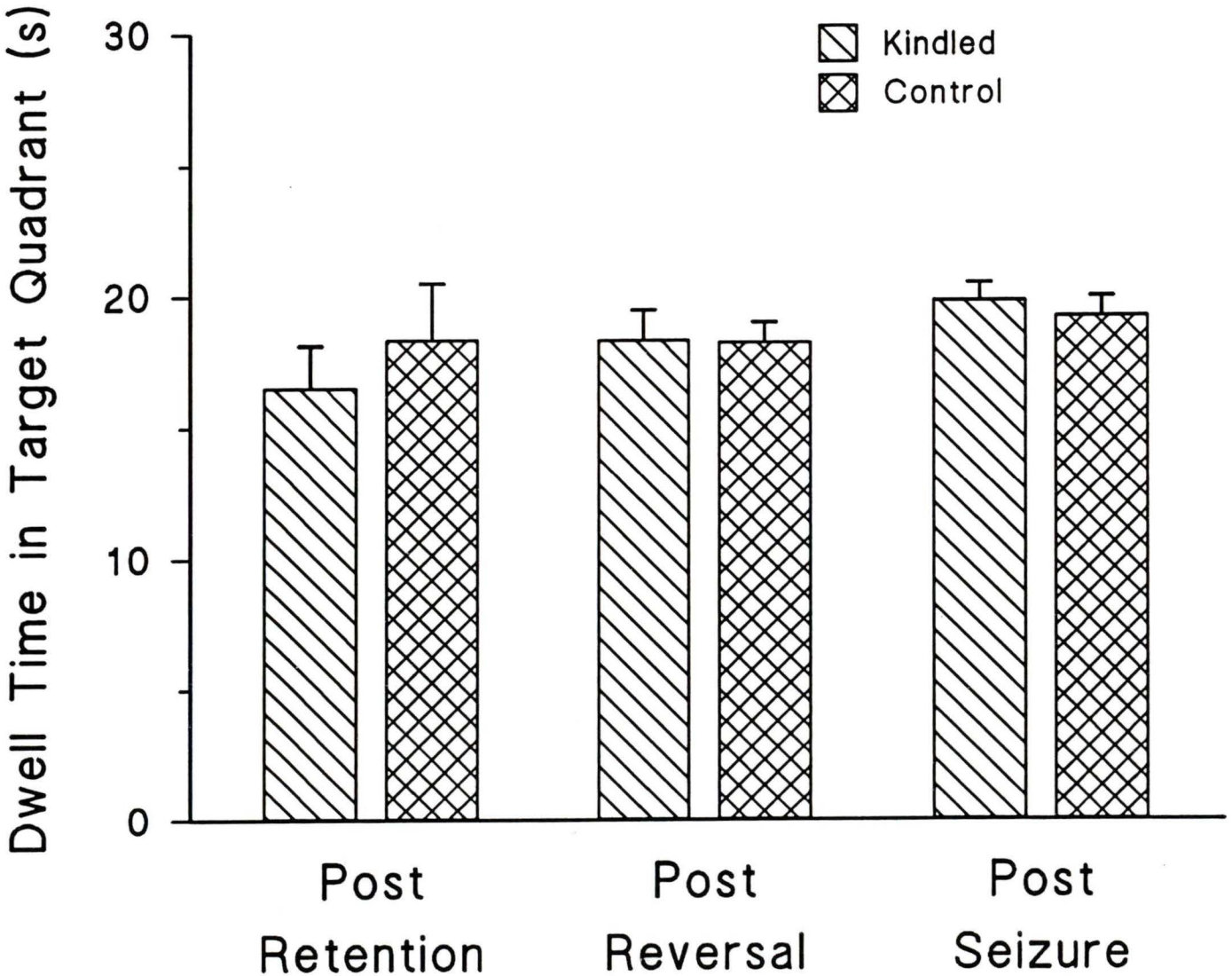
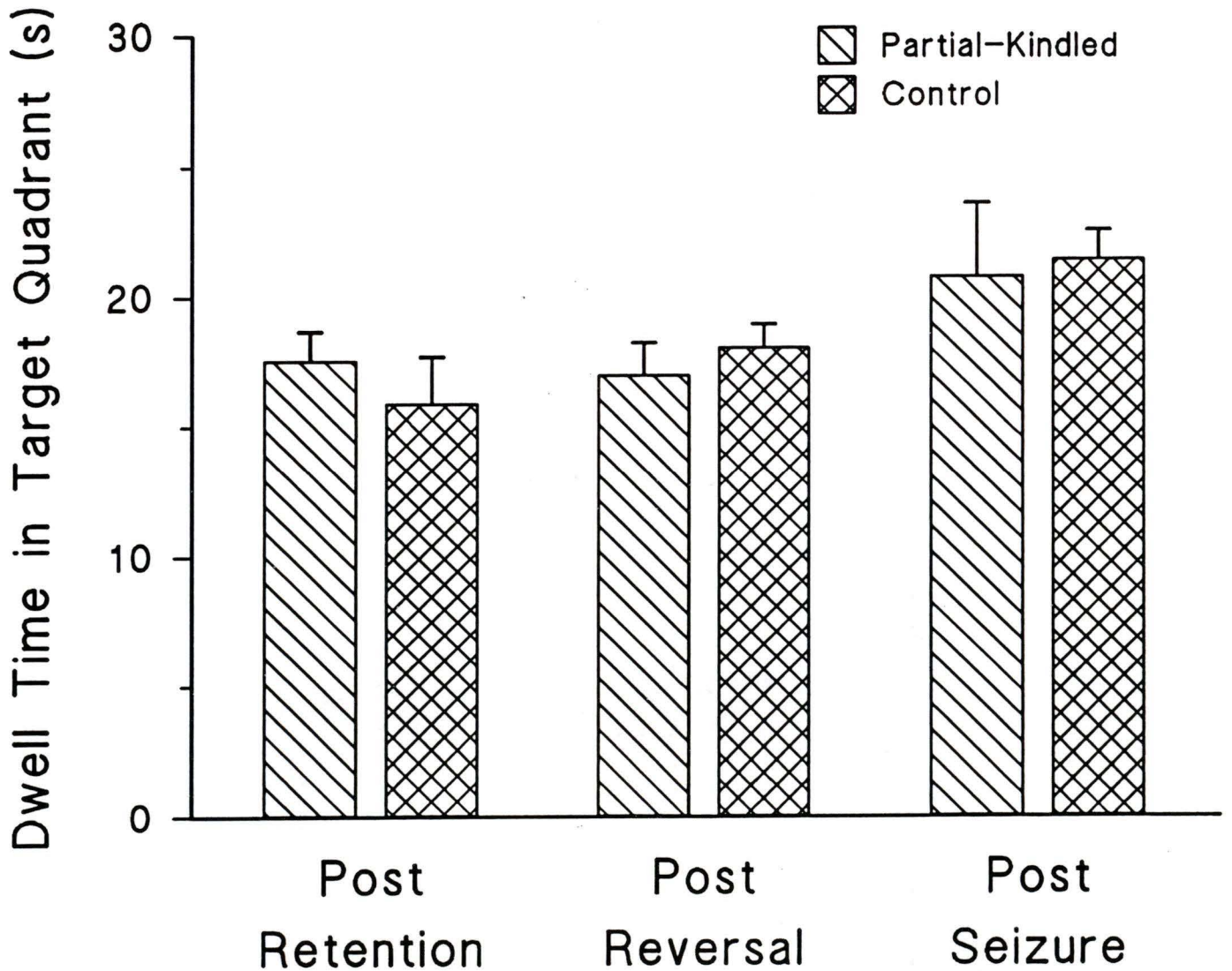

Dwell Time in Target Quadrant (s)
30
20
10
0
Kindled
Control
Post Retention
Post Reversal
Post Seizure

Figure 11. Probe trial performance following CA1 partial kindling (Experiment 2). Rats in the partial-kindled group demonstrated dwell times in the target quadrant similar to that of controls during all 3 probe trials (postretention, postreversal, and postseizure).


Dwell Time in Target Quadrant (s)
30
20
10
0
Partial–Kindled
Control
Post
Retention
Post
Reversal
Post
Seizure

function despite continuing daily ADs. However, the two studies differ in the interval in which the stimulation was implemented. I delivered stimulation to the rats 25-45 min prior to daily testing, whereas Leung et al. stimulated immediately following each daily trial. Thus it appears that kindling and short-term epileptiform activity initiated in field CA1 are responsible for disrupting performance on an already learned spatial task.

One should not put too much reliance on the lack of a significant performance deficit in the partial-kindled groups (Experiments 1 and 2) during the seizure phase. Due to the small number of subjects that completed this phase ($n = 3$ in Experiment 1; $n = 2$ in Experiment 2), the results may perhaps not be reliable.

EXPERIMENT 3

Several investigators have examined the short-term effects of hippocampal kindling on spatial memory (Feasey-Truger et al., 1993; McNamara et al., 1992; Olton and Wolf, 1981; Cain, Hargreaves, Boon, and Dennison, 1993; Lopes da Silva et al., 1986; Robinson et al., 1993). The general findings of all these studies, despite differences in procedure and site of stimulation, are that spatial memory is disrupted if it is measured at a relatively short interval after triggering epileptiform activity. To determine whether this result would be found in my paradigm, in Experiment 3 I therefore examined the short-term aftereffects of epileptiform activity triggered in field CA1 of the hippocampus during both acquisition and retention in the Morris water maze.

Methods

Twenty-seven rats were separated into 4 groups: (1) AD group (n = 9); (2) AD control group (n = 9); (3) retention-seizure group (n = 5); (4) retention-control group (n = 4). Rats in the AD group received stimulation that evoked an AD prior to daily training in the water maze. Over the course of acquisition, these rats experienced a total of 7 ADs. Rats in the retention-seizure group were trained in the maze prior to kindling. Twenty-four hrs after development of asymptotic performance in the water maze (4 trials per day for 7 days), maze testing was suspended and the rats received daily stimulation until three consecutive stage 5 seizures had been kindled. Subsequently, maze testing was resumed and the rats continued to receive stimulation prior to daily testing in the maze; over the course of retention they

experienced a total of 7 generalized seizures. Testing in the maze for all rats in this experiment began 25-45 min after induction of an AD or seizure. This interval has been shown to be sufficient for recovery of both behaviour and normal EEG activity (McNamara et al., 1992).

On the day after the completion of acquisition or retention, a cue task was introduced in which the rats were required to navigate to a visible platform located in the center of a different quadrant on each trial. The cue task was designed to assess whether any general sensorimotor or motivational factors interfered with the rats' ability to escape, in a simpler situation in which escape could be guided by proximal rather than distal spatial cues. Experimental rats received kindling stimulation 25-45 min prior to visible platform testing.

Recovery. On the day following the visible platform task, daily kindling stimulation was discontinued in all groups. The platform remained in the same quadrant (northwest) as in acquisition and retention; rats were given a further 4 trials daily for 7 consecutive days. Twenty-five to forty-five minutes prior to daily maze testing, all rats were placed in the plexiglass chamber used in the kindling procedure, but no stimulation was delivered. A 30 s probe trial was given after the final trial on the last day of recovery.

Layoff. On the day following the completion of the recovery phase, all groups were subjected to a period in which the rats were not handled, stimulated, or tested in the water maze. The period lasted 7 days for rats in the AD groups and 14 days for rats in the seizure groups. The longer interval was chosen because, in studies in the

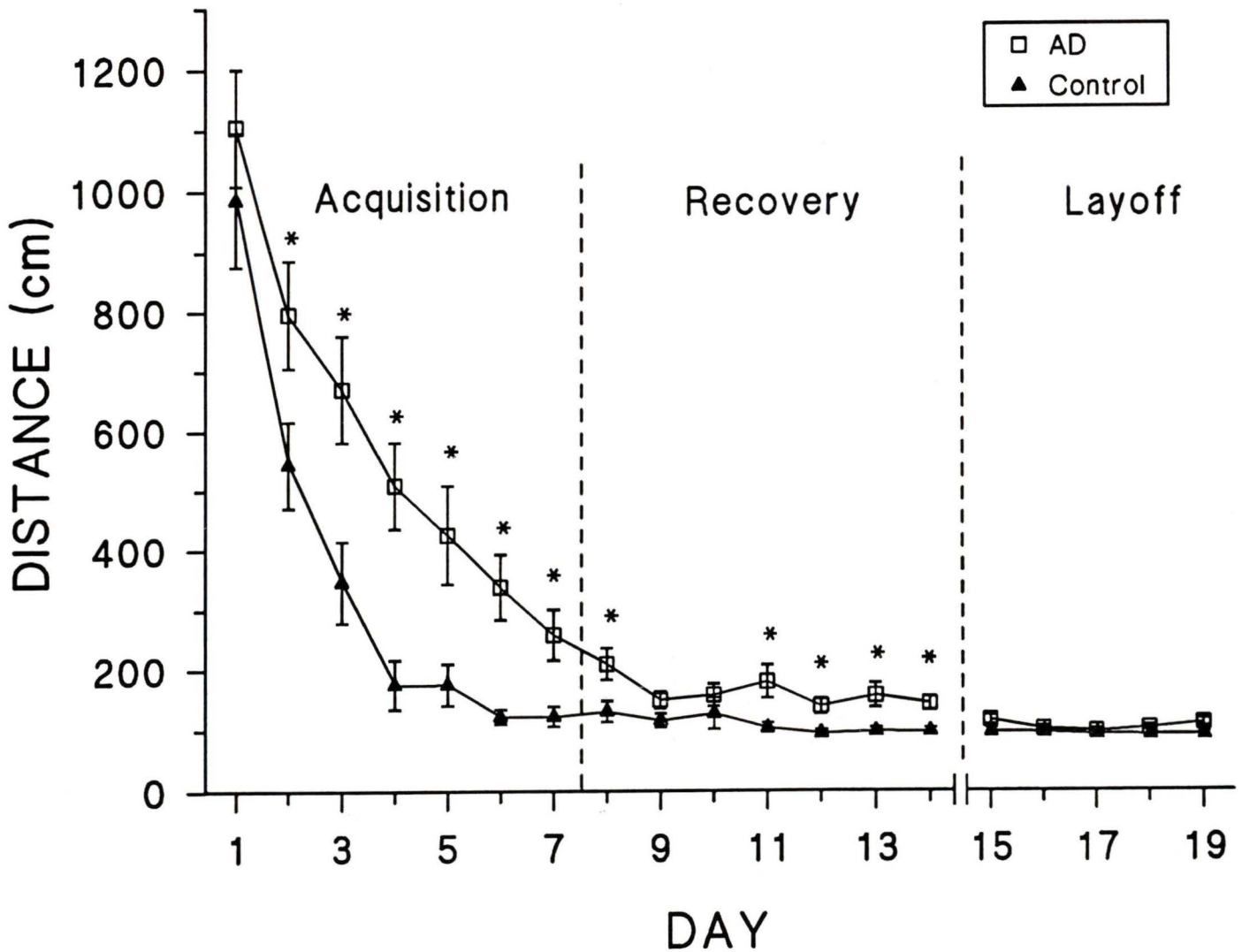
radial-arm maze, kindled rats showed impaired performance and controls showed asymptotic performance when tested at this interval (Leung et al., 1990). Following the period in which all testing was suspended, rats in all groups were tested in the water maze over the next 5 days at 4 trials per day. This was followed by a probe trial after the last trial on the last day. The submerged platform remained in the same quadrant (northwest) over the entire 3 phases of the Experiment.

Results

Kindling. As mentioned previously, rats in the *AD* group experienced a total of 7 ADs during acquisition and an additional AD just prior to visible platform testing. Rats in the *seizure* group required 29.4 $\pm$ 6.27 ADs before the first stage 5 seizure was evoked and 37.8 $\pm$ 5.82 ADs before three consecutive stage 5 seizures were evoked. These rats experienced a total of 7 generalized seizures during retention and an additional seizure just prior to visible platform testing. The initial ADTs for the AD and seizure groups were 22.22 $\pm$ 1.47 μA and 22.0 $\pm$ 1.49 μA, respectively.

Acquisition and Retention. Triggering an AD in field CA1 25-45 min before daily testing in the water maze produced a deficit in acquisition, as indicated by the longer swim distances required by the experimental group to locate the submerged platform (Fig. 12). An overall ANOVA on swim distance of the AD group during acquisition revealed a significant groups effect [F (1, 78) = 18.16, $P < 0.0001$] and days effect [F (6, 468) = 41.66, $P < 0.0001$], but a nonsignificant interaction between groups and days [F (12, 468) = 0.78, $P = 0.584$]. Post-hoc analysis revealed that the AD group swam significantly longer distances than controls on Days

Figure 12. Effects of CA1 evoked ADs on water maze performance during acquisition. Triggering an AD in field CA1 25-45 min before daily maze testing produced a deficit in acquisition, as indicated by the longer swim distances required by the AD group to locate the submerged platform. Note that maze performance in the AD group did not recover to control levels despite the discontinuation of stimulation. Note also that despite similar swim distances during the layoff phase, the differences were significant ($P < 0.03$). $*P < 0.05$.


AD
Control
Acquisition
Recovery
Layoff
DISTANCE (cm)
1200
1000
800
600
400
200
0
1
3
5
7
9
11
13
15
17
19
DAY

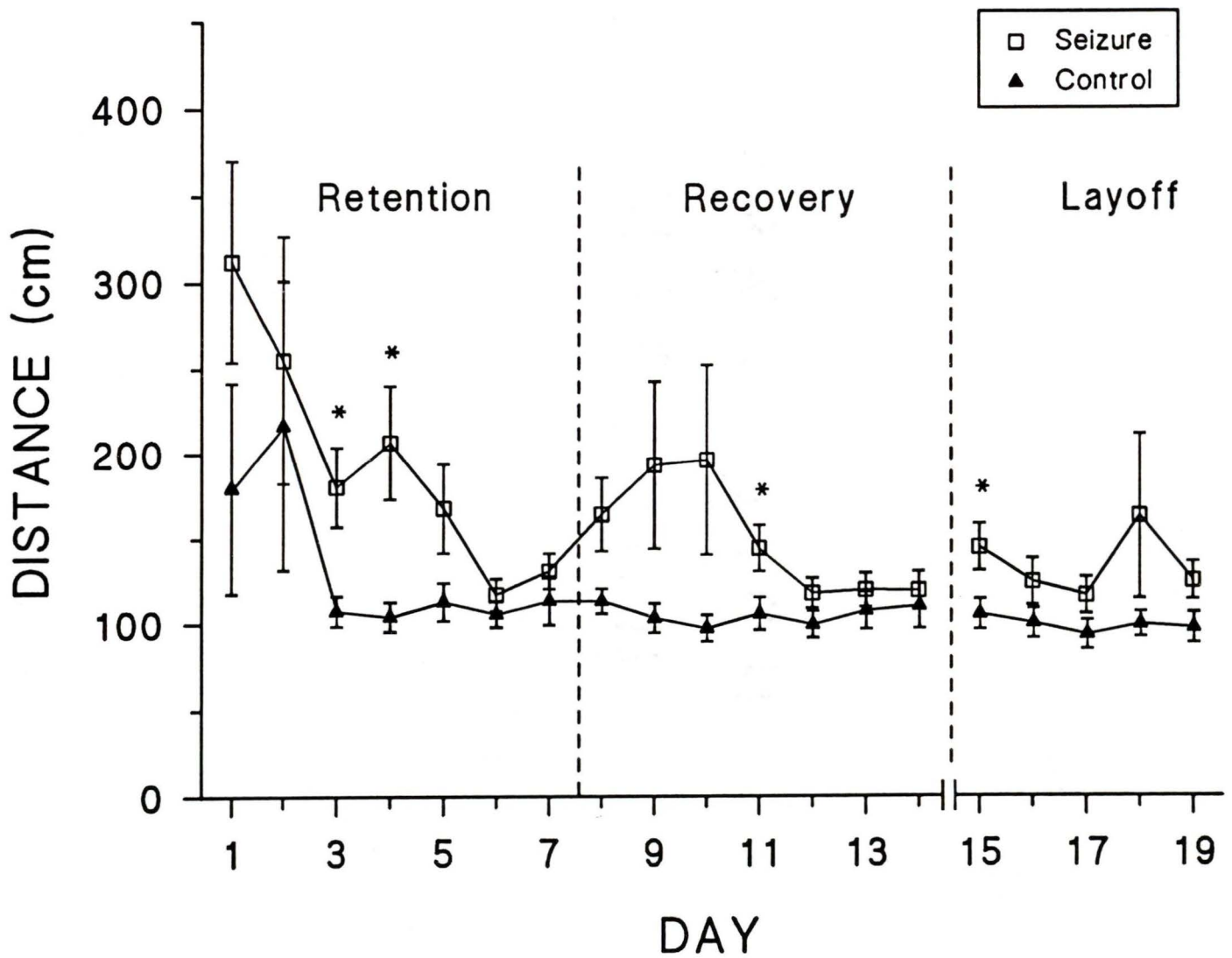
2-7 ($P < 0.040$).

Triggering a generalized seizure 25-45 min prior to daily testing in rats that had previously been trained in the water maze produced a deficit in performance (Fig. 13). An overall ANOVA on swim distance of the seizure group during retention indicated a significant groups effect [F (1, 34) = 5.05, $P < 0.031$] and days effect [F (6, 204) = 3.60 $P < 0.002$], but a nonsignificant interaction between groups and days [F (12, 204) = 0.62, $P = 0.716$]. Post-hoc analysis revealed that the seizure group swam significantly longer distances than controls on Day 3 and 4 ($P < 0.0135$).

Recovery. The performance of rats in the AD group did not recover to control levels during the one week free of stimulation following acquisition (Fig. 12). An overall ANOVA on swim distance during recovery revealed a significant groups effect [F (1, 58) = 12.61, $P < 0.001$] and days effect [F (6, 348) = 2.14, $P < 0.048$], but a nonsignificant interaction between groups and days [F (12, 348) = 0.62, $P = 0.714$]. Post-hoc analysis revealed that the AD group swam significantly longer distances than controls on Days 8, 11, 12, 13, and 14 ($P < 0.0211$).

The performance of rats in the seizure group also did not recover to control levels during the one week free of stimulation following retention (Fig. 13). An overall ANOVA on swim distance during recovery revealed a significant groups effect [F (1, 32) = 6.00, $P < 0.020$], but a nonsignificant days effect and a nonsignificant interaction between groups and days. Post-hoc analysis revealed that the seizure group swam significantly longer distances than controls on Day 11 only ($P < 0.0392$), with a marginally nonsignificant difference occurring on Day 8 ($P = 0.0551$).

Figure 13. Effects of CA1 kindled seizures on water maze performance during retention. Triggering a generalized seizure 25-45 min prior to daily testing in rats that had been previously trained in the water maze produced a deficit in performance, as indicated by the longer swim distances required by the seizure group to locate the submerged platform. Note that maze performance in the seizure group did not recover to control levels despite the discontinuation of stimulation. Note also that the seizure group did not perform to control levels following the period in which maze testing was suspended. $*P < 0.05$.


Seizure
Control
Retention
Recovery
Layoff
DISTANCE (cm)
400
300
200
100
0
1
3
5
7
9
11
13
15
17
19
DAY
*
*
*
*

Layoff. Rats in the AD group were not able to significantly perform to control levels following a one week period in which maze testing was suspended (Fig. 12). An overall ANOVA of swim distances during the layoff phase revealed a significant groups effect [F (1, 38) = 9.72, $P < 0.003$] but a nonsignificant days effect and a nonsignificant interaction between groups and days. Post-hoc analysis revealed no significant differences between control and experimental rats on any day during the layoff phase.

Rats in the seizure group also did not perform to control levels following the period in which maze testing was suspended (Fig. 13). An overall ANOVA on swim distance during the layoff phase revealed a significant groups effect [F (1, 34) = 6.17, $P < 0.018$] but a nonsignificant days effect and a nonsignificant interaction between groups and days. Post-hoc analysis revealed that the seizure group swam significantly longer distances than controls on Day 15 only ($P < 0.0350$).

Probe Trials. Experimental rats in both the AD and seizure groups demonstrated a bias for the target quadrant similar to that of controls during the probe trials. Analysis of the postacquisition, postretention, and postrecovery probe trials revealed nonsignificant differences between control and experimental rats in the amount of time spent in the target quadrant searching for the submerged platform (Figs. 14 & 15). However, during the postlayoff probe, rats in the AD group displayed a shorter dwell time in the target quadrant than controls (14.84 $\pm$ 0.68 s vs. 18.86 $\pm$ 0.89 s). Analysis of dwell time in the target quadrant during the postlayoff probe revealed a significant groups effect [F (1, 9) = 6.67, $P < 0.0325$].

Figure 14. Probe trial performance following CA1 evoked ADs (Experiment 3). Rats in the AD group demonstrated dwell times in the target quadrant similar to that of controls during the postacquisition and postrecovery probe trials. Note that during the postlayoff probe trial, the AD group displayed a shorter dwell time in the target quadrant than controls ($P < 0.0325$).

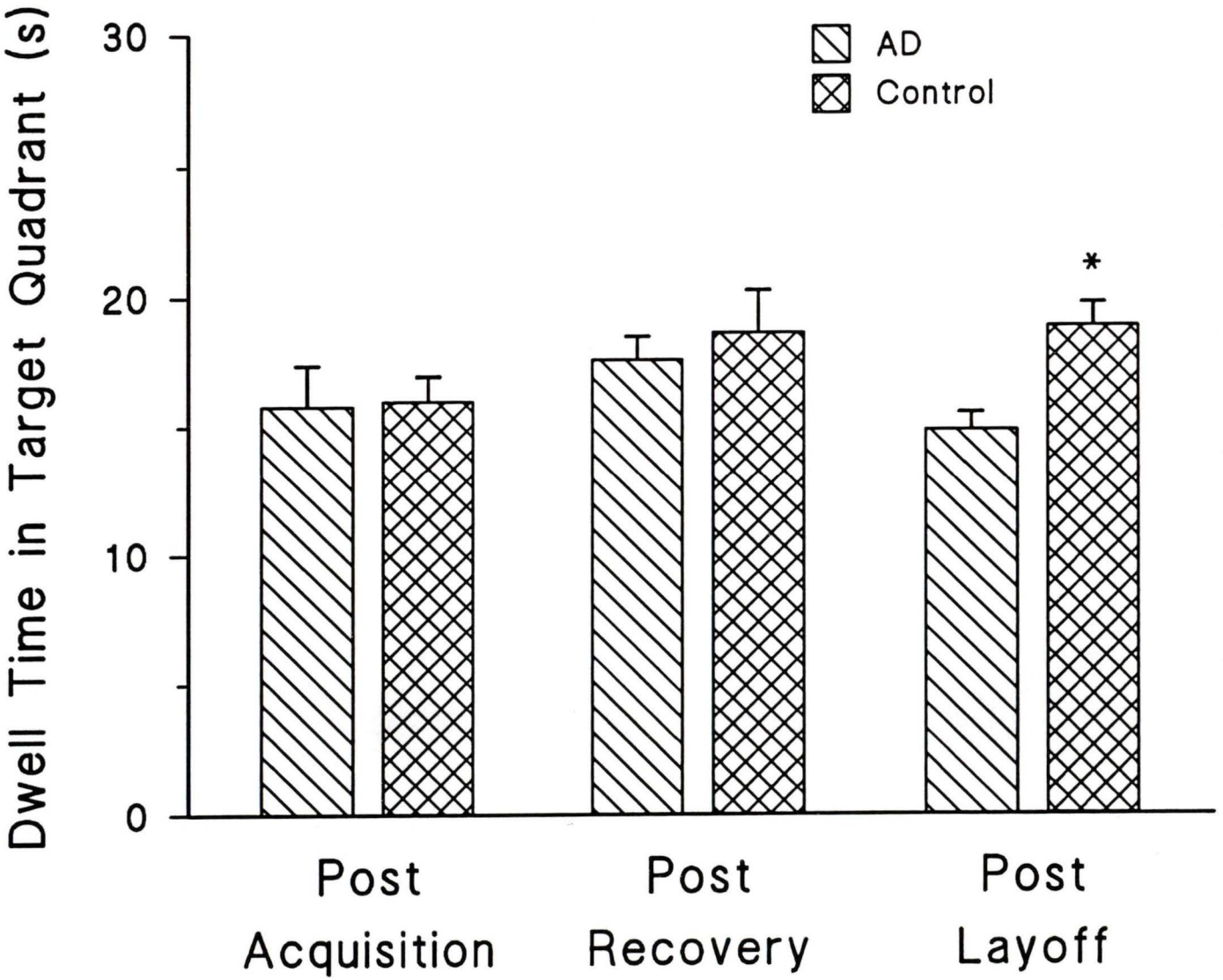
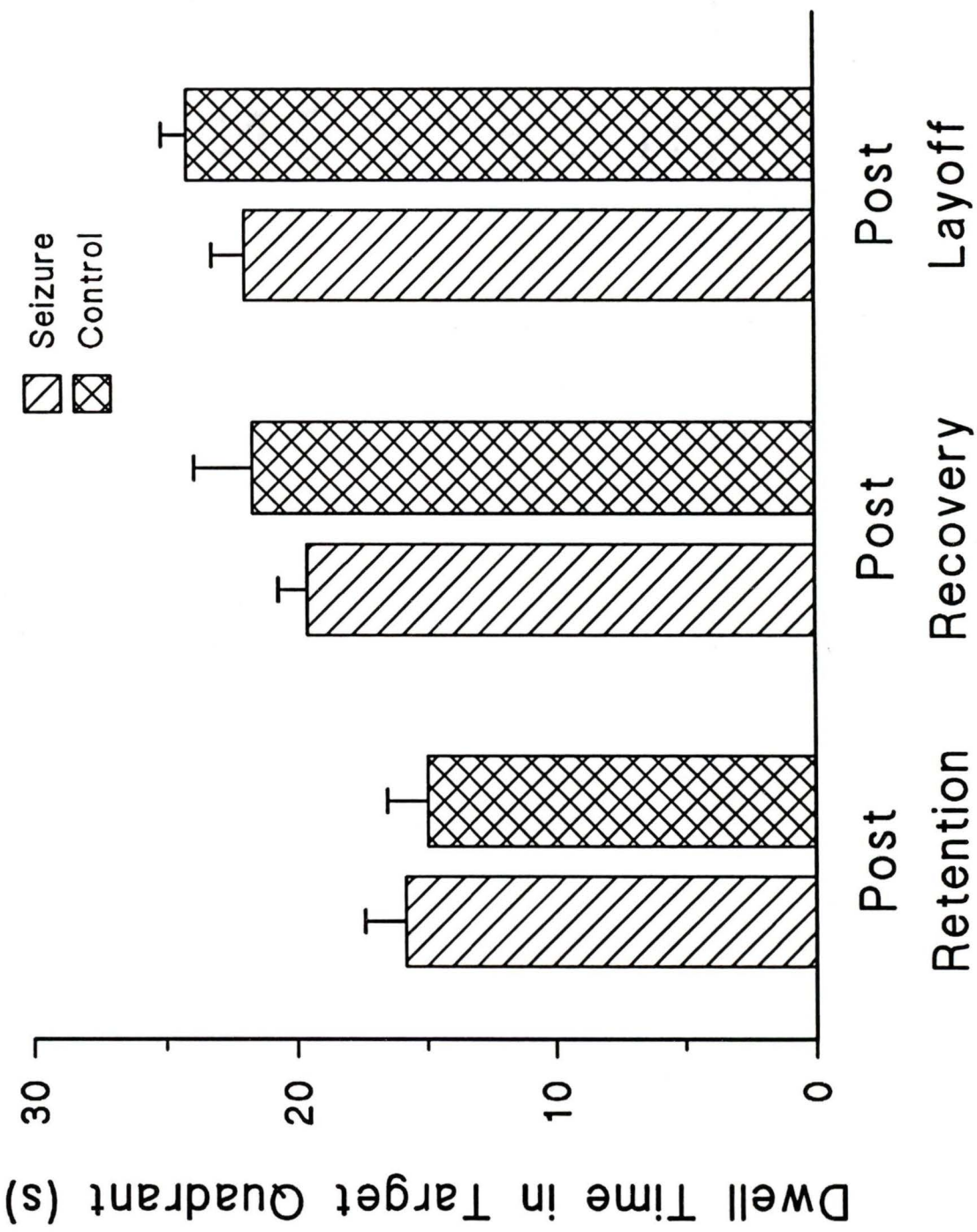

Dwell Time in Target Quadrant (s)
30
20
10
0
AD
Control
*
Post Acquisition
Post Recovery
Post Layoff

Figure 15. Probe trial performance following CA1 kindled seizures (Experiment 3). Rats in the seizure group demonstrated dwell times in the target quadrant similar to that of controls during the postretention, postrecovery, and postlayoff probe trials.


Dwell Time in Target Quadrant (s)
30
20
10
0
Seizure
Control
Post
Retention
Post
Recovery
Post
Layoff

Visible Platform. Triggering an AD or generalized seizure 25-45 min prior to maze testing did not have any significant effect on the rats' ability to escape to a visible platform. Rats in the AD group required 6.33 $\pm$ 0.44 s to escape to the visible platform, and controls required 6.51 $\pm$ 0.86 s. Rats in the seizure group required 8.47 $\pm$ 1.99 s to escape to the visible platform, and controls required 6.35 $\pm$ 1.02 s (Fig. 16). Neither ADs nor generalized seizures had any significant effects on swim speeds during visible platform testing. Rats in the AD group swam at a speed of 26.73 $\pm$ 1.08 cm/s, and controls swam at 26.52 $\pm$ 1.32 cm/s. Rats in the seizure group swam at a speed of 30.32 $\pm$ 1.70 cm/s, and controls swam at 29.51 $\pm$ 1.65 cm/s (Fig. 17).

Discussion

The results of Experiment 3 confirm previous reports that induction of ADs or generalized seizures prior to daily testing result in deficits in acquisition of the Morris water maze. Acquisition deficits were found in the Morris water maze (McNamara et al., 1992) and in the radial-arm maze (Robinson et al., 1993) when seizures were evoked in the PP. Furthermore, I found that triggering of seizures produced deficits in performance of previously established maze learning, which I have referred to as a measure of retention. These findings are consistent with the previous results from Leung et al. (1990).

In the present experiment, the deficits in performance persisted for at least one week after the last AD and at least three weeks after the last generalized seizure. This finding is consistent with previous reports that disruption in retention in the radial

Figure 16. Effects of CA1 evoked ADs and kindled seizures on latency to escape to a visible platform. Triggering an AD or generalized seizure 25-45 min prior to maze testing did not have any significant effect on the latency to escape to a visible platform.

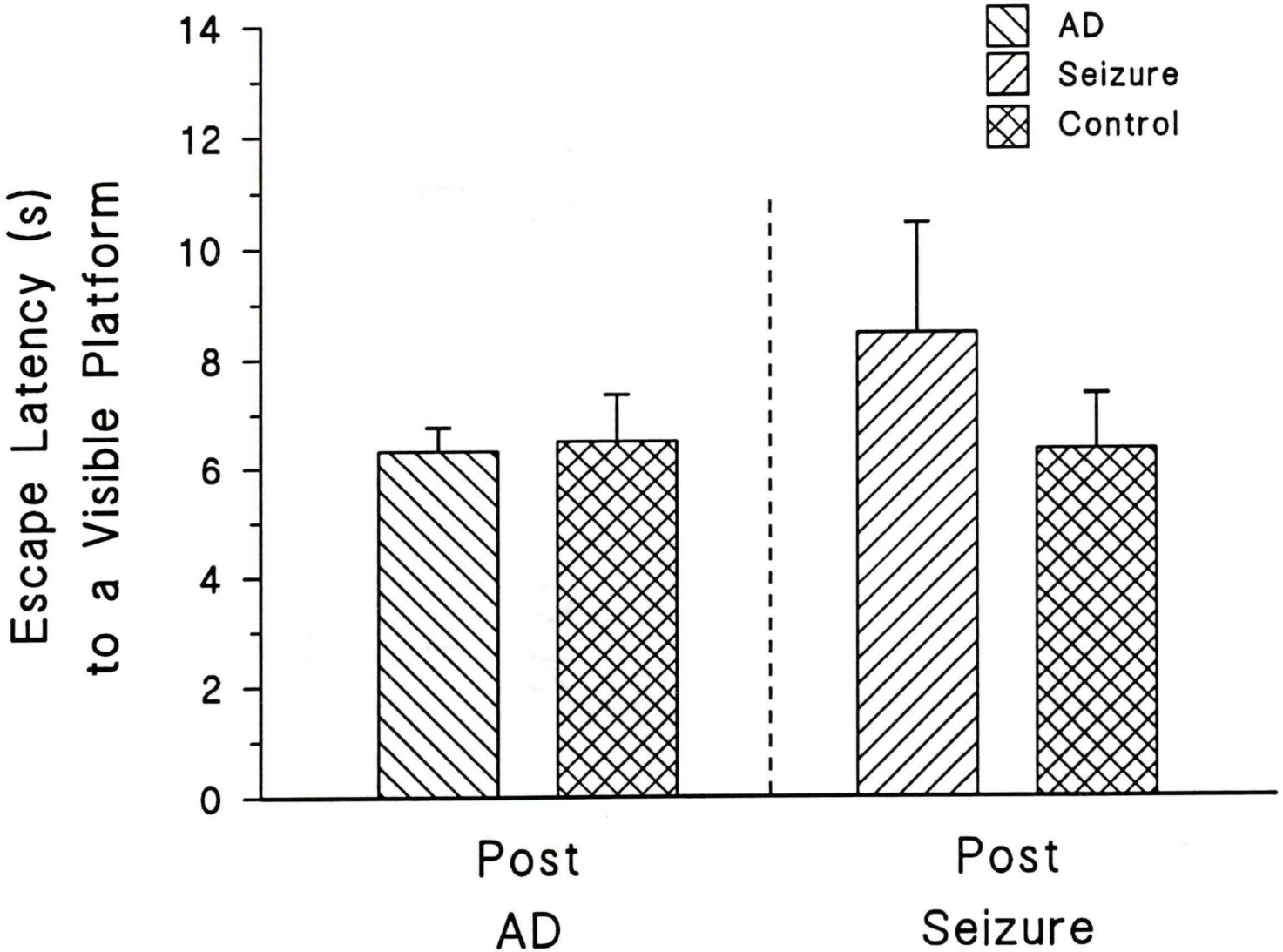
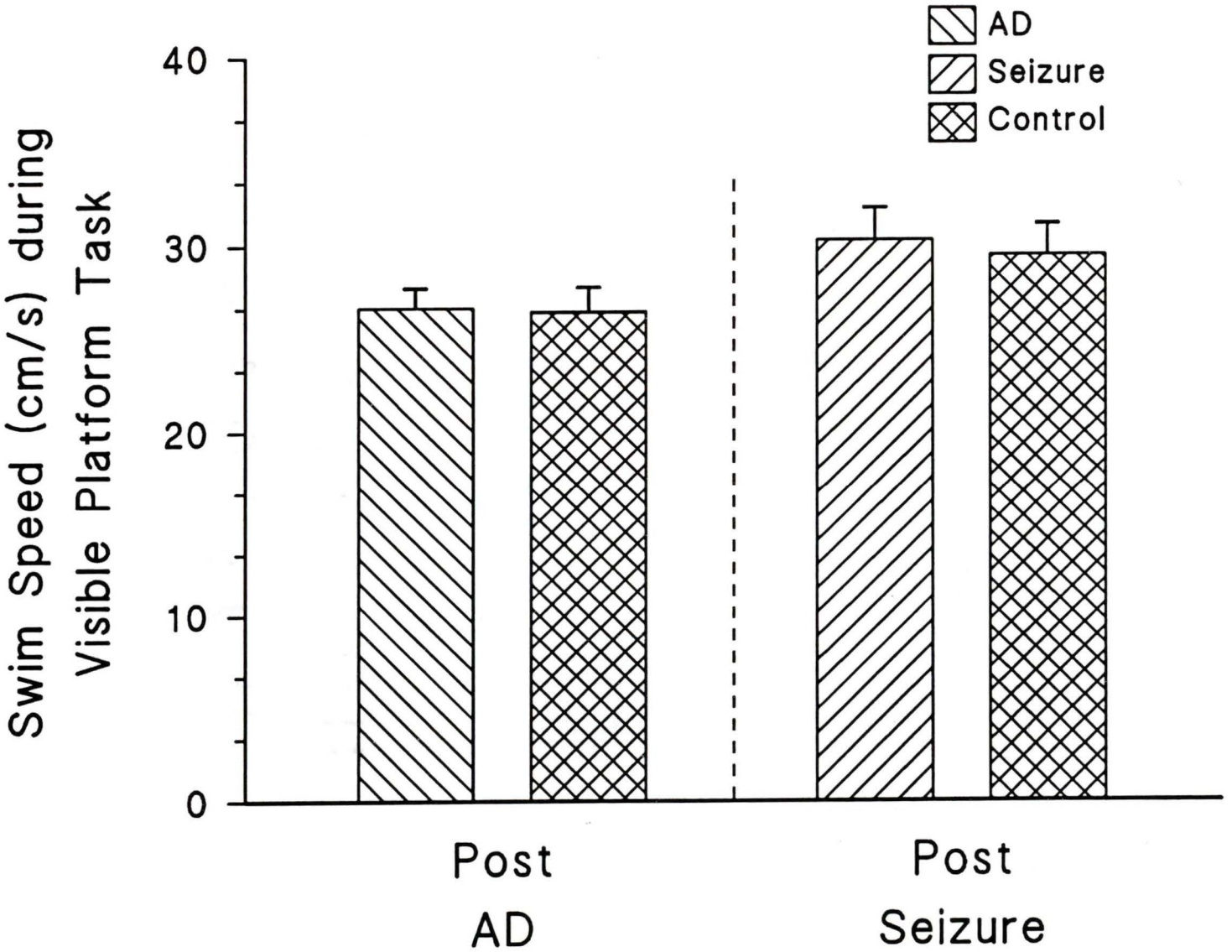

Escape Latency (s)
to a Visible Platform
14
12
10
8
6
4
2
0
AD
Seizure
Control
Post
AD
Post
Seizure

Figure 17. Effects of CA1 evoked ADs and kindled seizures on swim speed (cm/s) during visible platform testing. Swim speed did not differ significantly.


AD
Seizure
Control
Swim Speed (cm/s) during
Visible Platform Task
40
30
20
10
0
Post
AD
Post
Seizure

maze persisted at least 3 weeks after the last generalized seizure or AD (Leung et al., 1990), and up to 1 week postkindling (Lopes da Silva et al., 1986). In addition, the induction of generalized seizures in the DG produced deficits in the radial maze up to 3 weeks after the last kindling stimulation (Feasey-Truger et al., 1993). Thus the results of Experiment 3 strongly suggest that the acquisition and retention of spatial information is jeopardized by triggering transient epileptiform activity in field CA1. Furthermore, such deficits in spatial performance persist for some time even after kindling stimulation is discontinued.

General Discussion

The Morris water maze was used to assess the effects of hippocampal kindling on spatial learning and memory. I found that kindling of field CA1 until 3 consecutive generalized seizures were evoked was sufficient to produce significant performance deficits in the spatial task. The impairment in performance was indicated by the increased distances to locate the submerged escape platform during acquisition and retention. I found that kindling to subconvulsive seizures (partial-kindling) had differential effects on performance in the water maze. Partial-kindled rats in Experiment 1 (acquisition) performed to control levels during the acquisition and reversal phases, whereas partial-kindled rats in Experiment 2 (retention) showed an impairment in performance during the retention phase only. I found that ADs evoked 25-45 min prior to daily training in the water maze produced significant performance deficits that persisted at least one week following the discontinuation of kindling stimulation, followed by gradual recovery during the five day period in which testing was reinstituted. Generalized seizures also produced deficits in retention, as defined by the performance of rats that were trained in the water maze before kindling. The deficits in the seizure rats persisted during the one week free of stimulation period, and into the five day period in which testing was reinstituted.

Learning vs. behavioural deficit. Although the results suggest that hippocampal kindling produces deficits in spatial memory, other factors must be considered. It is possible that the observed deficits in performance could be due to general behavioural or motivational impairments, and not deficits in memory. I attempted to resolve this

in 3 ways. First, if a sensorimotor dysfunction was responsible for the performance deficit, swim paths might be expected to show thigmotaxis (i.e., wall following). This was not the case, as rats appeared to use circular search patterns to locate the escape platform. In other words, rats that showed impaired performance in fact eventually learned there was an escape platform, but they were simply slower to learn the exact location of it (Fig. 3). This was suggested by the purposeful swim patterns that included a substantial area of the water surface. Secondly, performance on a visible platform task, a cue task that should be sensitive to general behavioural deficits, would be expected to be be impaired. I found that triggering an AD or generalized seizure 25-45 min prior to visible platform testing did not significantly affect the time required to escape to the visible platform. Also, swim speed was not affected in the visible platform task, which suggests that the rats were motivated to escape. Thirdly, swim distances were still increased over those of controls up to 3 weeks after the last stimulation (Experiment 3), a period that would seem to be beyond the point at which simple sensorimotor deficits would occur.

The arguments presented above suggest that the performance deficit produced by kindling was unlikely to be a result of simple sensorimotor deficits. The findings are consistent with previous reports that nonmemory deficits are not necessarily associated with the impairment in maze performance. McNamara et al. (1992) found that neither swim speed nor visible platform training was significantly affected by the induction of seizures or kindling of the PP in the Morris water maze. Feasey-Truger et al. (1993) reported that motor deficits were unlikely to be responsible for the

reference memory impairment produced by dentate kindling in the radial maze. They found no deficit in working memory, which would also be expected if therewere general behavioural disturbance. They also found memory deficits 3 weeks after the cessation of kindling stimulation.

Although these results collectively suggest that sensorimotor or motivational deficits do not account for the effects of kindling and kindled seizures on maze learning and memory, they do not entirely rule out the possibility. Further work needs to be done to determine whether motor or sensory impairments can be detected either after kindling or in the postictal period, using appropriately sensitive and selective tests.

Probe Trials. The finding that rats in all kindled groups demonstrated dwell times similar to those of controls during probe trials is consistent with the findings of McNamara et al. (1992). They found that controls and kindled rats demonstrated a significant bias for the target quadrant during probe trials. However, McNamara et al. also found that their PP seizure group failed to show a quadrant bias, that is, the rats swam in the target quadrant at chance levels. This latter result differs from my finding that ADs and generalized seizures evoked by stimulation of field CA1 failed to produce significant differences in dwell times from that of controls during the postacquisition and postretention probe trials. The difference in results may be a function of the site of stimulation (CA1 vs. PP). Alternatively, although the dwell times of the CA1 kindled rats in the target quadrant were similar to controls', perhaps the kindled rats had learned not the specific location of the platform but only its

general location (i.e., the quadrant). This hypothesis suggests that tests more sensitive than the probe trial will have to be devised to unmask subtle deficits in spatial learning.

The finding that kindling of hippocampal field CA1 disrupts performance in the Morris water maze is consistent with previous reports that CA1 kindling produced errors in working memory (Leung et al., 1990) and reference memory (Lopes da Silva et al., 1986) in the radial-arm maze. However, Leung et al. also found impairments in performance following partial kindling, and reported that the disruption was caused primarily by ADs and not necessarily associated with generalized seizures. The results of Experiment 1 suggest that partial kindling of field CA1 does not disrupt acquisition in the water maze. The results of Experiment 2, that kindling and partial kindling both disrupt retention in the maze, suggest that retention (or relearning) is more vulnerable to disruption than initial acquisition.

The finding that ADs and generalized seizures evoked in hippocampal field CA1 produce deficts in water maze performance is consistent with other reports that seizures triggered from the PP produce deficits in the water maze (McNamara et al., 1992) and the radial-arm maze (Robinson et al., 1993). McNamara et al. found that pretraining or posttraining PP seizures impaired place learning in the Morris water maze. Robinson et al. reported learning impairments in rats that experienced an AD or generalized seizure before learning trials. However, these authors did not find a significant impairment in place learning in PP kindled rats when tested 24 hrs after the last seizure. Again, it is possible that the differences in results may be attributable to

the different site of stimulation (CA1 vs. PP). It is therefore possible that the susceptibility to deficits in certain components of spatial memory produced by hippocampal kindling may vary according to the site within the hippocampal formation that is kindled (CA1, PP, or DG), and the task used to assess the impairment (ie., water maze or radial maze). Why does the site of stimulation matter? Perhaps different neural circuits are involved. There is some evidence that PP kindling does not enhance subsequent susceptibility to kindling in the DG (Spiller and Racine, 1994), suggesting that PP kindling involves non-hippocampal circuitry. The electrophysiological correlates of PP and DG kindling also differ. PP kindling results in enhanced inhibition in the DG (Tuff, Racine, and Adamec, 1983), whereas CA1 kindling is accompanied by diminished inhibition in field CA1 (Kamphuis, Lopes da Silva, and Wadman, 1988). Conceivably, these electrophysiological differences are responsible for the differing behavioural consequences of PP and CA1 kindling.

The present results do not support the findings that hippocampal seizures specifically impaired working memory but not reference memory (Knowlton et al., 1989). The Morris water maze, as used in the present study, is viewed as requiring primarily reference memory (Morris, 1983). Therefore the present data confirm that reference memory as well may be compromised by epileptiform activity within the hippocampus (McNamara et al., 1992). In addition, the impairment produced by evoking ADs prior to daily testing does not concur with the results of Olton and Wolf (1981), who reported that hippocampal seizures did not produce anterograde deficits in working memory. In Experiment 3 on the other hand, the elicitation of hippocampal

ADs (in the absence of behavioural seizures) was sufficient to produce anterograde performance deficits. Olton and Wolf also induced electrophysiological seizures that were primarily nonconvulsive, similar to my procedure. Therefore the reason for the discrepancy in results is not obvious.

The finding that kindling, ADs, and generalized seizures produce performance deficits in the Morris water maze is consistent with hippocampal lesion data (Morris et al., 1982; Jarrard, 1983; Barnes, 1988) and effects of PP lesions (Skelton and McNamara, 1992). The present results provide further evidence that disruption of normal hippocampal functioning produces deficits in tasks that require spatial learning and memory for optimal performance. This supports the idea that hippocampal kindling may act as a functional lesion, altering neural activity throughout the hippocampal formation and any anatomically connected structures that may play a role in the processing and maintenance of spatial information. The effects of kindling and kindled seizures as discussed here provide a new approach to mapping the boundaries of experimental learning and memory deficits, and may eventually provide further insight into the memory disturbances associated with temporal lobe epilepsy.

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VITA

Surname: Gilbert Given Names: Trevor Howard

Place of Birth: Cranbrook, British Columbia, Canada

Educational Institutions Attended:

University of Victoria	1993-1995
University of Victoria	1988-1992

Degrees Awarded:

B.A. (Honours)	University of Victoria	1992

Honours and Awards:

NSERC (PGS B)	1995 - 1996
Graduate Teaching Fellowship	1994 - 1995
Graduate Teaching Fellowship	1993 - 1994

Publications:

Refereed Journal Articles:

Kirkby, R.D., Gilbert, T.H. and Corcoran, M.E. (1993). Kindling antagonism: mapping of susceptible sites. Brain Research, 616, 17-24.

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Author

Trevor H. Gilbert

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Date