KINDLING WITH STIMULATION OF THE HILUS OF THE DENTATE GYRUS

by

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ABSTRACT

Despite the large number of studies investigating neural plasticity in the dentate gyrus (DG), there is surprisingly little information regarding the susceptibility of this region to kindling. In addition, the findings in the literature regarding the role of long-term potentiation (LTP) in kindling are inconsistent. This study was, therefore, designed for two purposes. First, kindling with stimulation of the hilus of the DG was characterized, both electrographically and behaviorally. Second, changes in the amplitudes of population spikes evoked in dentate granule cells by stimulation of the perforant path (PP) were measured during kindling.

I found that hilar kindling possesses several unique characteristics that set it apart from typical limbic

kindling. These include low rate of kindling, marked instability of the seizures (i.e., many regressions of clinical stages and many stimulation days on which no AD was observed), and relatively little or no growth in AD duration. Kindling in the hilus did not produce permanent LTP of the PP-DG population spike. Rather, after a brief initial increase in population spike amplitude, there was a large decrease to below baseline and control levels.

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DEDICATION

This thesis is dedicated to my parents, who have never stopped encouraging and supporting me in all that I do.

INTRODUCTION

Kindling refers to the gradual and progressive appearance of seizure activity, both electrical and behavioral, due to the repeated administration of brief low-intensity electrical stimulation. This phenomenon was originally discovered and investigated by Goddard (1967) and further elaborated by Goddard, McIntyre, and Leech (1969). Since these landmark papers, literally hundreds of papers have been published on the subject. Through these investigations, kindling has been shown to be a transsynaptic event, not due to localized disturbances produced by edema, gliosis, poisoning, metallic ion deposits, or tissue damage at the tip of the stimulating electrode (Racine, 1972b; Wada, Sato, & Corcoran, 1974).

As mentioned above, kindling involves changes in both electrophysiology and behavior. Electrophysiologically, the subject displays afterdischarge (AD), which represents the repetitive discharge of a group of neurons and is evident in the EEG as several seconds of high-amplitude

rhythmic spiking. As kindling progresses, the AD increases in duration, amplitude, and frequency. Kindling does not depend on stimulus parameters such as frequency or intensity as long as the stimulation intensity is high enough to elicit AD, the occurrence of which is crucial for the development of motor seizures (Racine, 1972b). As the AD increases in duration, typically over a period of days or weeks, the subject displays a progressive evolution of clinical signs. One of the most widely used behavioral classifications for limbic kindling was developed by Racine (1972b) and consists of five clinical stages: mouth and facial movements (stage 1), head nodding (stage 2), forelimb clonus (stage 3), rearing (stage 4), and rearing and falling (stage 5). Once a fully generalized clonic seizure (stage 5) is kindled, the subject will typically respond to any subsequent stimulation with a stage-5 seizure.

One of the most interesting properties of kindling is that in order for it to proceed, the electrical stimulations must be spaced over time. In fact, the number of stimulations required for the kindling of generalized seizures is inversely related to the duration of the interval between stimulations, the optimal interval being 24 hours (Goddard et al., 1969). Another important

characteristic of kindling is that it is "an enduring if not permanent phenomenon" (Pinel & Van Oot, 1976). For example, rats that developed stage-5 seizures after amygdaloid stimulation demonstrated a generalized motor seizure on the fourth trial after a 12-week rest period (Goddard et al., 1969). Permanence of kindling has also been shown in cats that have been left unstimulated for as long as 12 months. They responded to either the first or second "rekindling" stimulation with a generalized seizure (Wada et al., 1974).

Although most of the early work in kindling involved stimulation of limbic areas, kindling has been demonstrated with stimulation of a wide variety of brain sites. However, some anatomical specificity exists whereby, for example, seizures can be readily kindled with amygdaloid stimulation whereas they cannot be kindled with stimulation of the substantia nigra or cerebellum (Goddard et al., 1969). After major seizures have been kindled with stimulation of one site, other sites within the brain will show a greater potential for kindling such that fewer ADs are needed to kindle with stimulation of the second structure. This effect, known as transfer (Goddard et al., 1969; Racine, 1972b), can occur between contralateral homologous regions (e.g. left and right amygdala) or

between ipsilateral nonhomologous structures (e.g. amygdala and septum) (Burnham, 1976). Kindling is not specific to a single species, but has been shown in rats, cats, rabbits, monkeys, baboons, dogs, mice, frogs, and reptiles, and possibly in humans (Goddard et al., 1969; McNamara, Byrne, Dasheiff, & Fitz, 1980).

Another characteristic of kindling is spontaneity of seizure activity. Perhaps the earliest sign of spontaneous activity is in the form of interictal spiking common in the early stages of kindling. If stimulation is continued long enough, spontaneous motor seizures will develop (Pinel, Mucha, & Phillips, 1975; Wada et al., 1974).

Given these properties of kindling, it is evident why kindling is considered one of the best animal models of human epilepsy (Gaito, 1974; McNamara et al., 1980; Pinel & Van Oot, 1976; Wada et al., 1974). Not only does kindling represent the clinical phenomenon of epilepsy, but it also allows for easy experimental control.

A great deal of kindling research is concerned with the search for the neuronal mechanism of kindling. It is important to discover the nature of the cellular activity that allows for the gradual evolution of such widespread epileptiform activity, and allows the susceptibility to

epileptiform activity to remain permanently. One of the suggestions for the cellular mechanism underlying kindling is long-term potentiation (LTP) (Douglas & Goddard, 1975; Goddard & Douglas, 1976).

The phenomenon of LTP was originally documented by Bliss and colleagues (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973) and can be defined as an increase in the efficacy of excitatory synaptic transmission after brief high-frequency trains of stimulation. The increased efficacy of synaptic transmission produced by LTP has been shown to last days (Bliss & Gardner-Medwin, 1973), weeks, and even months (Douglas & Goddard, 1975). LTP has a rapid onset, detectable within seconds after the stimulation. It also has the property of accumulation, such that repeated trains of stimulation lead to more LTP. Also, LTP seems to show some decay over time if stimulations are stopped. Stimulation effective in eliciting LTP has been found to be similar to the normal discharge patterns of certain brain sites, such as the hippocampus (Douglas, 1977). These various properties of LTP, especially immediacy and persistence, have led researchers to speculate that LTP may be the neurophysiological mechanism underlying human learning and memory (Bliss, Goddard, & Riives, 1983).

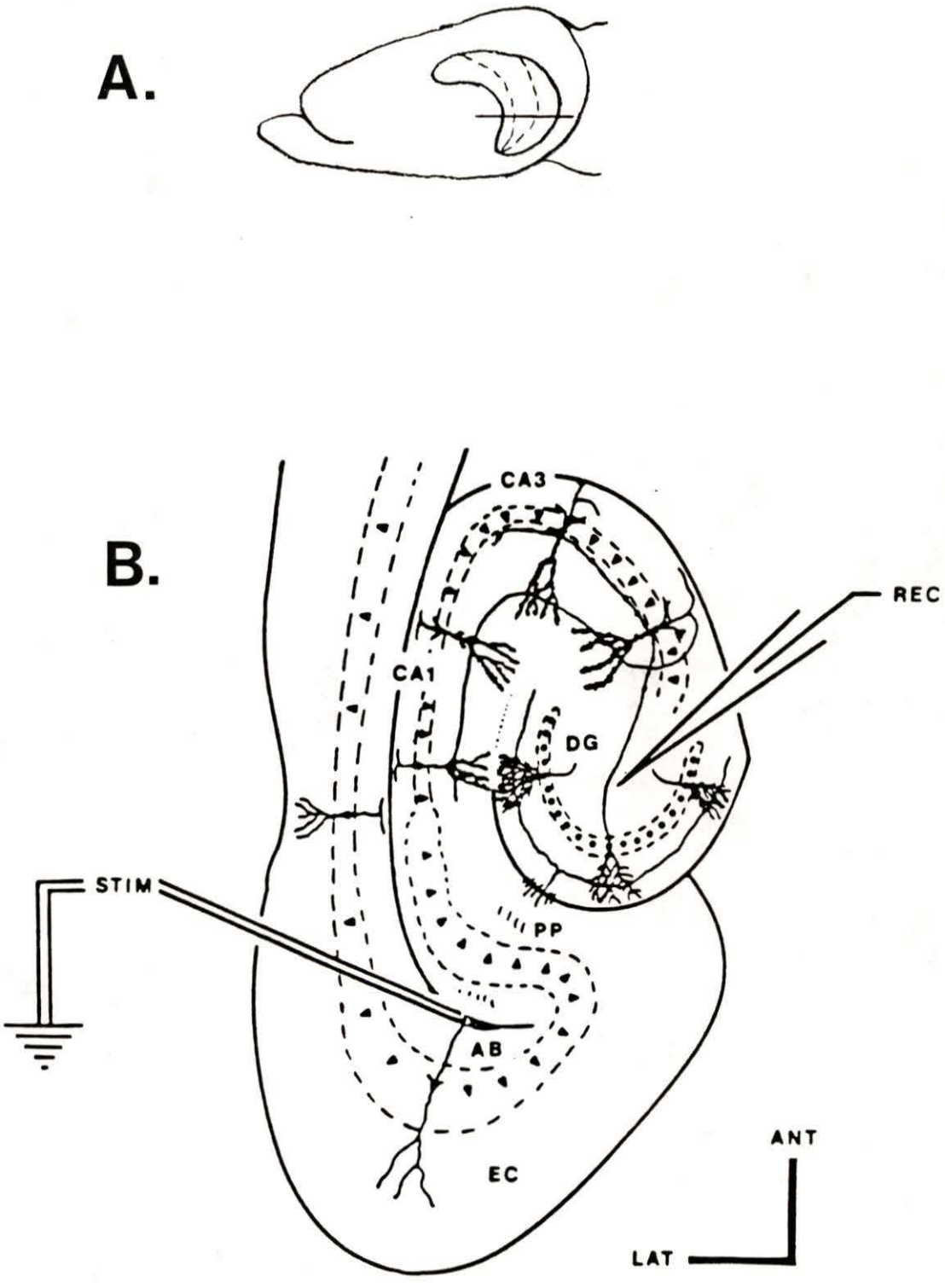
LTP was originally discovered and studied most extensively in the hippocampus, the most primitive structure of the cerebral cortex. The hippocampus is an ideal structure for electrophysiological studies since its anatomy (Lorente de No, 1934; Ramon y Cajal, 1911) and electrophysiology (Gloor, Vera, & Sperti, 1963) are so well-known and well-defined. As a whole, the hippocampal formation is a C-shaped structure that lies in a transverse orientation running along the inferior horn of the lateral ventricle in the rat brain. The structure is folded around itself in an S-shape, with the dentate gyrus forming a C-shaped cap along one free edge of the S (Swanson, 1979). A horizontal section of the structure best illustrates these features (Figure 1).

The eight major cytoarchitectonically distinct fields of the hippocampal formation can be divided into four areas: dentate gyrus (DG), ammon's horn (fields CA1, CA2, CA3, and CA4; CA = Cornu Ammonis), subicular complex (subiculum, presubiculum, and parasubiculum), and entorhinal cortex (Swanson, 1979). The hippocampus has a highly laminated organization, which makes the study of electrophysiological events relatively easy (Gloor et al., 1963; Lomo, 1971). Within the hippocampus proper, the large pyramidal cells of areas CA1 to CA4 together form

Figure 1: Hippocampal anatomy.

A. The orientation of the C-shaped hippocampus (left) within the rat brain. The dark line shows the approximate location of the schematic section in B (redrawn from Swanson, 1979).

B. Schematic horizontal section through the left hippocampus showing the laminar organization of neuronal components. The locations of the recording (REC) and stimulating (STIM) electrodes used in the thesis are shown. Abbreviations: AB, angular bundle; CA1, cornu ammonis field 1; CA3, cornu ammonis field 3; DG, dentate gyrus; EC, entorhinal cortex; PP, perforant path. Calibrations: anterior (ANT) and lateral (LAT) directions; 0.5 mm (from Skelton, 1982).


A.
B.
CA3
CA1
DG
REC
STIM
PP
AB
EC
ANT
LAT

the folded S-shaped sheet. The C-shaped sheet of DG granule cells is folded along one edge of the sheet of pyramidal cells. Within these sheets of granule and pyramidal cells, there is a laminar organization of cellular components such that each of the neuronal components (e.g. dendrites, cell bodies, and axons) are found in separate layers (Figure 1). Some afferent fibres (the perforant path; see below) travel up to these sheets and bifurcate, synapsing "en passage" with specific zones of the dendritic trees. This organization allows for the creation of huge extracellular field potentials (in the order of mV) since stimulation of afferent projections causes all dendrites in a certain region to depolarize simultaneously.

A type of lamellar organization also exists in which the hippocampus can be envisaged as a stack of plates each of which has its own intrinsic and extrinsic pathways. This form of organization is significant because it makes possible <u>in vitro</u> studies requiring the use of hippocampal slices. It is possible to elicit synaptic activity in the entire trisynaptic circuit with this technique (Swanson, Teyler, & Thompson, 1982).

The monosynaptic pathway in the hippocampus that perhaps has been studied the most in relation to LTP is

the perforant pathway (PP). These fibers originate in the entorhinal cortex and then pass through a "bottleneck" deep in the angular bundle (Lomo, 1971). The axons then perforate through the hippocampal fissure to the DG, where they synapse on the outer two-thirds of the apical dendrites of the granule cell molecular layer (Hjorth-Simonsen & Jeune, 1972).

The intricate anatomical organization of the hippocampus results in an equally intricate electrophysiological relation. When the PP is electrically stimulated, a large characteristic evoked field potential is elicited from the dentate granule cells (Lomo, 1971). The field potential consists of two distinct components, each representing a separate neuronal event. The population excitatory post-synaptic potential (pEPSP) represents the extracellular current flow resulting from the dendritic depolarization of the granule cells. Superimposed on this wave is a sharp population spike of the opposite polarity produced by the almost synchronous firing of a large number of granule cells. The population spike therefore represents the overall excitability of the cells, depending on both the number of cells firing and their synchronicity of firing. The opposite polarities of the two components reverse

depending on whether recording is done in the dendritic molecular layer (negative-going EPSP, positive population spike) or in the cell body/axonal layer called the hilus (positive-going EPSP, negative population spike) (Bliss & Lomo, 1973; Douglas, 1977; Lomo, 1971). These two layers, therefore, form a dipole due to the neuronal currents flowing between the "sink" at the dendrites and the "source" at the soma and axon hillock (Gloor et al., 1963). Figure 2 illustrates a characteristic evoked field potential in the hilus of the DG.

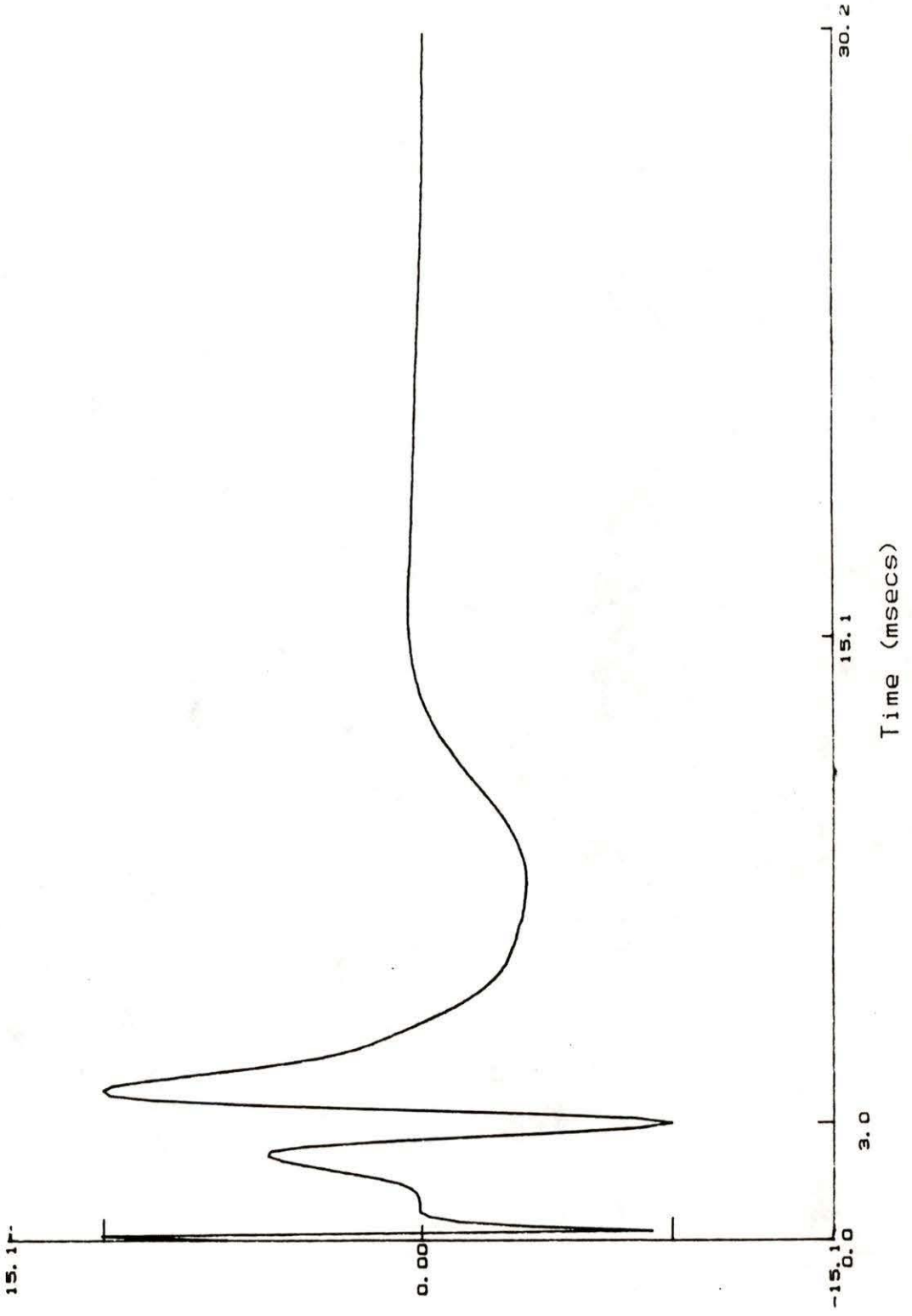
Many studies have demonstrated that when a brief train of tetanic stimulation is applied to the PP, the amplitude of the evoked potential in the dentate granule cells is reliably enhanced (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973; Douglas, 1977; Douglas & Goddard, 1975). More specifically, both the pEPSP and the population spike can undergo LTP.

Role of LTP in Kindling

There are several lines of support for the possible role of LTP in the cellular mechanism of kindling (McNamara et al., 1980):

1. Both kindling and LTP involve long-lasting enhanced

Figure 2: Typical field potential evoked by stimulation (0.1 msec pulse at 400 uA) of the PP recorded from the hilus of the DG.


15.1
0.00
-15.1
0.0
3.0
15.1
30.2
Time (msecs)
Amplitude (mV)

responsiveness to a fixed electrical stimulation (Giacchino, Somjen, Frush, & McNamara, 1984).

2. Both kindling and LTP are widespread phenomena in the limbic system (Goddard et al., 1969; Racine, Milgram, & Hafner, 1983).

3. One of the most effective stimulation patterns for producing LTP is similar to the high-frequency cellular activity that occurs during siezures and interictal spiking (Douglas, 1977). For example, stimulation bursts designed to mimic the discharge of neurons during AD resulted in LTP of forebrain evoked potentials (Racine, Newberry, & Burnham, 1975). These findings suggest that the epileptiform activity of the neurons undergoing AD may cause a long-term enhancement of synaptic transmission similar to LTP.

4. After LTP with amygdaloid stimulation, amygdaloid kindling occurs at a facilitated rate (i.e. a significant reduction in the number of ADs to a stage-5 seizure) (Racine et al., 1975). It has recently been shown that prior LTP of the PP-DG synapse results in a facilitation of kindling with stimulation of the entorhinal cortex (Sutula & Steward, 1984).

5. Both field potential and single cell recording studies have shown that kindling does produce a strong

long-lasting increase in excitatory responses. For example, amygdaloid kindling resulted in LTP of evoked potentials in several brain areas (e.g. hippocampus, preoptic area) that are monosynaptically or bisynaptically connected to the amygdala (Racine, Gartner, & Burnham, 1972). In addition, partial kindling with stimulation of the PP is associated with LTP of the monosynaptic EPSP and population spike in the DG elicited by PP stimulation (Douglas & Goddard, 1975; Goddard & Douglas, 1976).

However, there are also some lines of evidence that challenge the proposed role of LTP in kindling, or at least challenge the notion that LTP is the sole mechanism of kindling (Goddard, 1981; Racine, 1981):

1. Large amounts of prior LTP produce only a partial savings effect during subsequent kindling (Racine et al., 1975). If LTP is the sole mechanism of kindling, kindling should be much more facilitated after LTP.

2. There is an inverse relation between the amount of potentiation that a pathway will yield and the rate at which it kindles. Thus stimulation of the olfactory cortex results in rapid kindling, but it is very difficult to demonstrate LTP with this site. Stimulation of the hippocampus results in a very slow rate of kindling, however, yet LTP is a reliable and robust phenomenon here

(Racine, 1981).

3. Finally, if LTP is subserving kindling, it should parallel the time course of kindling. However, evoked potentials do not remain potentiated throughout the entire course of kindling; rather, potentiation occurs only during the early stages of kindling and then decays (Douglas & Goddard, 1975; Goddard & Douglas, 1976).

One possible explanation for these contradictions regarding the role of LTP in kindling is that most of the studies have concentrated on only one aspect of the evoked potential, the pEPSP. Typically, AD is induced in the PP and field potentials in the DG are monitored before and during the course of kindling. The consensus of these studies is that LTP of the pEPSP does not occur or occurs only during the early stages of kindling and is followed by a decrease. For example, Giacchino et al. (1984) examined LTP in the monosynaptic pathway between the lateral entorhinal cortex (LEC) and the DG in rats in which seizures were kindled with stimulation of the LEC. Kindling was not accompanied by evidence of LTP in either the slope or amplitude of the pEPSP recorded in the hilus. In this study, only EPSPs were monitored because stimulation of the LEC does not elicit a population spike.

In a similar study (Maru, Tatsuno, Okamoto, & Ashida,

1982) seizures were kindled with stimulation of the PP, and although the EPSPs evoked in the DG were greatly increased during the early stages of kindling, the increase gradually decayed as kindling progressed. However, it is interesting that in the majority of subjects in their study, the dentate population spike was found to be highly potentiated and remained so throughout the course of kindling. Another example of this apparent dissociation between LTP of the EPSP and the population spike comes from an investigation of the time course of decay of LTP in the PP-DG synapse (Racine et al., 1983). LTP of the population spike was found to be larger and longer lasting than LTP of the EPSP.

These studies suggest that there may be two types of LTP, one that increases the strength of excitatory synapses as measured by growth of the EPSP, and another that increases cellular excitability as reflected by growth of the population spike (Bliss & Lomo, 1973; Goddard, 1981; Swanson, Teyler, & Thompson, 1982). Due to this dissociation, it has been suggested that the role of LTP in kindling may be different than was originally thought. Since kindling appears to result in an increase in postsynaptic cellular excitability (Maru et al., 1982), then perhaps it is enhanced cellular excitability that is

responsible for kindling. Goddard believes that the observed increases in cellular excitablility are sufficiently permanent to play a role in the mechanism of kindling (Goddard, 1981).

Another line of research provides additional support for the notion that kindling may result specifically from LTP of cellular excitability. It is now a well-known finding that kindling is facilitated following forebrain depletion of noradrenaline (NA) (Corcoran & Mason, 1980). If LTP is involved in kindling and kindling is facilitated by NA depletion, then LTP should be enhanced by NA depletion (Goddard, 1981). Therefore, Bliss et al. (1983) depleted NA in rats and found that although LTP of the pEPSP was reduced by more than 50%, LTP of the population spike was not affected by depletion of NA. Therefore, although LTP of the EPSP seems to depend on forebrain NA, LTP of the population spike does not. This drug-induced dissociation between potentiation of the two components of the evoked potential adds further support to the notion that LTP of the DG evoked potential has two distinct and independent components, potentiation of the EPSP and potentiation of the population spike (Bliss et al., 1983).

Although the above studies appear to support the proposal that kindling may result from a permanent

potentiation of cellular excitability, other studies completely contradict this theory. Maru and Goddard (1984) found that kindling with stimulation of the PP resulted in a complex series of changes in field potentials: a long-lasting increase in the pEPSP, an initial increase and then a decrease in the population spike, and a temporary increase of commissural inhibition of the population spike. These results are the opposite of those obtained by Maru et al. (1982) described earlier. Goddard (1982) kindled seizures with stimulation of the hilus and found the amplitudes of the PP-DG pEPSP to be larger following kindling. However, the amplitude of the population spike tended to increase following development of stage-1 to 3 seizures and decrease following development of stage-5 seizures. Finally, after partial kindling with stimulation of the PP (up to 10 days), Racine et al. (1983) found initial increases in the amplitude of the population spike and then a decline back to baseline levels after 10 days. However, the EPSPs remained potentiated as of day 10. These findings suggest that kindling results in decreased cellular excitability.

One consistant finding in the literature is that kindling and LTP do seem to co-occur. However, some results point to LTP of the population spike and transient

increases in the pEPSP during kindling, whereas other experiments suggest the reverse findings. These differences may be attributed to flaws in the design of some of the studies. For example, in some studies evoked potentials were induced using only one stimulus intensity (Goddard, 1982; Maru et al., 1982). Such a lack of input-output (I/O) curve analysis does not allow for a thorough characterization of LTP. Also, many of these studies did not monitor field potentials throughout the entire course of kindling or for reasonably long periods of time (Goddard, 1982; Racine et al., 1983). Finally, some of the experiments did not monitor field potentials in control rats that were not stimulated for kindling (Goddard, 1982). Another quite serious problem with almost all of the studies concerns the measurement of pEPSPs recorded from the hilus. It has been suggested that the EPSP as observed in the hilus is not an accurate reflection of distal dendritic events and may in fact better represent proximal electrophysiological events occurring in the hilus. Therefore, the method of assessing changes in the pEPSP by recording only in the hilus is certainly questionable if not invalid (Abraham & McNaughton, 1984).

Objective

The objective of the present study was twofold. First, I wanted to characterize kindling with stimulation of the hilus, both electrographically and behaviorally. Despite the large number of studies investigating neural plasticity in the DG, there is surprisingly little information regarding the susceptibility of this region to kindling. Although kindling has been demonstrated most often with stimulation of limbic areas, especially the amygdala, the hippocampus has been the subject of many fewer kindling studies. Recently two studies have described differences between the dorsal and ventral hippocampus in rate and pattern of kindling (Lerner-Natoli, Rondouin, & Baldy-Moulinier, 1984; Racine, Rose, & Burnham, 1977). However, a detailed description of kindling specifically with stimulation of the hilus has not been carried out. I therefore examined hilar kindling in terms of rate; threshold of AD; frequency, amplitude, and duration of AD; number of days off (i.e., stimulation days resulting in no AD after AD has been previously elicited); and behavioral effects.

Secondly, the present study was designed to assess more carefully the effects of kindling on evoked field

potentials. Over the course of hilar kindling, evoked potentials were monitored in the hilus in response to test pulses applied to the PP in the angular bundle. Seizures were not kindled in control rats, but field potentials in controls were monitored at times comparable to the experimental group. I decided to monitor only the population spike component of the evoked field potentials due to the reasons previously discussed. In addition, test pulses consisted of an ascending series of six current intensities so that I/O curves could be constructed.

I realized that the results of this aspect of the study cannot be directly compared to the results of the studies in which kindling stimulation was applied to the PP rather than to the hilus (with the exception of Goddard, 1982). However, I felt that it was worthwhile to assess the effect of direct stimulation of the granule cells on the PP-DG population spike. There is evidence that kindling results in a nonspecific heterosynaptic change in the responsiveness of the stimulated cells (Racine, Kairiss, & Smith, 1981) such that they become "epileptic" or highly excitable. I therefore predicted that kindling stimulation directly applied to the dentate granule cells would result in an increased excitability of

the cells, not unlike the increased excitability of the granule cells reported after kindling with stimulation of the PP. If the amplitude of the population spike can be taken as an index of cellular excitability, then an increase in the PP-DG population spike should be evident at least during some stage of kindling.

METHOD

Subjects

Male hooded rats of the Long-Evans strain attained from Charles River Canada Inc. (St. Constant, Quebec) were used, weighing between 300 and 375 grams at the time of surgery. The rats were housed in individual stainless steel cages with food and water available *ad libitum*. A 12 hour light/dark cycle was in effect throughout the study.

Surgery

Under sodium pentobarbital (Nembutal) anesthesia (60 mg/kg), rats received implantation of a recording electrode in the hilus of the DG and a stimulating electrode in the PP. The recording and stimulating electrodes were identical, consisting of a single strand of stainless steel wire 76 um in diameter coated with

teflon (114 um total diameter). Cemented to each electrode approximately 5 mm above the tip was a small plastic bead that served to anchor the electrode in the dental acrylic. Between 1/4 and 1/2 mm of insulation on the tips of the electrodes was removed. Two skull screws, connected to uninsulated stainless steel wire, served as current and reference electrodes. Gold-plated pins (Amphenol 220-S02) were soldered to the ends of the four wires, linked to a microconnector, and fixed to the skull using dental acrylic and two additional anchoring skull screws.

Electrodes were implanted in the left side of the brain using both stereotaxic and electrophysiological guidance. With the skull level between bregma and lambda, coordinates for the hilus were 4.0 mm posterior to bregma, 2.0 mm lateral to the midline, and 3.3 mm ventral to the surface of the cortex; coordinates for the PP were 8.1 mm posterior to bregma, 4.3 mm lateral to the midline, and 2.4 mm ventral to the surface of the cortex. As both electrodes were lowered, a storage oscilloscope displayed field potentials recorded from the electrode in the hilus, evoked by single square wave pulses delivered to the PP electrode (duration of 0.1 msec, frequency of 0.2 Hz, and intensity of 400 uA). The positions and depths of the

electrodes were adjusted to produce a hilar evoked potential with a maximum population spike (recording electrode adjustment) with minimum current threshold (stimulating electrode adjustment). If an adequate field potential could not be found after approximately one hour of searching, the rat was sacrificed by cervical fracture. Rats with stable evoked potentials were allowed 12 days to recover from surgery. After recovery, population spike amplitudes were measured in response to a stimulus intensity of 400 uA. Rats with amplitudes less than 6 mV were excluded from the study. The remaining rats were randomly assigned to experimental and control groups.

Determination of AD Threshold

Electrical stimulation for kindling in the hilus consisted of a 1 sec train of constant current balanced biphasic square-wave pulses, with a pulse width of 1.0 msec, delivered at a frequency of 60 pps. On the first day, a train of 30 uA (base-to-peak) stimulation was applied, and the intensity was increased in steps of 20 uA every 3 min until AD was elicited. The intensity was then decreased by increments of 10 uA on the next day and subsequent days until the AD was no longer evoked.

Threshold was defined as the minimum intensity sufficient to evoke AD.

Kindling

Rats from the experimental group were stimulated at their threshold intensities 6 days a week (Monday to Saturday) at approximately the same time each day. When stage-5 generalized seizures were noted on 3 consecutive stimulation days, stimulation was discontinued for a period of 3 weeks. After this rest period, rats were again stimulated once daily until one stage-5 seizure was displayed (rekindling), in order to test for permanency of kindling. Rats that did not exibit any stage-5 seizures were stimulated up to an arbitrary cutoff of 60 days, at which time they were removed from the experiment. Monopolar EEG from both the hilus and PP was recorded before and after each daily stimulation, and the duration of the evoked AD and the frequency and amplitude of epileptiform spikes were measured. Amplitude of AD was recorded as the mean amplitude of the spikes within the 4-sec block containing the largest amplitude spikes in any day's record. This method was also used for determination of frequency of AD. Behavioral responses during kindling

were carefully observed and recorded. Control rats did not receive kindling stimulation, but simply remained in their home cages during the kindling sessions.

Evoked Potentials

At least 1 hour prior to every second kindling session, evoked potentials in the DG were induced by stimulation of the PP with 3 single 0.1 msec pulses of cathodal stimulation at each of 6 current intensities applied in an ascending series: 20, 50, 100, 150, 250, and 400 uA. These 18 pulses were delivered at a frequency of 0.033 Hz (1/30 sec). This low frequency was used because it has been shown that LTP can occur at frequencies as low as 0.1 Hz (1/10 sec) in freely moving rats (Skelton, 1982). The field potentials evoked by stimulation were amplified, filtered between 0.1 to 10 kHz, digitized by Isaac model 91A, coupled to an Apple II Plus computer, and stored on disk for later analysis. The potentials were also monitored on a storage oscilloscope during the recording. In rats that developed stage-5 seizures, evoked potentials were monitored throughout the 3-week rest period after kindling and for 2 additional days after rekindling.

Evoked potentials were later averaged at each current intensity, and I/O curves were constructed each day for each subject. This was done by plotting the peak-to-peak population spike amplitude (output) against the six stimulation intensities (input). The geometric area bounded by the I/O curve and X-axis was then quantified using the formula for the area of a polygon (Mamelak, 1964). Changes in synaptic efficacy were assessed by using a percentage score calculated by comparing the area under each I/O curve with the area under a baseline I/O curve (Skelton, 1982). The baseline curve was averaged over 3 days of I/O curves collected from each subject prior to kindling. This method of assessing changes in synaptic efficacy is sensitive to both decreases in threshold and increases in population spike amplitudes at all intensities, which are both important measures of LTP (Skelton, 1982).

During the evoked potential recording sessions, behavioral responses were carefully observed and recorded.

Histology

At the end of the experiment, rats were given an overdose of sodium pentobarbital, and locations of the electrode tips were marked by passing 1 mA of anodal

current for 5 sec through each of the two electrodes. The rats were then perfused intracardially with 0.9% saline followed by 10% formalin. After perfusion, the brains were placed in formalin for at least 1 month before they were sectioned into 40 um slices, mounted on slides, and stained with thionin.

Analysis of Data

Because of the small number of subjects that completed the study, the data were not analyzed statistically. Rather, differences between groups are expressed as tendencies, with no implication that these represent statistically significant differences.

RESULTS

Of a total of 32 rats that underwent surgery, field potentials over criterion value (6 mv) were found in 20. After the 12-day recovery period, 15 of these 20 rats remained, with stable population spike amplitudes ranging from 9 mv to over 20 mv in response to a current intensity of 400 uA. One rat lost its electrode pedestal just 16 days into the study, and its data were excluded. The experimental (N=8) and control (N=6) groups consisted of the remaining 14 animals. Two of the six control rats were removed from the study, both at day 46, due to either a dislodged electrode pedestal or an infection under the pedestal. Data from these subjects were used up to 4 days prior to their removal from the experiment. The other four control rats remained in the study for the full 60 days.

I. Characterization of Hilar Kindling

Kindling Rates

Of the eight experimental rats, only three developed generalized stage-5 seizures. Although one of these rats displayed three consecutive stage-5 seizures immediately, the other two rats took 23 and 24 stimulation days after the first stage-5 seizure in order to show three consecutive seizures. The pattern of seizure development was abrupt: All three rats displayed a progression from stage-1 seizures directly to stage-5 seizures, without developing the intermediate stages of seizure characteristic of limbic kindling. Two of the rats did exhibit stage-2 seizures, but there was a regression back to stage-1 before a generalized seizure was exhibited. The topography of the generalized seizures was also unusual. During AD, the rats often became prostrate and exhibited generalized clonic jerking; they omitted the rearing behavior commonly seen in an amygdaloid stage-5 seizure. After the clonic component of the convulsion, the rats would typically remain still, appearing to be in a semiconscious state. This was followed by several minutes of automatisms, during which the rats would appear

to be trying to climb the walls of the recording box. For several minutes after the automatisms, the rats showed signs of hyperirritability. During rekindling, two rats developed a stage-5 seizure after two ADs, although one of these rats demonstrated 4 days off (i.e., no AD in response to stimulation) in the process. The third rat lost its electrode pedestal just prior to rekindling and thus could not be tested for persistence of seizure (Table 1).

The other five experimental rats developed only stage-1 (N=1) or stage-2 (N=4) seizures despite the 60-day stimulation period. One rat lost its electrode pedestal on day 54 of stimulation and could not be tested further. Due to the post-hoc observation that there appeared to be two distinct groups within the experimental animals, I decided to do all analyses separately for the two subgroups (Group A: rats that developed stage-1 and 2 seizures; Group B: rats that developed stage-5 seizures) to allow for comparison between them.

There was no apparent difference between Groups A and B in the number of ADs to the first stage-1 seizure. However, rats in Group B tended to require fewer ADs to develop stage-2 seizures than Group A. No rats in either group developed stage-3 or stage-4 seizures, with the

TABLE 1

Number of ADs to 1st Stage-5 Seizure, to 3rd Consecutive Stage-5 Seizure, and to Rekindling in Rats Kindled to Stage-5 Seizures

Rat	1st Stage-5	3rd Consecutive Stage-5	Rekindling
985	40	42	2
987	13	36	2
999	13	37	-*

* Rat lost electode pedestal just prior to rekindling.

exception of one occurrence of a stage-3 seizure in one subject. Regression to an earlier behavioral stage once a certain stage was reached was equally common in both subgroups (Table 2).

AD Threshold

The mean threshold for AD in the experimental group was 49 uA (SEM=14, range=15-130). Rats in Group B appeared to have slightly lower thresholds ($\overline{X}$=37, SEM=12, range=20-60) than rats in Group A ($\overline{X}$=57, SEM=21, range=15-130).

General Morphology of AD

The AD recorded in both the PP and hilus typically began 2-4 sec after the kindling train had ended. In all experimental rats, hilar AD usually subsided first wheras the AD recorded from the PP continued for several more seconds. The AD in the hilus was almost always followed by a flat EEG recording and then gradual recovery. Although the EEG recorded from the PP did show some decrement in amplitude from baseline after the AD, it never became flat as that of the hilar recording. There

TABLE 2

Number of ADs to each Stage of Seizure and Number of Regressions during Kindling for all Experimental Subjects

	Stage of Seizure					
Group A	1	2	3	4	5	Number of Regressions
982	5	46	-	-	-	9
990	5	44	-	-	-	6
004	6	36	-	-	-	12
006	4	-	-	-	-	2
007	5	49	46	-	-	12
$\bar{X}$	5.0	43.8				8.2
SEM	0.3	2.8				1.9
Group B						
985	8	-	-	-	40	3
987	6	24	-	-	13	8
999	5	14	-	-	13	11
$\bar{X}$	6.3	19.0			22.0	7.3
SEM	0.9	5.0			9.0	2.3

was usually a secondary episode of AD, occurring in both the hilus and PP for half of the rats and only in the PP for the other half. The secondary AD was generally shorter in duration and of lower frequency than the primary AD.

The frequency of the primary AD in the PP and hilus measured over the 1st AD, the 30th AD, and the final AD tended to increase, especially for Group B (Table 3). Amplitudes of AD measured in the PP did not appear to change over time. However, amplitude of the hilar AD did tend to increase in Group A, although not in Group B (Table 4). Low-frequency postictal spiking was seen in 5 out of 8 rats including all 3 rats in Group B.

The wave form of the AD also changed with repeated stimulation. Initially, AD was composed of regularly spaced simple biphasic spikes. However, with further stimulation the waveform became more complex and variable, with periods of notched or double spike configurations or very high frequency bursts. This progression of waveform complexity has also been noted in amygdaloid AD (Racine, 1972b).

TABLE 3

Frequency of AD (spikes/sec)

Group		1st AD		30th AD		Final AD	
		PP	Hilus	PP	Hilus	PP	Hilus
A	$\bar{X}$	5.0	3.4	5.0	9.2	6.9	7.7
	SEM	0.8	0.2	0.9	2.0	0.8	1.3
B	$\bar{X}$	5.5	4.3	9.8	13.0	12.0	9.5
	SEM	0.3	0.8	1.7	3.3	1.7	0.3

TABLE 4

Amplitude of AD (uV peak-to-peak)

Group		1st AD		30th AD		Final AD	
		PP	Hilus	PP	Hilus	PP	Hilus
A	$\bar{X}$	938	4206	936	4500	908	4714
	SEM	44.1	228.9	40.9	181.4	73.6	307.8
B	$\bar{X}$	977	4270	956	3990	1056	4310
	SEM	103.0	191.4	61.3	158.8	67.0	185.0

Growth of AD

Total duration of AD (i.e., addition of all episodes of AD per recording) in Group A remained fairly stable over the first 30 days of kindling, but generally appeared to decrease in the final 30 days. In contrast, duration of AD in Group B increased as the rats developed stage-5 seizures. Group B also showed higher overall AD durations, with greater variability than in Group A (Figures 3 and 4).

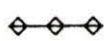
Days Off

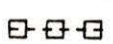
Days off are defined as days when rats did not exhibit any AD in response to kindling stimulation after AD had previously been evoked. There was a tendency for Group A to display more days off than Group B (Table 5). When stimulated on the day after a day off, the rat would usually respond with an AD.

Ictal and Postictal Behaviors During Kindling

A number of behaviors were observed during kindling, some during AD but before the behavioral seizure (ictal

Figure 3: Growth of AD duration in the nilus.

 Group A (N=5)

 Group B (N=3)

Note that on stimulation day 38 Group B consisted of only 2 rats, and on stimulation day 40 only 1 rat remained in Group B.

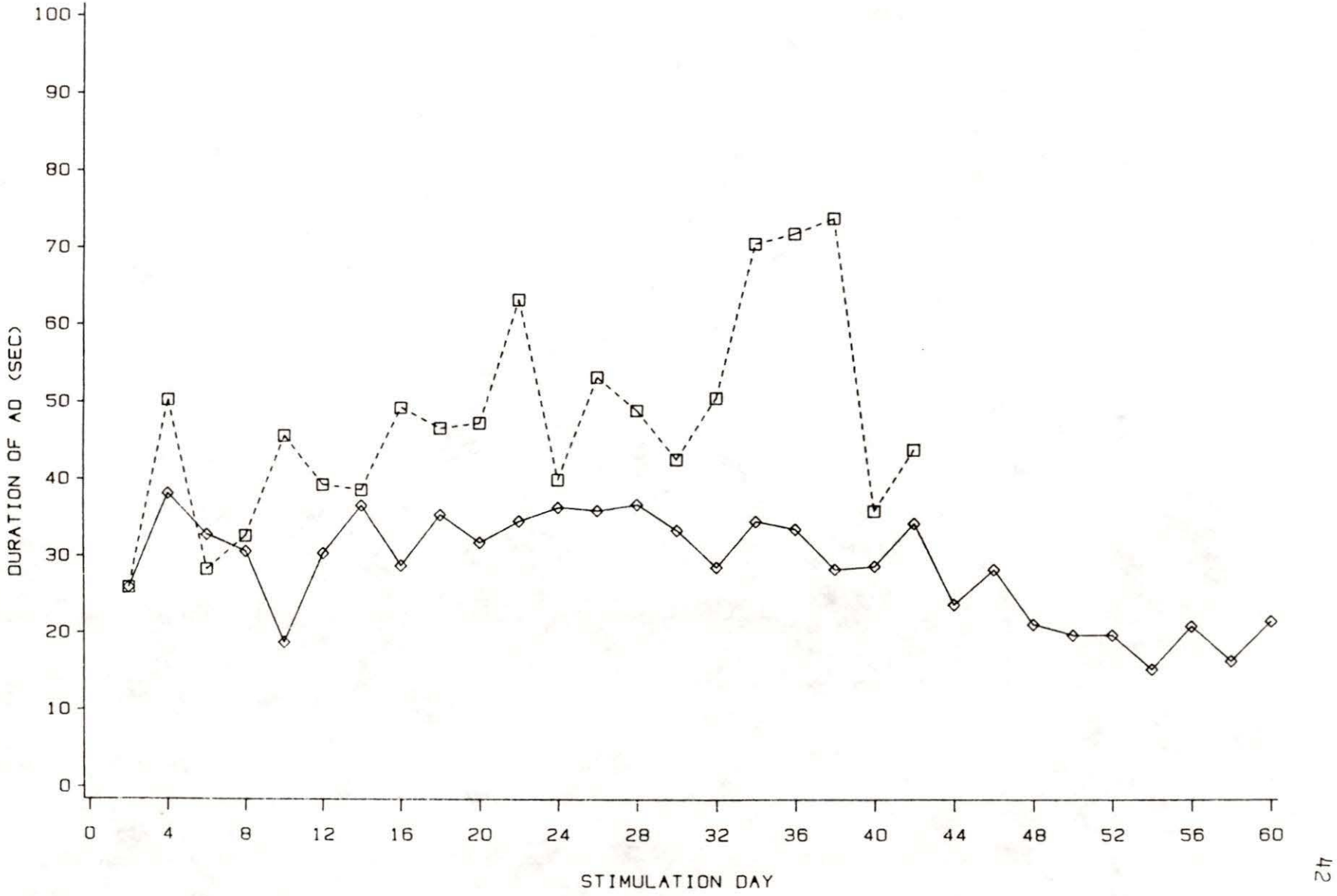

DURATION OF AD (SEC)
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
STIMULATION DAY

Figure 4: Growth of AD duration in the perforant path.

 Group A (N=5)

 Group B (N=3)

Note that on stimulation day 38 Group B consisted of only 2 rats, and on stimulation day 40 only 1 rat remained in Group B.

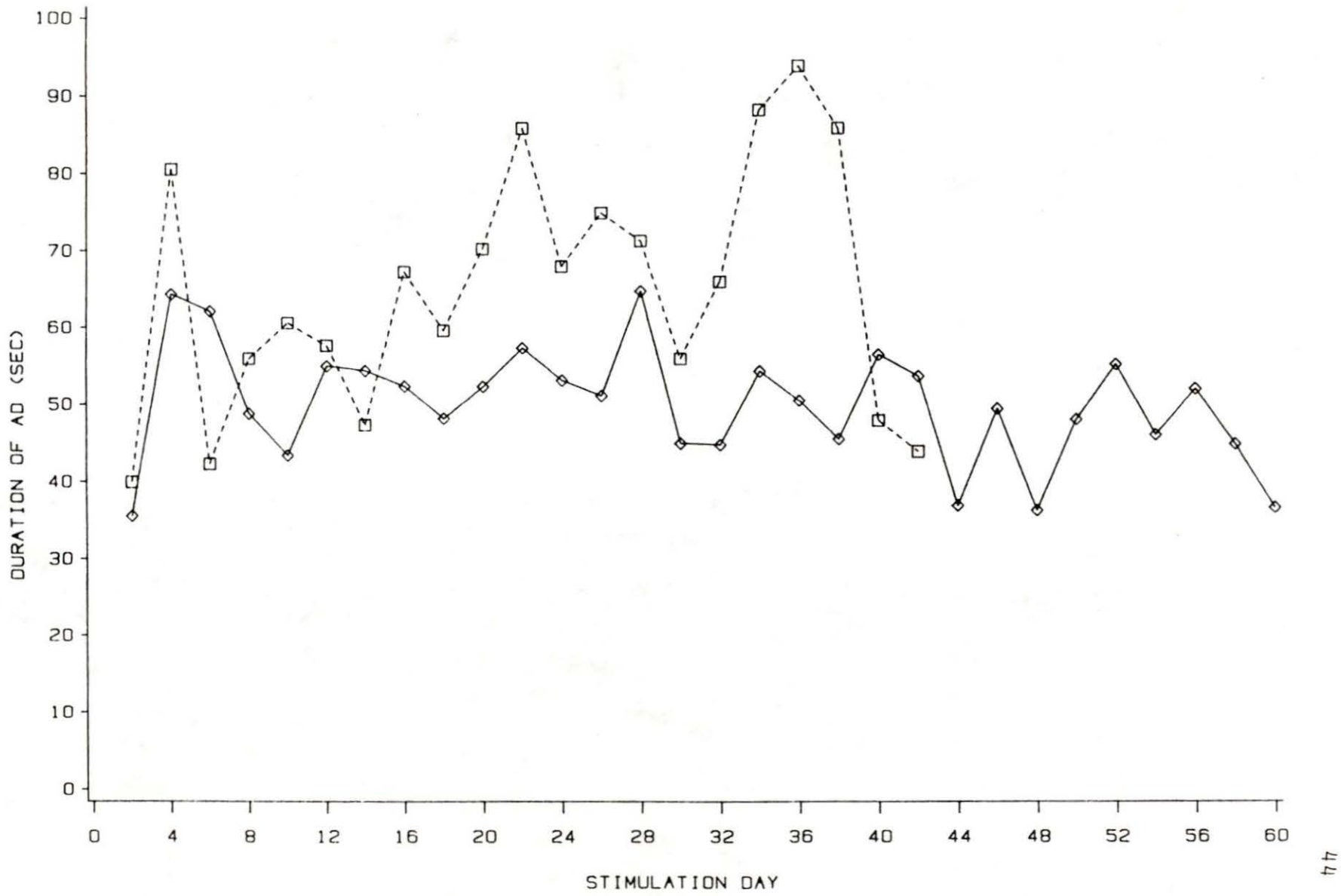

DURATION OF AD (SEC)
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
STIMULATION DAY

TABLE 5

Number of Days Off During Kindling

Group	Number of Days Off		% of Stimulation Days AD Present	
A	$\bar{X}$	3.2	$\bar{X}$	94.6
	SEM	1.6	SEM	2.5
	range	0-8	range	86.7-100
B	$\bar{X}$	0.67	$\bar{X}$	98.3
	SEM	0.33	SEM	0.9
	range	0-1	range	97.4-100

behaviors) and others after termination of the AD and seizure (postictal behaviors). The behaviors noted most frequently in both groups of experimental rats were grooming, wet dog shakes (WDSs), and stretching. WDSs are shaking movements of the body such as those made by a wet dog and commonly occur in most fur-coated and feathered animals after skin irritation or sensations of cold. On the average, rats groomed on 22% of the stimulation days, showed WDSs on 19% of the days, and stretched on 10% of these days (Table 6). Group A groomed less but displayed more WDS and stretching behavior than Group B. The incidence of these behaviors seemed to be evenly distributed throughout the course of kindling; that is, there was no obvious tendency for the behaviors to occur in a given rat during a particular stage of kindling. Yawns and sneezes were also observed on occasion, but were much less frequent than the other behaviors.

II. Effects of Kindling on Evoked Potentials

During the initial stages of kindling (prior to stimulation day 8), mean population spikes in both Groups A and B were increased in amplitude, with Group A showing a greater increase than Group B (Figure 5). As kindling

TABLE 6

Percentage of Stimulation Days that Grooming, WDSs, and Stretching were Observed

Group		Grooming	WDSs	Stretching
A	$\bar{X}$	15.4%	21.2%	12.8%
	SEM	4.4	8.7	4.6
	range	2-28%	3-52%	0-27%
B	$\bar{X}$	33.3%	15.0%	4.3%
	SEM	16.4	4.9	3.4
	range	8-64%	6-23%	0-11%
Overall	$\bar{X}$	22.1%	18.9%	9.6%
	SEM	6.8	5.6	3.3
	range	2-64%	3-52%	0-27%

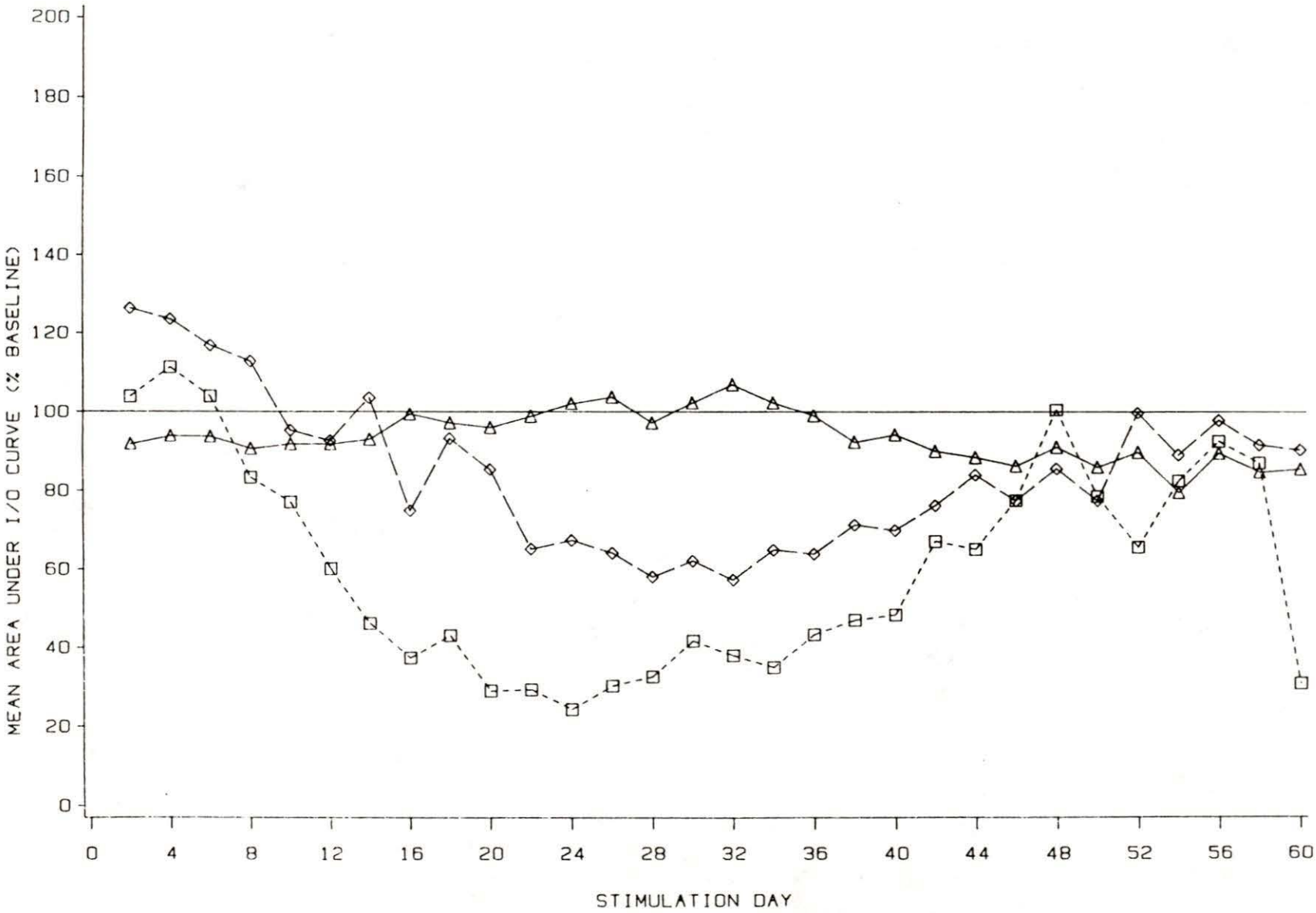
Figure 5: Mean changes in I/O curve areas over the course of kindling.

◇—◇—◇ Group A (N=5)

□—□—□ Group B (N=3)

△—△—△ Control (N=6)

Note that on stimulation day 46 Group B consisted of only 2 rats, and on stimulation day 60 only 1 rat remained in Group B.


MEAN AREA UNDER I/O CURVE (% BASELINE)
200
180
160
140
120
100
80
60
40
20
0
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
STIMULATION DAY

progressed, however, the mean population spikes decreased in amplitude, with I/O curve areas returning to baseline levels, and then decreasing far below these levels. Group B showed a greater decrease than Group A. Toward the end of the 60-day stimulation period, mean population spike amplitudes for both groups gradually increased, although they never fully recovered to baseline levels. Control rats showed no change in I/O curve area except for a slight decrease toward the end of the 60 days. The I/O curve area records for each of the three rats in Group B are shown in Figure 6. All three rats displayed an initial increase and a subsequent marked decrease in area under the I/O curve during the course of kindling. Whereas the population spikes and the associated area of the I/O curve in one of the rats remained at a very depressed level (approximately 25% of baseline area), the population spikes and the associated area of the I/O curve in another rat recovered to levels above baseline. The persistence of the decline in the population spikes and the associated area of the I/O curve in the third rat could not be assessed, because it dislodged its electrode pedestal on day 44.

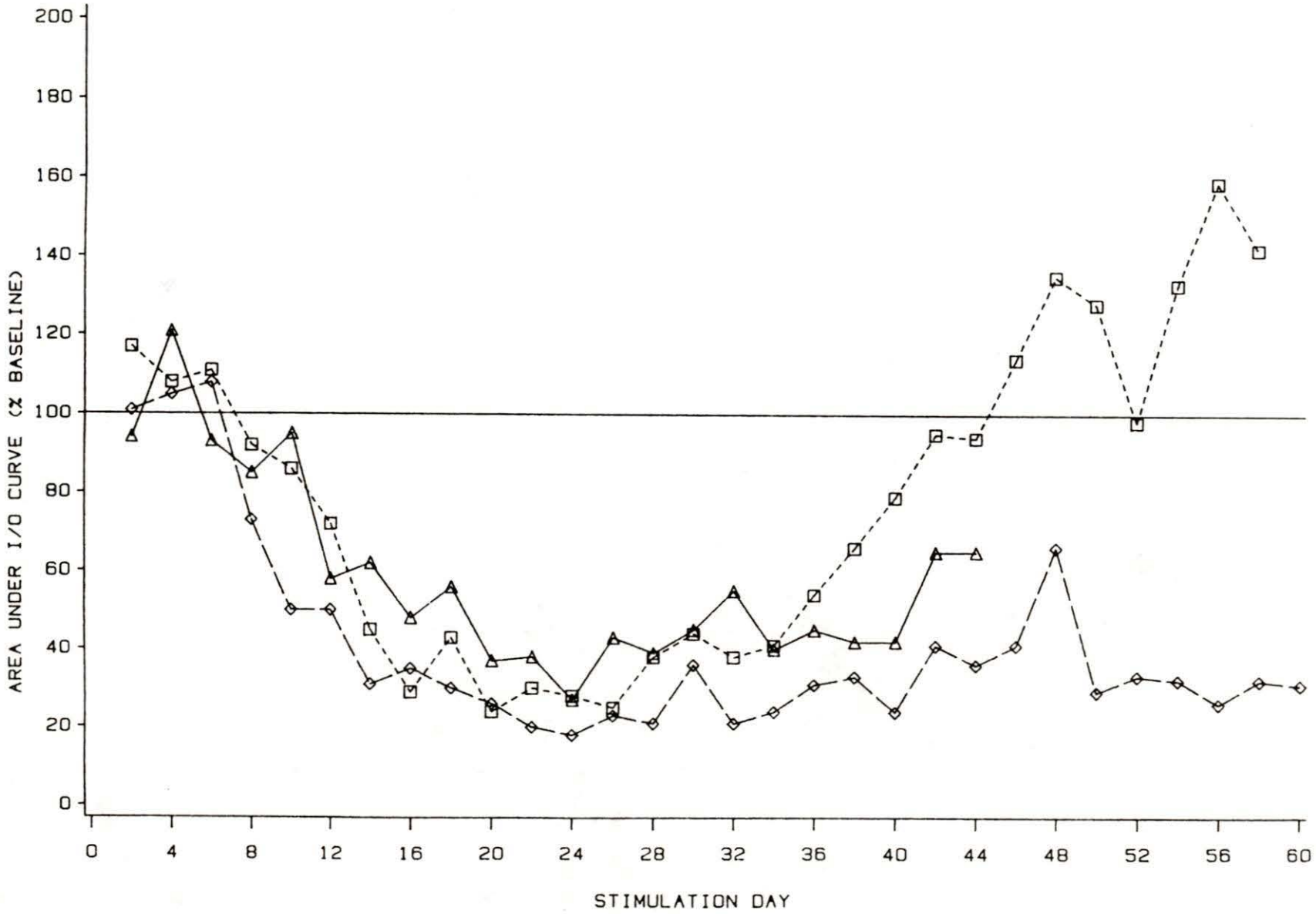
Figure 6: Changes in I/O curve areas over kindling for Group B, the 3 rats that developed stage-5 seizures.

◇—◇—◇ Rat 985

△—△—△ Rat 999

□—□—□ Rat 987

Note the following days on which the rats developed their 3rd consecutive stage-5 seizure: Rat 985, day 42; Rat 987, day 36; Rat 999, day 37.


AREA UNDER I/O CURVE (% BASELINE)
200
180
160
140
120
100
80
60
40
20
0
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
STIMULATION DAY

Behavioral Observations During Recording of Evoked Potentials

During the period in which evoked potentials were recorded (8.5 min per session), several behaviors that were commonly seen during kindling often occurred, including grooming, WDSs, stretching, and yawning (Table 7). The most common behavior was grooming, which occurred on 52% of the recording sessions for the experimental group and on 58% of the sessions for the control group. WDSs were not as common as during kindling, and appeared to occur more frequently in the control rats. The frequency of the behaviors did not appear to change across sessions. Similarily, within a given session, the frequency of the behaviors did not appear to increase or decrease; that is, the occurrence of the behaviors did not seem related to current intensity, which was presented in an ascending series within each session. The behaviors were often displayed immediately after a stimulation, appearing driven by it. Grooming was measured in seconds for each 8.5-min recording session (Figure 7). There did not appear to be any consistent change in the duration of grooming measured over the recording sessions for either the experimental or control groups. However, the control

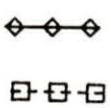
TABLE 7

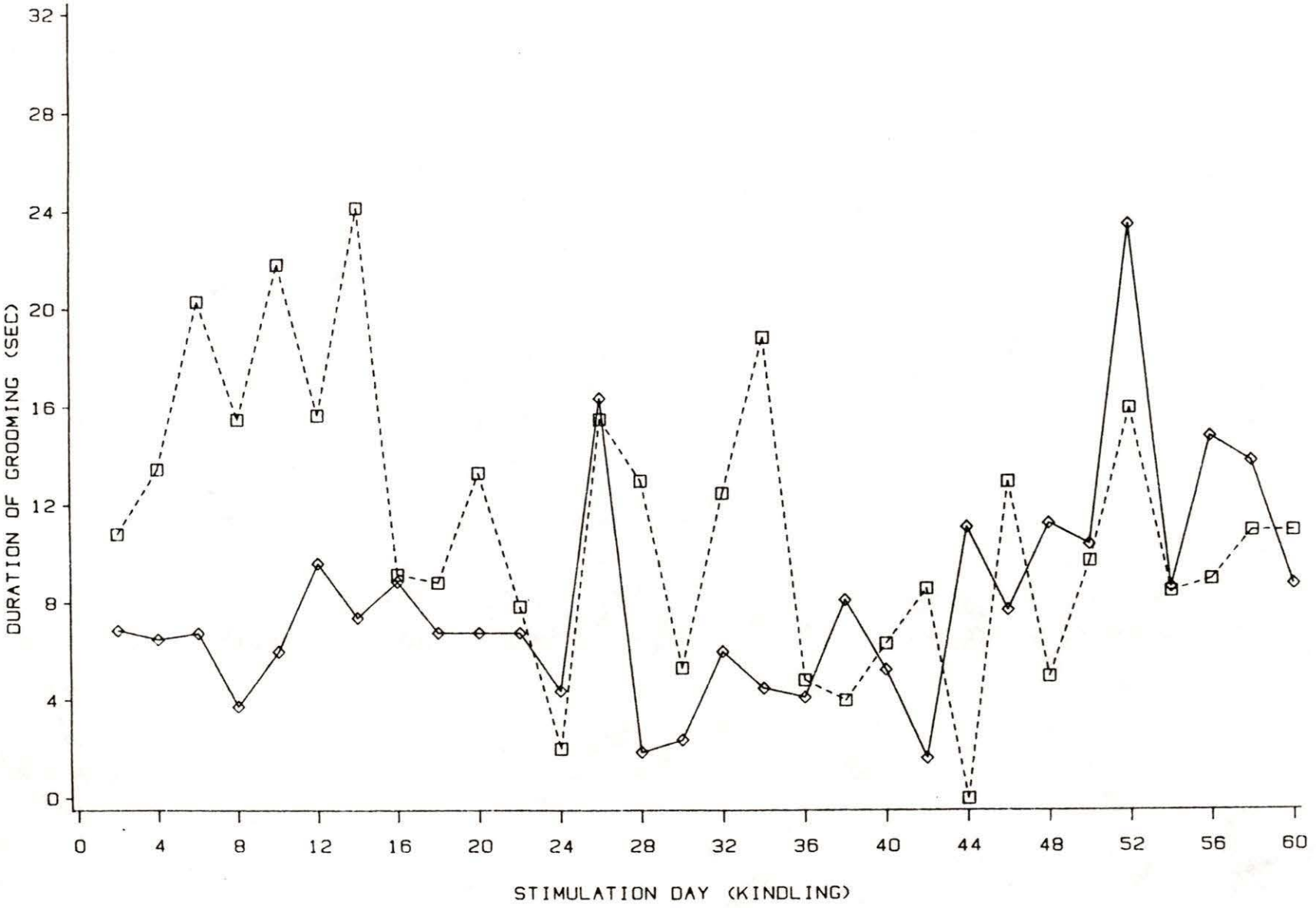
Percentage of Evoked Potential Recording Days that Grooming, WDSs, Stretching, and Yawning were Observed

Group		Grooming	WDSs	Stretching	Yawning
Exp *	$\bar{X}$	52%	0.25%	19%	7%
	SEM	9.5	0.1	5.0	3.9
	range	13-83	0-5	0-40	0-28
Con **	$\bar{X}$	58%	8.0%	11%	3%
	SEM	7.8	4.9	4.1	1.2
	range	30-82	0-30	3-27	0-7

* Experimental Group
** Control Group

Figure 7: Duration of grooming during recording of evoked potentials measured over kindling.




DURATION OF GROOMING (SEC)
0
4
8
12
16
20
24
28
32
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
STIMULATION DAY (KINDLING)

rats showed higher durations of grooming during the first 14 days than did the experimental rats.

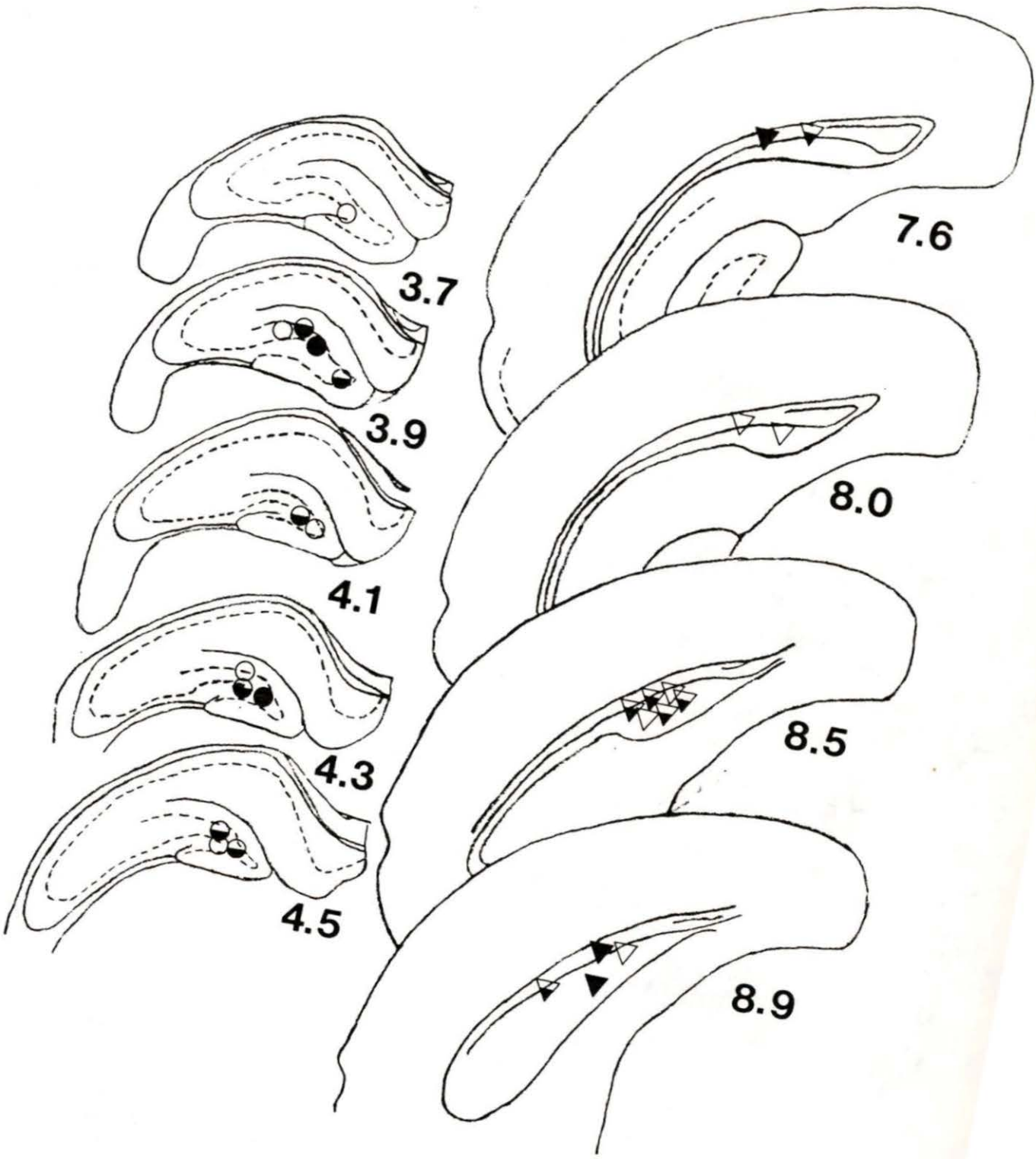
Another behavior that was displayed by about half of the rats during recording of evoked potentials was apparent aggressive behavior. The rats would typically bite and tear at the litter paper covering the floor of the recording box. Also, during this biting and tearing the rats displayed increased levels of locomotor activity.

Histology

The recording and stimulating electrodes from all rats were found to be in the hilus of the DG and in the angular bundle, respectively, with the exception of the electrode directed at the hilus in one rat from Group B whose location could not be determined. There were no obvious differences in electrode placement between Groups A and B or between experimental and control rats. In particular, hilar electrodes from rats in Group B (excluding the subject whose hilar placement could not be identified) were not located nearer to the convergence of the mossy fibers than electrodes from rats in Group A (Figure 8).

Figure 8: Histological verification of electrode placement.

Placements of recording (○ Group A; ● Group B; ◒ Control) and stimulating (▽ Group A; ▼ Group B; ▽ Control) electrodes are shown on partial coronal sections of the dentate gyrus and posterior hippocampus (redrawn from Konig and Klippel, 1963). The approximate distance of each section from Bregma is shown.


3.7
3.9
4.1
4.3
4.5
7.6
8.0
8.5
8.9

DISCUSSION

The present study was designed to characterize kindling of the hilar region of the DG and to examine its effects on the population spike component of the PP-DG evoked potentials. Each feature of the study will be discussed separately.

I. Characterization of Hilar Kindling

Post-hoc analyses on the rate of kindling revealed that the experimental group comprised two distinct subgroups: those rats that developed stage-5 seizures (Group B), and those that developed only stage-1 or stage-2 seizures (Group A) in spite of the 60-day stimulation period. Although there was high intersubject variability on most measures, the two subgroups did differ on a number of measures. Compared to rats in Group A, rats in Group B tended to:

1. require fewer ADs to reach the first stage-2

seizure.

2. have lower threshold for AD.

3. have higher overall duration of AD and, unlike Group A, tended to show an increase in AD duration over kindling.

4. demonstrate fewer days off.

5. groom more, but tended to show fewer WDSs and less stretching behavior.

6. all show postictal spiking, compared to only 2 out of 5 rats in Group A.

Many of these differences suggest that the three rats in Group B were simply more susceptible to developing fully generalized seizures. However, the reasons for this differential susceptibility can only be speculated. Since there were no obvious histological differences in electrode location between the two groups, it seems likely that the mechanism is of a more refined nature, possibly neurochemical.

In general, although kindling with stimulation of the hilus was typical of limbic kindling in some ways (e.g., threshold for AD, relatively late development of motor seizures), it also had several unique characteristics. One of the most obvious differences was in rate of kindling. Whereas amygdaloid kindling progresses to

stage-5 seizures after about 10-15 ADs (Burnham, 1978), Group B in this experiment took an average of 22 ADs to develop stage-5 seizures. Furthermore, the other five experimental rats had developed only stage-1 or stage-2 seizures despite 60 days of stimulation.

Related to the low rate of kindling was the high instability of kindling (i.e., the many regressions and days off). Even after a stage-5 seizure had been produced, regressions back to stage-1 seizures were common. Instability of kindled seizures has also been noted with anterior neocortical kindling (Burnham, 1978; Seidel & Corcoran, submitted for publication), but has not been reported for amygdaloid kindling. If the phenomenon is due to a transient increase in the threshold for AD, it is not consistent with Racine's (1972a) finding that limbic kindling results in a permanent lowering of AD threshold. In addition, unlike the rapid incremental growth of AD seen in amygdaloid kindling, both hilar and PP AD grew gradually (Group B) or not at all (Group A). Also, the occurrence of flat EEG in the hilus after the primary AD is not characteristic of limbic sites, except for the hippocampus. Many of these hilar traits could be taken to suggest the presence of a strong local inhibitory process affecting hilar kindling. This suggestion is

supported by studies that have found an increase in paired-pulse depression in the PP-DG response after kindling (Racine et al., 1983; Tuff, Racine, & Adamec, 1983). A recurrent inhibitory circuit with GABAergic transmission is known to exist in the hippocampus. It is tempting to speculate that this system may become potentiated during kindling, resulting in reduced propogation of AD to other structures (Lerner-Natoli et al., 1984; Tuff, Racine, & Adamec, 1983). In further support of this speculation, it has been reported than an increase in GABAergic inhibition in the DG occurs following amygdaloid kindling (Tuff, Racine, & Adamec, 1983; Tuff, Racine, & Mishra, 1983). This possibility is certainly consistent with the low rate of kindling and high number of days off observed in this study. Furthermore, this hypothesized inhibition could have been so great in Group A that it completely prevented the triggering of generalized seizures. However, although still showing signs of apparent inhibition (e.g., days off, regression), rats in Group B nonetheless developed generalized seizures. The reasons for these differences between the groups are not clear.

Goddard et al. (1969) proposed that the rate of kindling is related to the strength of the anatomical

connections between the stimulated structure and the amygdala. In line with their proposal, another possible explanation for the instability and low rate of hilar kindling is that there are relatively weak anatomical connections between the dorsal hippocampus and the amygdala (see Lerner-Natoli et al., 1984; Racine et al., 1977). It might also be hypothesized that the characteristics of hilar kindling are due to the relatively weak anatomical connections between the dorsal hippocampus and the proposed brainstem or midbrain motor sites that are thought to be responsible for driving motor seizures (Racine, Okujavo, & Chipashvili, 1972). Of course, the above proposals are not mutally exclusive, and all may be correct.

Some of the behaviors observed during kindling and in some cases during the recording of evoked potentials (including WDSs, grooming, and apparent aggression) have been previously observed during or after stimulation of the hippocampal formation (Damiano & Connor, 1984; Lerner-Natoli et al., 1984; Racine et al., 1977). In fact, WDSs appeared to be directly driven by stimulation of the hippocampal formation that did not evoke AD (Damiano & Connor, 1984). The high frequency of the other behaviors frequently observed in the present study, in

particular grooming and stretching, might indicate that these also are responses that occur as a consequence of nonepileptiform activity in the hippocampal formation.

II. Effects of Kindling on Evoked Potentials

Hilar kindling in both Groups A and B resulted in an initial increase in population spike amplitudes, followed by a decrease to far below baseline levels, and finally a return to near baseline level. Population spike amplitudes in controls remained at baseline except for a slight decrease at 60 days. These findings are similar to Goddard's (1982), who also kindled with stimulation of the hilus, and are also similar to the findings of Maru and Goddard (1984) and Racine et al. (1983), who all stimulated the PP. It appears, therefore, that either direct or indirect stimulation of the dentate granule cells results in an initial increase in population spike amplitudes, followed by a large decrease. Unfortunately, the present study does not allow for any clear explanation of these results, and the findings can be interpreted in several ways, any of which or a combination of which may be correct. I shall offer three reasonable speculations that might explain the results. First and probably most

superficially, these results may be taken to indicate that the time courses of kindling and of the observed increase in the amplitude of the population spike differ, with kindling ultimately resulting in a decrease in the amplitude of the population spike. If the amplitude of the population spike can be taken as an index of cellular excitability, these results then suggest that a long-lasting increase in the excitability of granule cells is not the major mechanism of hilar kindling. Second, the possibility exists that LTP does subserve kindling, but the pathways involving the LTP do not include the particular one measured in this study (i.e., PP-DG). For example, it is possible that all afferents to the stimulated hilar cells would show potentiated responses during the initial stages of kindling as the hilar cells become increasingly excitable. As kindling progresses, however, certain afferents with greater intrinsic "plastic" properties might take precedence over others, and the former would become potentiated. As these preferred afferents are potentiated, other converging afferents (e.g., PP-DG afferent) might be suppressed via long-term heterosynaptic depression (Abraham & Goddard, 1983). This speculation accounts for both the brief initial potentiation of the population spike and the later

decrease in the population spike seen in the present study. The third speculation also does not preclude a major role of LTP in kindling. The large decrease in population spike amplitude observed during kindling might also suggest the possible existence of a strong tonic inhibitory mechanism that becomes potentiated during hilar kindling. In support of this hypothesis, hippocampal kindling resulted in a temporary increase in the inhibitory effects of stimulating commissural fibers arising from the contralateral hippocampus (Goddard, 1982; Maru & Goddard, 1984). Another possibility is that the proposed increase in tonic inhibition may be somehow mediated by the GABAergic recurrent inhibitory system (Tuff, Racine, & Adamec, 1983). Whatever the mechanism, an increase in tonic inhibition due to kindling could result in a decreased number of cells firing, and thus a decrease in the population spike amplitude.

Further differences between Groups A and B were indicated in this second aspect of the study, with Group B showing a more marked decrease in population spike amplitude as kindling progressed. This finding is not consistent with the previous speculation that rats in Group B may be predisposed to less hilar inhibition. It is not readily apparent why rats that kindled to fully

generalized motor seizures would show a more striking decrease in population spike amplitude than rats that did not.

Summary and Conclusions

There are two major findings in the present study. First, hilar kindling possesses several unique characteristics that set it apart from typical limbic kindling. These include low rate of kindling, marked instability of the seizures (i.e., many regressions and days off), and relatively little or no growth in AD duration. Second, kindling in the hilus did not result in permanent LTP of the PP-DG population spike. Rather, after a brief initial increase in population spike amplitude, there was a large decrease to below baseline and control levels.

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