KINDLING AND PLASTICITY OF THE HIPPOCAMPAL INHIBITORY SYSTEM

by

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Kindling and Plasticity of the Hippocampal Inhibitory System

Kindling-induced changes in the ease with which a signal is transmitted from one set of neurons to the next were studied in this thesis. Kindling involves the application of epileptogenic stimulation to brain sites until fully generalized behavioural seizures occur. It is known that hippocampal inhibition is increased as a result of kindling hippocampal neurons or pathways. Experiment 1 of this thesis showed that the kindling-induced increase in inhibition was not associated with the permanent change produced by the kindling stimulation but was dependent on the stimulation itself. If kindling stimulation was terminated, inhibition returned towards pre-kindling levels. Experiment 2 indicated that an increase in benzodiazepine receptors may underly the kindling-induced increase in inhibition. These plasticity characteristics of the hippocampal inhibitory system are similar to those of a form of neural plasticity observed in the hippocampal excitatory system named long-term potentiation (LTP). LTP is of special interest to psychologists because of its potential involvement in information storage processes. The results of the experiments in this thesis raise the possibility that plasticity of inhibitory systems may also play an important role in information storage processes.

ABSTRACT

Kindling involves the repeated application of epileptogenic stimulation to certain brain sites until fully generalized behavioral convulsions occur. Input/output relationships (determined by recording responses evoked at different stimulation intensities) and paired-pulse facilitation/depression effects were monitored in the hilus of the hippocampal dentate gyrus as stimulation pulses were applied to the perforant path. These effects were measured before, during and after kindling the perforant path or the hilus of the dentate gyrus.

After kindling was completed, the perforant path-kindled animals showed an increase in population spike height (related to the number of cells firing) at the higher intensities but a decrease at lower intensities. A small increase in the slope of the rising phase of the population EPSP was observed at the higher intensities. The dentate gyrus-kindled animals showed a progressive decrease in population spike height during kindling, while the slope increased in magnitude. All of these kindling-induced effects, except for the increase in slope, appeared to decay towards pre-kindling levels over a 4 week stimulation-free period after the completion of kindling.

Paired-pulse depression of the population spike height was "potentiated" in both groups as a result of kindling. Paired-pulse effects returned towards pre-kindling levels over a 4 week stimulation-free period post-kindling. This decay was especially clear in the perforant path-kindled group.

Kindling the perforant path resulted in a significant increase in number of benzodiazepine receptors. This increase in receptor number is believed to underly the kindling-induced increase in paired-pulse depression. Results indicated that the increase in receptor number may decline with time after kindling is terminated.

It was concluded that almost all kindling-induced effects studied disappear over time after kindling is terminated. They are dependent on the occurrence of epileptogenic stimulation. Characteristics of potentiation of the hippocampal inhibitory system appear to be similar to those of potentiation of the hippocampal excitatory system.

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List of Abbreviations

AD:	Afterdischarge
Bz:	Benzodiazepine
CNS:	Central Nervous System/
E1:	Conditioning Slope (Population EPSP)
E2:	Test Slope (Population EPSP)
EEG:	Electroencephalogram
EPSP:	Excitatory Postsynaptic Potential
GABA:	Gamma AminobutyricAcid
GAD:	Glutamic Acid Decarboxylase
IM:	Intramuscular
IPI:	Interpulse Interval
IPSP:	Inhibitory Postsynaptic Potential
LTP:	Long-Term Potentiation
P1:	Conditioning Population Spike Height
P2:	Test Population Spike Height
PNS:	Peripheral Nervous System

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CHAPTER 1

PLASTICITY IN THE CENTRAL NERVOUS SYSTEM

1.1 Introduction

Hebb (1949) wrote in the introduction of his book "The Organization of Behavior":

> ... that behavior and neural function are perfectly correlated, that one is completely caused by another. (p. xiii),

and

> Psychologist and neurophysiologist thus chart the same bay - working perhaps from opposite shores, sometimes overlapping and duplicating one another, but using some of the same fixed points and continually with the opportunity of contributing to each other's results. (p. xiv).

The two main points of Hebb's theory about information storage in the nervous system were that:

1. Synaptic connections can be strengthened as a result of repeated and persistent use (a strengthened synaptic connection implies an increased efficiency in transmission from one nerve cell to another). Hebb proposed a growth of synaptic terminals as the mechanism for the change in synaptic strength but did not believe that the type of mechanism underlying synaptic change was crucial for his theory.

2. Cell assemblies are formed in which neural activity can reverberate for varying lengths of time. Which cells will be part of any particular assembly depends on the external stimulus conditions and on the state of the central nervous system (CNS) at the time of learning or trace formation.

The details of the theory are not as important as the effect it had on subsequent thinking about the nervous system and its storage capabilities. The notion that "memory" is determined by synaptic alteration was attractive because of the enormous number of synapses present in the human brain, a structure with an enormous memory capacity. Furthermore, synaptic modifiability is amenable to experimental analysis. The idea of cell assemblies was important, in part, because of its implicit warning against a simple equation of specific anatomical structure with function.

Even prior to the publication of Hebb's book, there were numerous examples of short-term increases in synaptic strength as a result of stimulation. Most of these effects were found in the peripheral nervous system (PNS). Sechenov (1863) described how pinching one of the toes of a decapitated frog's hind leg led to flexion in all joints. After this reflex had subsided, Sechenov observed that the leg remained bent in all joints for a period of half an hour. This effect was particularly clear in the joint between shank and foot. Sherrington (1906) found that certain reflexes could be triggered if stimuli were repeatedly administered at a moderate frequency (11.3 Hz). Identical stimuli applied in isolation would not

trigger these reflexes. Schaefer et al. (1938) and Feng (1941) reported an increase in endplate potentials after repeated activation of the pre-synaptic axon. The action potential traveling along this axon did not appear to be affected by the stimulation. These potentiation effects at the neuromuscular junction were later studied by del Castillo and Katz (1954a,b,c) using quantal analysis. But it was Liley (1956a,b) who provided crucial evidence showing that post-activation phenomena observed at the neuromuscular junction were the result of a stimulation-dependent increase in output of transmitter substance. These findings have been confirmed by numerous other investigators (see Martin, 1977). Only a few experiments investigated post-activation potentiation phenomena in the CNS. Hughes et al. (1956) reported such potentiation effects in the lateral geniculate nucleus after stimulating the optic tract. Campbell and Sutin (1959) found that "spike-like" events could be recorded in the hippocampal gyrus after stimulating the fornix. Gloor et al. (1964) reported that repeatedly activating the perforant path led to short-lasting (about 10 s) potentiation effects in the hippocampal dentate gyrus.

Short-lasting increases in synaptic strength at the neuromuscular junction could be divided into three types. Depending on the strength, duration and frequency of stimulation, one of 3 post-activation potentiation effects could be induced: facilitation, augmentation or potentiation. These increases in synaptic efficacy decayed exponentially with time constants ranging from milliseconds, seconds, to minutes respectively (Barrett and Stevens, 1972; Magleby

and Zengel, 1975a,b, 1976a,b,c; Magleby, 1973). After McNaughton (1980) described similar events at the perforant path-dentate granule cell synapse in the hippocampus, Racine and Milgram (1983) found that such short-term potentiation phenomena could be induced in a large number of limbic forebrain pathways. Although these synaptic changes did not last long enough to account for long-term memory, they do fulfill the temporal requirements for the basis of at least some types of short-term memory (Goddard, 1980).

Although most post-activation potentiation effects appeared to be short-lasting (seconds or minutes), there were some reports of longer-lasting effects. Eccles and McIntyre (1951) found that potentiation of a monosynaptic reflex arc in the spinal cord could last for hours. After applying long trains of stimulation (over 1/2 hour) to spinal cord inputs of cats, Spencer and Wigdor (1965) observed potentiation effects lasting several hours. Similar, and even longer-lasting, changes could be induced in certain parts of the CNS by electrical stimulation of nerve pathways. Such changes were named "long-term potentiation" (LTP). This effect could remain for weeks, sometimes months, after the last stimulation was applied (Bliss and Gardner-Medwin, 1973; Douglas and Goddard, 1975; Barnes, 1979; Racine et al., 1983). The duration of LTP makes it one of the more attractive candidates for the neural basis of long-term memory (Goddard, 1980). Since this is the phenomenon of interest in the present thesis, it will be discussed in more detail in the following section.

1.2 Long-Term Potentiation (LTP) of Excitatory Synapses

1.2.1 The Phenomenon and its Characteristics

In 1971, Lomo analyzed the monosynaptically evoked response that could be recorded in the hippocampal dentate gyrus when a stimulating pulse was applied to the perforant path. The perforant path conveys input to the dentate gyrus from the entorhinal cortex (Figs. 1, 2). The first component of this response was thought to reflect the sum of synaptic current flow in a population of neurons, because its onset time was identical to the onset time of the intracellularly recorded EPSP (Excitatory Post-Synaptic Potential). Lomo (1971) also found that if the intensity of stimulation was increased, a compound spike appeared superimposed on the "population EPSP" (Fig. 3). He proposed this "population spike" was due to the synchronous discharge of granule cells and that size of the spike reflected the number of cells firing. This was confirmed by Andersen et al. (1971b).

Bliss and Lomo (1973) stimulated the perforant path with a train of pulses (10-20 Hz for 10-15 s or 100 Hz for 3-4 s) and observed a potentiation of test responses evoked in the granule cells. This potentiation lasted from 30 minutes up to 10 hours and was reflected in a decrease in latency of the population spike and an increase in the amplitude of the population EPSP and spike height (Bottom of Fig. 3). No such potentiation was observed in the contralateral control pathway. Bliss and Gardner-Medwin (1973) found

Figure 1.

A top view of the rat hippocampal formation after removing the left cortex. As the hippocampus extends posteriorly, it curves ventrolaterally into the temporal lobe.

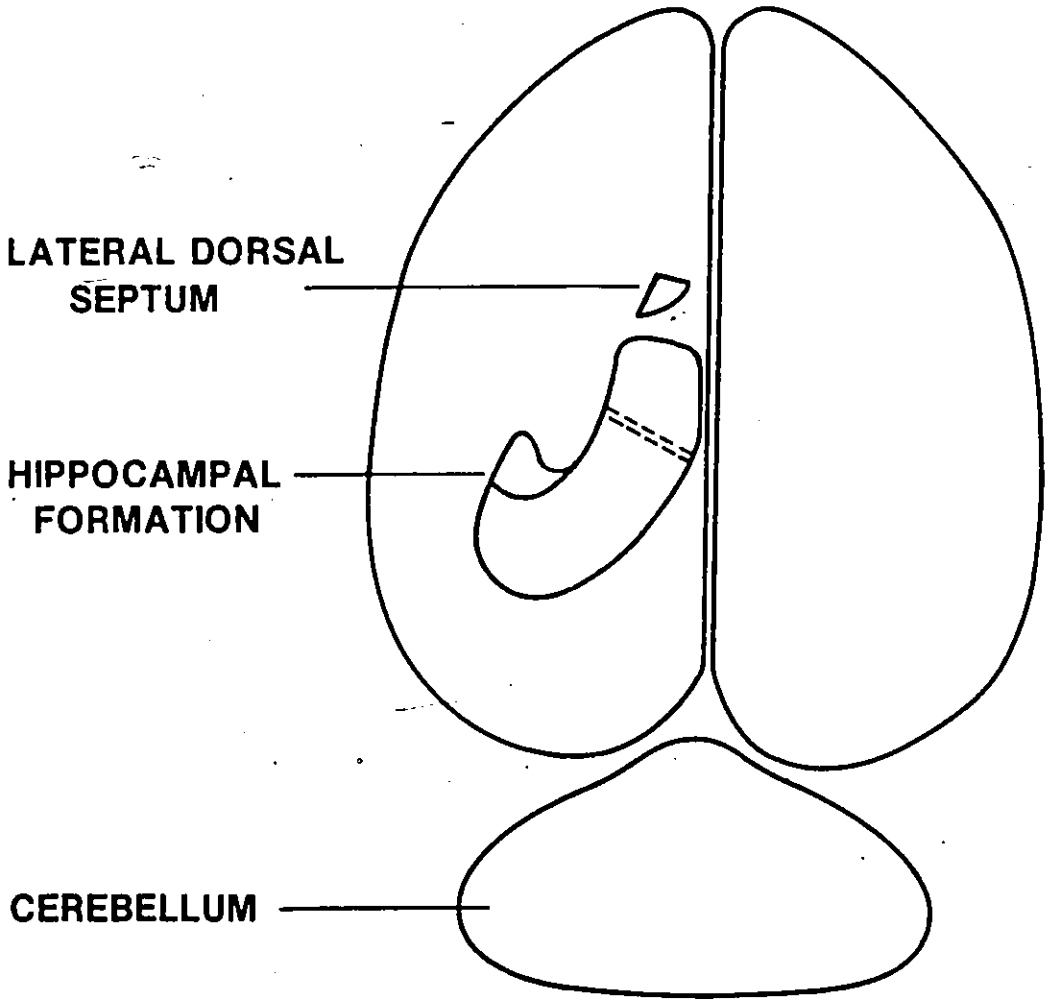

LATERAL DORSAL
SEPTUM
HIPPOCAMPAL
FORMATION
CEREBELLUM

Figure 2.

A diagram of the internal circuitry of the hippocampal lamella oriented at right angles to the longitudinal axis (as indicated by the dashed lines in the previous figure). Perforant path (pp) fibres, arising in the entorhinal cortex (ENTO), make contact with granule cells (Gr). Axons of the granule cells, the mossy fibres (mf) innervate CA3 pyramidal cells. Axons from CA3 neurons, the Schaffer collaterals (Sch), project to the CA1 pyramidal cells. CA1 and CA3 cells send their axons via the alveus (alv) into the fimbria (fim).

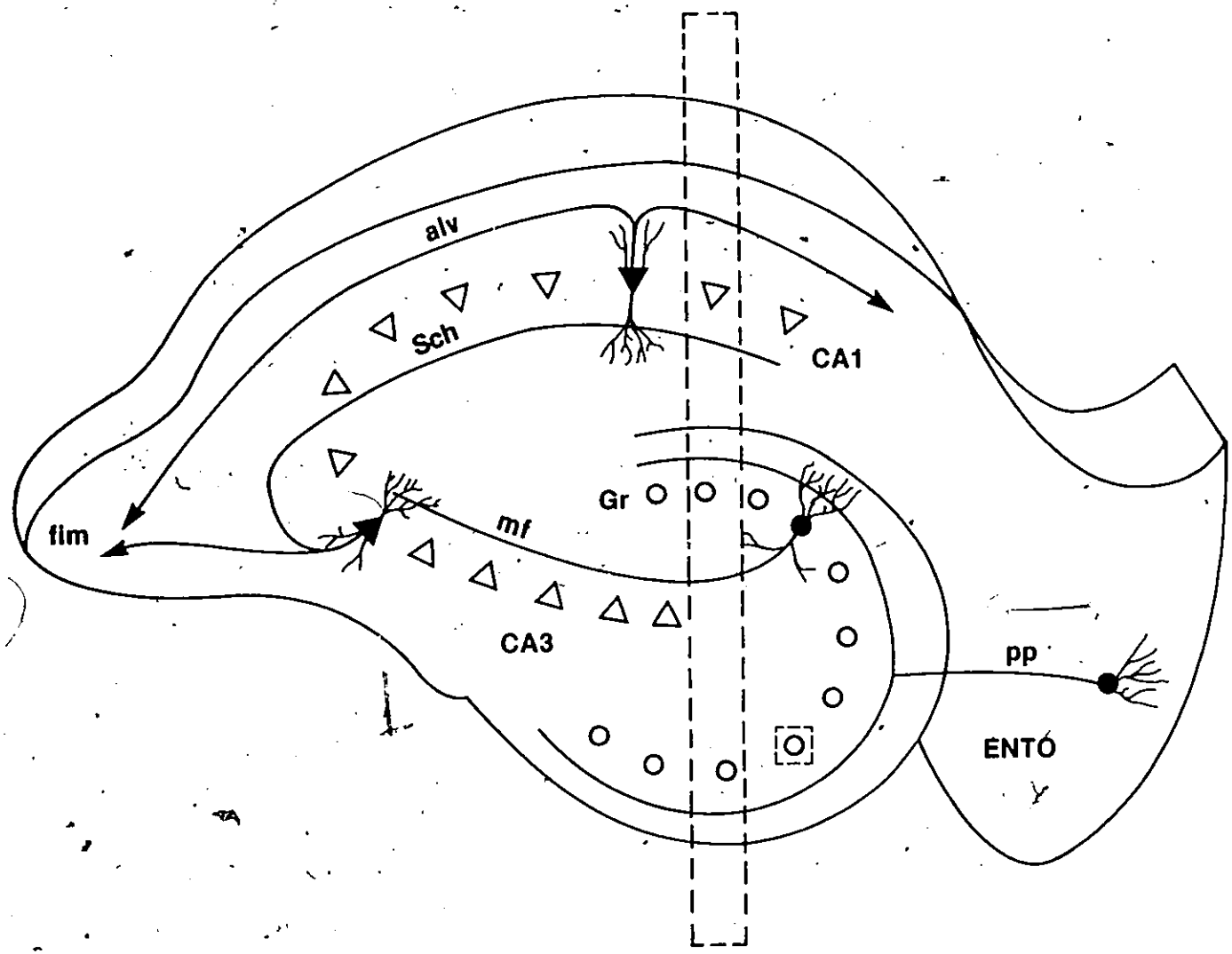
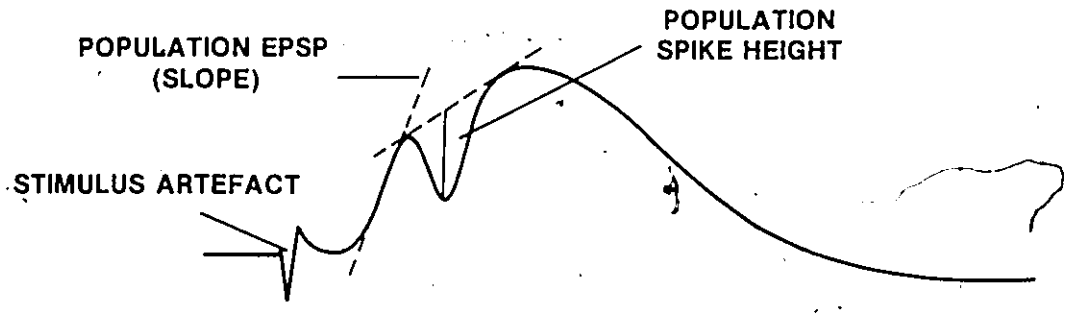
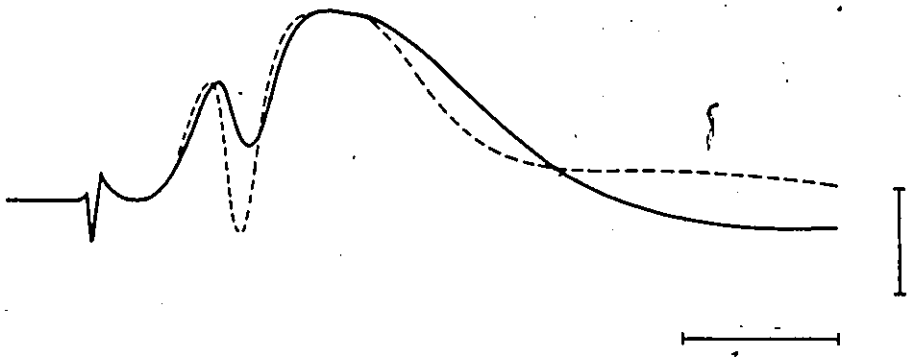

alv
Sch
CA1
fim
mf
Gr
CA3
pp
ENTO

Figure 3.

Top: A typical response evoked in the hilus of the dentate gyrus by perforant path stimulation. The first 3 components are the stimulus artifact, the population EPSP (slope), and the population spike.

Bottom: The solid line is the same response as above. The dashed line represents the response, evoked by an identical stimulus pulse to the perforant path, after high frequency stimulation of the perforant path. Vertical calibration: 3 mv; Horizontal calibration: 4 ms.


POPULATION EPSP
(SLOPE)
POPULATION
SPIKE HEIGHT
STIMULUS ARTEFACT



that this long-term potentiation (LTP) of the perforant path-dentate granule cell synapse could last from 3 hours to 3 days in freely moving, unanaesthetized rabbits.

It has now been established that LTP develops within minutes of stimulation and reaches peak values 5-20 minutes post stimulation. LTP-related amplitude increases in the EPSP range from 0-100%, typically 50%. The population spike amplitude can increase from 0-1200% with an average of 250% (Swanson et al., 1982).

A wide variety of stimulation parameters can be used to produce LTP. The most effective stimulation pattern appears to be repeated high frequency trains. Douglas (1977) found trains of 8-10 pulses delivered at a frequency of 400 Hz to be optimal. LTP, however, has been produced by frequencies from 1 Hz to 500 Hz (see Swanson et al., 1982). The trains are usually delivered at intervals of seconds or minutes. The optimal frequency with which the individual pulses are delivered (i.e. 400 Hz) corresponds rather closely to the normal burst discharge patterns of hippocampal cells (Fox and Ranck, 1975; Kandel and Spencer, 1961; Ranck, 1973). Although the stimulation frequency is an important variable, the intensity of the stimulation appears to be a more salient factor. There is a threshold intensity below which no LTP can be induced (McNaughton et al., 1978). This implies that a minimum number of fibres must be activated in order to produce LTP since stimulation intensity determines the number of activated nerve fibres. Furthermore, it appears that the intensity also determines peak LTP levels (McNaughton et al., 1978).

The development of LTP is not dependent on massive discharge of the granule cells. In fact, LTP of the perforant path-granule cell synapse can be obtained even if firing of the granule cells is inhibited. This inhibition was accomplished by application of a single pulse to the contralateral hilus prior to the LTP-inducing stimulation of the perforant path (Douglas et al., 1982). Release of neurotransmitter substance from the terminals of the stimulated pathway, however, must occur in order to establish LTP (Dunwiddie et al., 1978).

1.2.2 Mechanisms of LTP

At this point in time some researchers believe that (hippocampal) LTP is caused by an increased transmitter output from the pre-synaptic terminals resulting from high frequency stimulation of the afferent pathway (Bliss and Dolphin, 1982; Dolphin et al., 1982; Skrede and Malthe-Sorenssen, 1981; Feasey et al., 1985). Others advocate an increase in the number of post-synaptic receptors as the principle mechanism underlying LTP (Baudry and Lynch, 1982; Lynch and Baudry, 1984). Proponents of either position agree on the importance of Ca++ influx as a link in the chain of events leading to LTP. It has been demonstrated that Ca++ plays a crucial role in the production of LTP (Lynch et al., 1983; Baimbridge and Miller, 1981; Dunwiddie and Lynch, 1979). There is disagreement on where the critical Ca++ influx occurs and what sequence of events it triggers. For example, those who advocate a post-synaptic mechanism, propose that Ca++ influx into

post-synaptic dendrites (Kuhnt et al., 1985) activates a proteinase termed calpain. Calpain in turn changes the properties of a protein (fodrin) which affects the post synaptic membrane structure. One of these effects would be to uncover latent glutamate receptors (Baudry and Lynch, 1982; Lynch and Baudry, 1984; Siman et al., 1985). The blockade of LTP in pyramidal cells, by intracellular injections of EGTA, a Ca++ chelator, supports this hypothesis (Lynch et al., 1983). A problem for the post-synaptic theory, in which the transmitter glutamate plays an important role, is the evidence showing that glutamate may not be critically involved in the production of LTP (Fagni et al., 1983; Hori et al., 1982; Dolphin, 1983; Goh and Sastry, 1984). Advocates of a pre-synaptic mechanism propose that Ca++ influx into the pre-synaptic terminal leads to the activation of processes that eventually increase transmitter output. However, the proponents of a pre-synaptic mechanism have not been able to give a satisfactory explanation of cooperativity effects (see below).

Calmodulin also appears to play an important role in the production of LTP. Calmodulin is a protein which becomes active when bound with Ca++. Active calmodulin can increase levels of phosphorylation (phosphorus incorporation) of certain proteins (Cheung, 1980). Finn et al. (1980) found that trifluoperazine, a neuroleptic which interferes with calmodulin activity, blocked hippocampal LTP. This was later confirmed in a study by Mody et al. (1984).

Recently, Chang and Greenough (1984) found that high frequency activation of hippocampal CA1 cells, leads to an increase in

the number of shaft and sessile spine synapses within a period of 15 minutes after stimulation! These results are consistent with both the pre-synaptic and the post-synaptic mechanism underlying LTP. An increase in the number of synapses necessarily implies an increase in post-synaptic receptors as well as an increase in pre-synaptic transmitter output.

Finally, it was found that hippocampal LTP-inducing stimulation, and no other type of stimulation, leads to an increase in phosphorylation of a 47kD (kD: 1000 x Dalton, a measure of molecular weight) protein (F1). The amount and perseverance over time of the increased phosphorylation appeared to correlate well with extent and duration of LTP respectively (Nelson and Routtenberg, 1985; Routtenberg, 1985). As the F1 protein is believed to be localized to synaptic regions, this phenomenon might be involved in any of the mechanisms described above. There is some evidence, however, that this protein is very active in growth cones (Nelson et al., 1985) supporting the possibility that new synapses develop.

1.3 LTP, Memory and Learning

Although LTP in itself is an interesting phenomenon, most psychologists study LTP with the belief it may be one of the physiological mechanisms underlying memory formation. The evidence supporting this belief is mainly circumstantial. A review of LTP would not be complete without a summary of that evidence even though the experiments in the present thesis do not deal with this aspect of LTP.

If LTP has anything to do with memory and learning it should occur in other sites as well. In fact, LTP can be found in several sites within the CNS. LTP has been induced in the cortex (Wilson and Racine, 1983; Lee, 1982), in most of the limbic forebrain (Racine et al., 1983; Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973; Alger and Teyler, 1976; Andersen et al., 1977; Lynch et al., 1977; Schwartzkroin and Wester, 1975), in the superior cervical ganglion (Brown and McAfee, 1982), and in the magnocellular medial geniculate nucleus (Gerren and Weinberger, 1983). However, LTP in the hippocampus is largest, develops with the smallest magnitude of stimulation and appears to last longer in comparison to other limbic forebrain structures (Racine et al., 1983). The hippocampus is believed to play an important role in the storage of knowledge (Scoville and Milner, 1957; Douglas, 1967; O'Keefe and Nadel, 1978; Thompson et al., 1980; Berger et al., 1980; Olton et al., 1980). One might expect to find less LTP nearer the "sensory side" of the CNS and PNS. Sensory transmission must be relatively stable if it is to be of any value to an organism. The lateral olfactory tract does not appear to support LTP of the monosynaptic response in the pyriform cortex (Racine et al., 1983). This supports the argument that little LTP should be supported by sensory projection pathways. However, no studies have systematically addressed that hypothesis.

Most people tend to think of memory as relatively permanent, whereas LTP decays over time. How permanent is memory? It is possible that the so-called permanent memories are actually "refreshed"

frequently, even if the read out of their contents does not reach a level of conscious awareness. On the other hand, it is possible that the duration of LTP might be increased by certain manipulations. Perhaps the pattern of activation plays an important role in determining the duration of LTP. With the present techniques employed, the optimal stimulation parameters might not, as yet, have been determined. Finally, it is possible that some "reinforcing" input is required at the time of high-frequency stimulation to make LTP permanent. The following experiments partially address these questions.

Douglas and Goddard (1975) were able to induce hippocampal LTP in rats lasting from 12 days up to 2 months. They also found that successive trains of stimulation had a cumulative potentiation effect, even when spaced as long as 24 hours apart, until saturation was reached. However, stimulation parameters used in this study elicited afterdischarge (epileptogenic activity) in most of their animals. It is known that triggering afterdischarge (AD) produces additional changes in neural function that are not, apparently, related to LTP effects (Kairiss et al., 1984). McNaughton et al. (1978) showed that high frequency stimulation at a fixed intensity and applied at 6 minute intervals did not lead to additional increases in LTP levels. Rather, the amount of LTP tended to asymptote after a few stimulations. However, increasing the intensity of the high frequency stimulation did lead to higher levels of LTP. De Jonge and Racine (1985) were also unable to detect residual effects on LTP levels when

LTP was repeatedly induced. Although LTP decayed somewhat more slowly with subsequent LTP-inducing stimulation, the decay times were well within the normally observed range and the effects were definitely not permanent. This is also true for other studies in which LTP was found to decay at a slower rate when repeatedly induced (e.g. Barnes, 1979).

It is now clear that the activation of one input can influence the levels of LTP induced by concurrent activation of a separate input. Such heterosynaptic interactions could underly such phenomena as associative memory, effects of attention and arousal on memory formation and, perhaps, trace consolidation through "reinforcing" input. The levels of LTP achieved by stimulating either the medial or the lateral perforant path were lower than those observed when stimulation was applied to both pathways at the same time (McNaughton et al., 1978; McNaughton, 1980). Levy and Steward (1983) found that LTP could not be induced at the contralateral perforant path-ipsilateral granule cell synapse if only this crossed pathway was stimulated. However, if stimulation of the crossed pathway was followed by stimulation of the ipsilateral perforant path, LTP of the crossed input could be obtained. Delays of at least 20 ms between stimulation of both pathways did not interfere with the effect. Combining septal stimulation with perforant path stimulation also led to increased peak LTP levels of the perforant path-dentate evoked response. In fact, this cooperativity effect could still be observed if septal activation preceded perforant path activation by 2 s!

The strongest effects, however, were obtained with delays of 100 ms or less between activation of the septum and the perforant path (Robinson and Racine, 1982; Robinson, 1986). Bloch and Laroche (1985) were able to increase the magnitude and duration of LTP at the perforant path-granule cell synapse by stimulating the mesencephalic reticular formation within 10 s after the activation of the perforant path. Finally, high frequency stimulation of the contralateral hilus of the hippocampal dentate gyrus 50 ms before or 1 ms after high frequency stimulation of the ipsilateral perforant path prevents LTP from developing at the perforant path-granule cell synapse (Douglas et al., 1982).

Few studies have directly addressed the involvement of LTP in memory and learning. Landfield et al. (1978) reported that old rats, as compared with young rats, showed synaptic depression (a decrease in the ease of synaptic transmission) when repetitively stimulated. More importantly, synaptic plasticity was impaired under certain circumstances. It is not clear whether some of the observed plasticity phenomena should be labeled LTP. These older rats were previously shown to have memory deficits. A study by Barnes (1979) showed that there is a difference between senescent and mature adult rats in the ability to maintain LTP. This difference appeared to be correlated with performance on a number of tasks. For example, older rats showed poorer memory for a rewarded place on a circular platform than younger rats. Also, the induction of hippocampal LTP impaired the performance of older rats on a spontaneous alternation task in a T-maze. Somewhat

contradictory were the findings by Berger (1984). He found that conditioning of the nictitating membrane response to a tone was accelerated in animals that received LTP-inducing stimulation in the perforant path before training was started. This result could be due to an increased salience of the relatively simple stimulus (tone) as a result of the stimulation.

Thompson et al. (1980) described an increase in amplitude of the perforant path-dentate population spike during the course of nictitating membrane conditioning. They suggested that this might be equivalent to an LTP effect. As previously mentioned, Chang and Greenough (1984) found an increase in number of synapses and dendrites as a result of high frequency stimulation. Similar changes were found in animals brought up in a stimulus-rich environment as opposed to a stimulus-poor environment (Turner and Greenough, 1985; Sirevaag and Greenough, 1985). The former tend to outperform the latter on several tasks (see Hebb, 1966) indicating a possible relationship between LTP and memory and learning. It has also been mentioned that administration of leupeptin, which is assumed to prevent LTP, led to a decrease in performance on a spatial maze task (Staubli et al., 1984). Finally, inducing epileptogenic activity in the hippocampus shortly after LTP had been produced (in CA1), strongly reduced the amount of LTP. If, after a recovery period ranging from 20-50 minutes, LTP-inducing stimulation was applied again, LTP levels increased but never reached pre-epileptogenic-stimulation levels (Hesse and Teyler, 1976). It has been known for a long time that animals receiving an

electroconvulsive shock after learning trials do not learn the task (see McGaugh, 1966). More specifically, inducing epileptiform activity in the hippocampus was found to interfere with the acquisition of several tasks (McGaugh and Herz, 1972; McGaugh and Gold, 1976; Salafia et al., 1977). It is also well established that memory impairments occur in humans after suffering an epileptic attack (Aird et al., 1984).

1.4 LTP of (Inhibitory) Local Circuit Systems

Surprisingly little is known about the plasticity of interneurons or local circuit neurons. The absolute and relative number of local circuit neurons increases systematically in phylogeny, peaking in the human brain. The same is true for the elaboration of these cell's processes and the frequency with which they synapse onto projection cells (see Rakic, 1975). This raises the possibility that the increased processing and storage capacity of the human brain might be attributable, in part, to this increase in local circuit neurons and the increased complexity of their connective patterns. Birth trauma and anoxic events at the neonatal stage often lead to retardation of intellectual ability and to an abnormal pattern of connectivity of the inhibitory interneurons of the hippocampus (local circuit neurons are believed to be mostly inhibitory; see Rakic, 1975).

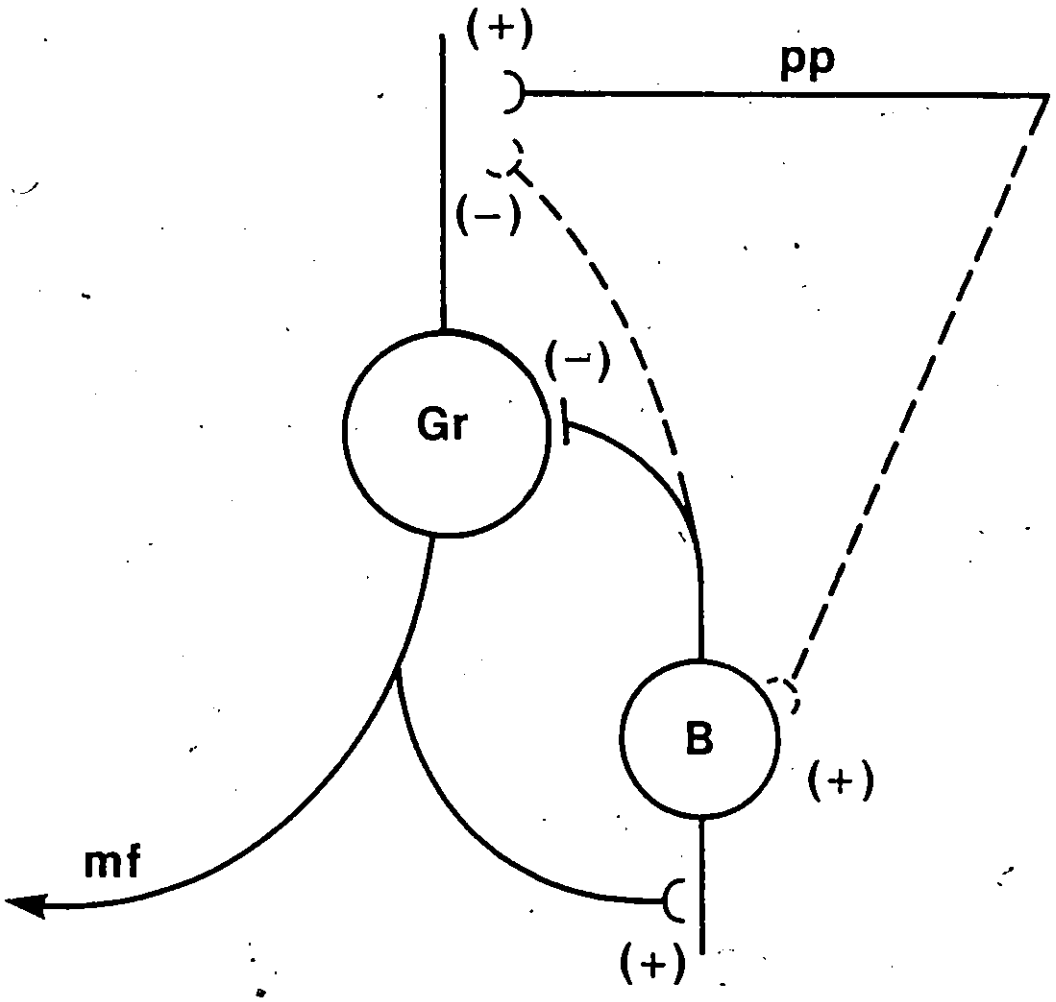
Hippocampal inhibitory interneurons, the basket cells, are known to receive collaterals from principal cells (granule and

pyramidal cells) and are responsible for recurrent inhibition (Andersen et al., 1964). It now appears that these basket cells also receive direct input from several afferent pathways to the hippocampus and, therefore, can act in a feedforward manner (Buzsaki and Eidelberg, 1982; Buzsaki, 1984; Fig. 4). These findings support the idea that inhibitory interneurons play a major role in information processing, since they can participate in information transfer as well as in feedback loops. Furthermore, the possibility of direct activation of basket cells via afferent pathways greatly simplifies the study of plasticity of the input portion of the inhibitory circuit. Target cells generally respond with only one or a few action potentials if high-frequency stimulation is applied to an afferent pathway (Lomo, 1971). Thus, if basket cells were innervated only by collaterals of principal cells, they might not be strongly activated and, consequently, might demonstrate little plasticity. Attempts to stimulate these cells directly often results in the triggering of epileptogenic activity (Kairiss et al., 1984).

Misgeld et al. (1979) were among the first to report long-lasting changes in inhibitory post-synaptic potential (IPSP) amplitudes. These were recorded from CA3 neurons before and after repetitive activation of the mossy fibres in guinea pig hippocampal slices. These increases in IPSP amplitudes, however, disappeared with subsequent trains of stimulation. Also, many cells actually showed a decrease in IPSP amplitude as a result of the repetitive stimulation. Yamamoto and Chujo (1978) reported only a depression of the IPSP in

Figure 4.

A schematic diagram of the small dashed area in figure 2. Perforant path (pp) fibres innervate granule cells (Gr). The latter send projection axons, the mossy fibres (mf), to CA3 cells. Collateral axons project to the inhibitory basket cells (B) which feed back onto the granule cells. The dashed lines represent pathways that are not well characterized at present (e.g. the feedforward inhibition believed to be mediated by the perforant path). Excitatory synapses are indicated by (+), inhibitory synapses by (-).


(+)
pp
(−)
(−)
Gr
B
(+)
mf
(+)

CA3 neurons using the same preparation and similar stimulation parameters as used by Misgeld et al. (1979). Buszaki and Eidelberg (1982) applied direct afferent stimulation (40 Hz for 5 s) to eleven interneurons, presumably basket cells, located in the (rat) hippocampal slice. They found a post-train increase in probability of cell discharge to subsequent stimulation. This increase in discharge probability could last from 2 minutes up to 2 hours (the median was 30 minutes). Three of those interneurons required more intense stimulation than the other eight to induce the LTP-like effect (Buzsaki and Eidelberg, 1982). Douglas et al. (1982) applied high frequency stimulation to the hilus. This was believed to activate contralateral basket cells, but no changes were found in the dentate field potentials. The authors stated that the intensity of the stimulation was such that it would not have produced much LTP in, for example, the perforant path-dentate granule cell (excitatory) synapse (low intensity stimulation was used in order to prevent AD). The stimulation was, however, able to prevent LTP of the ipsilateral perforant path-dentate synapse if contralateral hilus stimulation was applied within a certain time period of initiating ipsilateral perforant path stimulation. Abraham and Goddard (1983) described a small, heterosynaptic depression that could last for at least 3 hours. High frequency stimulation of either the medial or lateral perforant path led to a depression of synaptic transmission of the non-stimulated pathway. This effect might be due to potentiated inhibition. In our laboratory, Tim Kennedy was unable to show a change

in paired-pulse inhibition in the hippocampal dentate gyrus after high frequency activation of the perforant path. It was expected that if the perforant path activated basket cells in a feedforward manner, those synapses might increase in strength. Only the perforant path-dentate granule cell synapses potentiated. Abraham and Goddard (1983) found that they could not obtain heterosynaptic depression if LTP was induced in both components of the perforant path. These results are consistent with those of Tim Kennedy, who was probably stimulating both components of the pathway.

Inducing LTP via the ipsilateral perforant path decreases the amount of potentiation previously induced via the contralateral perforant path, possibly via potentiated feed forward inhibition (Levy and Steward, 1979; Wilson et al., 1981). Recently, Stripling et al. (1984) found that high frequency stimulation of the olfactory bulb in chronically implanted rats did not lead to LTP of its connections with the pyramidal cells in the pyriform cortex but did produce LTP in an electrophysiologically recorded late component believed to reflect inhibition in the pyriform cortex.

From the above it is clear that the evidence related to plasticity of (hippocampal) inhibitory synapses is fragmentary and not always convincing. Also, for most of the reported changes in the strength of inhibitory synapses, the duration is not yet sufficiently long to merit the term LTP (e.g. Buzsaki and Eidelberg, 1982). In some of the experiments the LTP-inducing stimulation was of a low intensity in order to prevent AD from occurring (Douglas et al., 1982). It is

well known that a minimum number of fibres must be activated to produce LTP of excitatory synapses (McNaughton et al., 1978). Since the stimulation intensity determines the number of fibres activated, it is possible that the hippocampal basket cells have not been activated strongly enough to produce LTP at their synapses. Another possibility is that the pattern of activation of the basket cells is crucial in determining whether long-term plastic changes will occur.

At present, the only reliable way to induce long-term changes at inhibitory synapses is to kindle an animal (Tuff et al., 1983a; Oliver and Miller, 1985). As mentioned above, triggering AD leads to LTP effects as well as neural changes that do not appear related to LTP effects. Therefore, it is important to compare the characteristics of the increased inhibition due to kindling to those of LTP. This was the aim of the present thesis. Before discussing the phenomenon of kindling-induced potentiation of inhibition, a short review of the kindling phenomenon will be provided.

CHAPTER 2

KINDLING

2.1 The Phenomenon and its Characteristics

Kindling can be defined as a progressive increase in epileptogenic responsivity produced by spaced and repeated epileptogenic stimulation of certain brain structures (Racine, 1978). This increase in neural responsivity is reflected in the animal's behavior as well as in its electroencephalogram (EEG). Except for behavioral arrest followed by exploratory behavior, there is little behavioral response to the first few electrical stimulations. With continued stimulations, however, the animals develop fully generalized motor seizures. The term kindling is used to refer to the process during which animals receive repeated epileptogenic brain stimulation as well as to the phenomenon itself. Racine (1972b) observed a consistent pattern of behavioral change during the course of amygdala kindling. During the 1st phase of kindling, mouth movements appeared (Stage 1). As stimulation was continued the following behaviors were observed: Head clonus (Stage 2), forelimb clonus (Stage 3; initially on the contralateral side to that of stimulation but eventually spreading to the ipsilateral side), a seizure driven clonic rearing (Stage 4), and finally a loss of postural control (Stage 5). Each later stage also included the behavioral responses of the preceding stages. An animal is generally said to be fully kindled when 2 or more

Stage 5 seizures have occurred successively. During the interictal period (the period between seizures) long-lasting changes in emotional behavior can occasionally be observed as measured by standard psychological tests (e.g. Boast and McIntyre, 1977: passive avoidance; Adamec, 1975: predatory behavior).

The epileptiform afterdischarge (AD) evoked by the first epileptogenic stimulation is relatively brief (about 10 s) and the EEG "spikes" that characterize the AD occur at a low frequency (1-2 Hz). At a more advanced stage of kindling, the AD spike frequency increases to 4-5 Hz and the AD duration increases to about 60-100 s. The spike amplitude, and morphological complexity also increases as does the propagation to other brain sites (Racine, 1978). It is possible to continue the stimulation treatment until animals show spontaneous seizures. The occurrence of spontaneous seizures does not appear to depend on stimulation of any specific brain area. A large number of stimulations (an average of 348) are required to reach that stage in rats (Pinel and Rovner, 1978; Pinel, 1981). However, cats (Wada et al., 1974) and baboons (Wada and Osawa, 1976) only required about 50 ADs until seizures appeared spontaneously.

The EEG during the interictal period is characterized by the spontaneous occurrence of isolated epileptiform spikes. The frequency of occurrence of these interictal spikes declines to low levels within days (Goddard, 1983) or weeks (Racine and Burnham, 1984) of the last stimulation.

A wide variety of stimulation parameters will produce kindling (e.g. Cain, 1981; Goddard et al., 1969). The most important

requirement appears to be that stimulation produces an AD in the affected brain site (Racine, 1972a). However, stimulation not eliciting AD can affect the AD threshold and thus render previously ineffective stimulation effective in triggering AD at a later point in time (Cain, 1981; Racine, 1972a). It has been found that massed stimulation is less successful and often fails to produce kindling (Goddard et al., 1969). The smallest interstimulation interval not interfering with the development of kindling is about 2 hours for amygdala kindling in the Long-Evans rat (Racine et al., 1973).

Obvious similarities exist between the kindled state of an animal and various forms of human epilepsy (Adamec et al., 1981b; Racine and Burnham, 1984). Consequently, kindling has been accepted as a model for epilepsy. The kindling effect has also generated interest because of its implications for the plasticity of the central nervous system. Kindling appears to involve permanent changes in brain function, because 12 months after the last kindling treatment only a few kindling stimulations are required to bring the animal once more to a fully kindled state (Wada et al., 1974). The inhibitory circuitry of the hippocampus also shows long-term changes as a result of the kindling process (Tuff et al., 1983a; Oliver and Miller, 1985; Adamec and Stark-Adamec, 1983).

2.2 Mechanisms of Kindling

2.2.1 Anatomy

It is generally accepted that kindling does not produce any observable tissue damage. Using Nissl stains, Goddard et al. (1969)

were unable detect tissue damage directly under the stimulating electrode. In fact, if the tissue underneath the stimulating electrode was intentionally damaged via electrolytic lesions, kindling was unaffected (except that higher current intensities were required). Racine et al. (1975a), using Golgi stains, did not observe any changes in morphology of cortical pyramidal cells due to kindling. Although changes in dendritic spines as a result of non-epileptogenic LTP-inducing stimulation (see chapter 1) have been reported (Fifkova and van Harreveld, 1977; Fifkova et al., 1982), such changes have not been found in the kindled preparation (Racine et al., 1975a; Crandall et al., 1979; Goddard and Douglas, 1975). Alterations in synaptic terminals have been reported by Goddard and Douglas (1975) and by Racine and Zaide (1978), but these findings have not been replicated and it is still uncertain whether kindling produces any observable changes in neuron morphology.

2.2.2 Neurochemistry

Racine and Burnham (1984) stated that experimental support for a biochemical mechanism of kindling must include evidence that "i) induction of the abnormality accelerates kindling; ii) the abnormality exists in the kindled brain tissue; and iii) that correction of the abnormality antagonizes kindled seizures" (p. 158). From existing reviews on this topic it is evident that no conclusive evidence related to the above criteria is available about any known neurotransmitter system (McNamara et al., 1980; Kalichman, 1982;

Peterson and Albertson, 1982). Disruption of norepinephrine (NE) systems does reliably facilitate kindling (Corcoran, 1981; McIntyre, 1981). It is not yet clear whether kindled animals show abnormal NE levels (Kalichman, 1982; see, however, McIntyre and Roberts, 1983).

Cain (1981) found that the development of kindling could be prevented by the inhibition of protein synthesis even though background neural activity and AD were not disrupted by the treatment. The AD, though reliably triggered, did not develop in animals treated with anisomycin (a protein synthesis inhibitor). Preliminary results from the same laboratory (Cain, 1981) point to a role for the cyclic nucleotide systems in kindling. When activated by hormones or by neurotransmitters, cyclic nucleotides direct a cell's response to these substances. After altering cyclic nucleotide levels, Cain observed a significant decrease in the number of ADs required to induce motor seizures.

2.2.3 Disinhibition

AD is triggered by an excess of neural excitation. This excess could arise as a result of excessive excitatory input or a lack of inhibitory input. Since GABA (Gamma Amino Buteric Acid) is an important inhibitory neurotransmitter in the CNS, a dysfunction in the GABA system could underly epilepsy and kindling (Ribak et al., 1979; Ribak, 1985). Reports have appeared in the literature showing that hippocampal inhibition can indeed fail (transiently) during periods of excess activation (Ben-Ari et al., 1979; Dingledine and Gjerstad,

1980). This could provide a mechanism for the triggering of AD and further failure in these systems could account for increases in the strength and propagation of the discharge. After replicating the above "failure of inhibition" findings using paired-pulse stimulation, however, Tuff et al. (1983a) found that the latency to AD onset was actually increased rather than decreased after kindling. These results, together with the finding that paired-pulse inhibition itself is increased after kindling (Tuff et al., 1983a), suggest that kindling produces an increase in GABA-mediated inhibition in the dentate gyrus of the hippocampus (Tuff et al., 1983a,b).

2.2.4 Kindling and Long-Term Potentiation (LTP)

LTP refers to the increase in synaptic strength which develops after the application of high frequency stimulation trains to an afferent pathway (Bliss and Lomo, 1973; Douglas, 1977; Douglas and Goddard, 1975; see chapter 1). The kindling-induced increase in the amplitude of AD spikes in secondary sites suggest a possible link to LTP. This link was confirmed , in part, by Racine et al. (1975b). Furthermore, kindling is facilitated when non-epileptogenic LTP-inducing stimulation is applied prior to the kindling stimulation (Racine et al., 1975b). For several reasons, however, LTP is unlikely to be the primary mechanism underlying kindling. First, it was found that kindling resulted in the development of additional components in evoked response measures. These components, the evoked epileptiform spikes, developed independently of the LTP-induced increase in the

amplitude of monosynaptically evoked responses (Racine et al., 1981; Racine and Milgram, 1980). Second, structures which kindle easily (e.g. the lateral olfactory tract) do not produce much LTP in their target structures when appropriately stimulated. The reverse also seems to be true. Structures which do not kindle very rapidly support large LTP effects (e.g. the dorsal hippocampus; Racine et al., 1983). Third, LTP invariably decays over time (Racine et al., 1983; de Jonge and Racine, 1985) while kindling appears to last indefinitely (see Racine, 1978). Fourth, inducing LTP in several different pathways did not facilitate subsequent kindling to a greater extent than did LTP induced in a single pathway (Racine et al., 1975b). Fifth, certain drugs (e.g. diazepam, reserpine), shown to affect kindling, do not appear to influence LTP. Finally, animals specifically bred to either kindle rapidly or slowly do not differ in their expression of LTP (Steingart and Racine, personal communication). Although LTP may play a role in the propagation of AD to secondary sites, it is not likely to be the mechanism underlying kindling.

2.2.5 Abnormal Bursting Cells

Racine and McIntyre (1986) recently formulated their latest hypothesis about the causes of kindling. They reviewed evidence showing that cells in the pyriform lobe are most sensitive to the kindling treatment compared to other cells studied. Interictal spikes develop more rapidly in the pyriform cortex, and this site appears to remain the dominant generator, regardless of which site is kindled

(Kairiss et al., 1984). Pyriform cells from kindled animals frequently displayed abnormal bursting responses believed to underly the interictal spikes (McIntyre and Wong, 1985). Assuming that such bursting cells are responsible for the development of seizures, kindling would develop as follows: AD, triggered in a particular brain site, will spread, possibly due to LTP of activated synapses, and eventually reach the pyriform cortex. Response characteristics of pyriform cells are altered in such a manner that they become bursting cells. The authors point to the possibility of a decrease in tonic inhibition (probably a decrease in NE levels or receptors), a change in membrane properties of the cells, or a change in cation-buffering mechanisms. Pyriform burst generators induce similar alterations in target neurons, possibly after prolonged and intense activation. After the level of forebrain activation reaches a critical level, activity is projected to motor nuclei in the brain stem which in turn drive the skeletal motor response. The type and strength of the convulsive response would depend on the amount of excitation reaching the brain stem motor nuclei.

2.2.6 Biochemistry

Although no one has advanced a specific biochemical hypothesis for epilepsy or kindling, some biochemical correlates do exist. Wadman et al. (1985) found an increase in dendritic Ca++ conductance in hippocampal slices of kindled animals. Wasterlain and Farber (1984) reported that septal kindling decreased levels of

Ca++/calmodulin induced phosphorylation (phosphorus incorporation) of certain membrane proteins of the hippocampus. Finally, Baimbridge and Miller (1984) showed a decrease in Ca++ - binding protein levels after kindling.

CHAPTER 3

THE HIPPOCAMPUS

3.1 Gross Anatomy

The hippocampus starts medially at the septum and curves posteriolaterally, along the medial wall of the inferior horn of the lateral ventricle, into the temporal lobe (Carpenter, 1972; Fig. 1, p. 6). The hippocampal formation has a peculiar anatomy. It can be considered as a series of parallel lamella oriented at right angles to the longitudinal axis of the hippocampus. Each of these lamella contain a very similar neural circuitry (Andersen et al., 1971a; Fig. 2, p. 7).

The "hippocampus proper" refers to the area containing the pyramidal cells while the area containing the granule cells is termed the "dentate gyrus". In this thesis the term "hippocampus" refers to both the hippocampus proper and the dentate gyrus. The area between the upper and lower granule cell body layers is called the "hilus" (Figs. 2, 5).

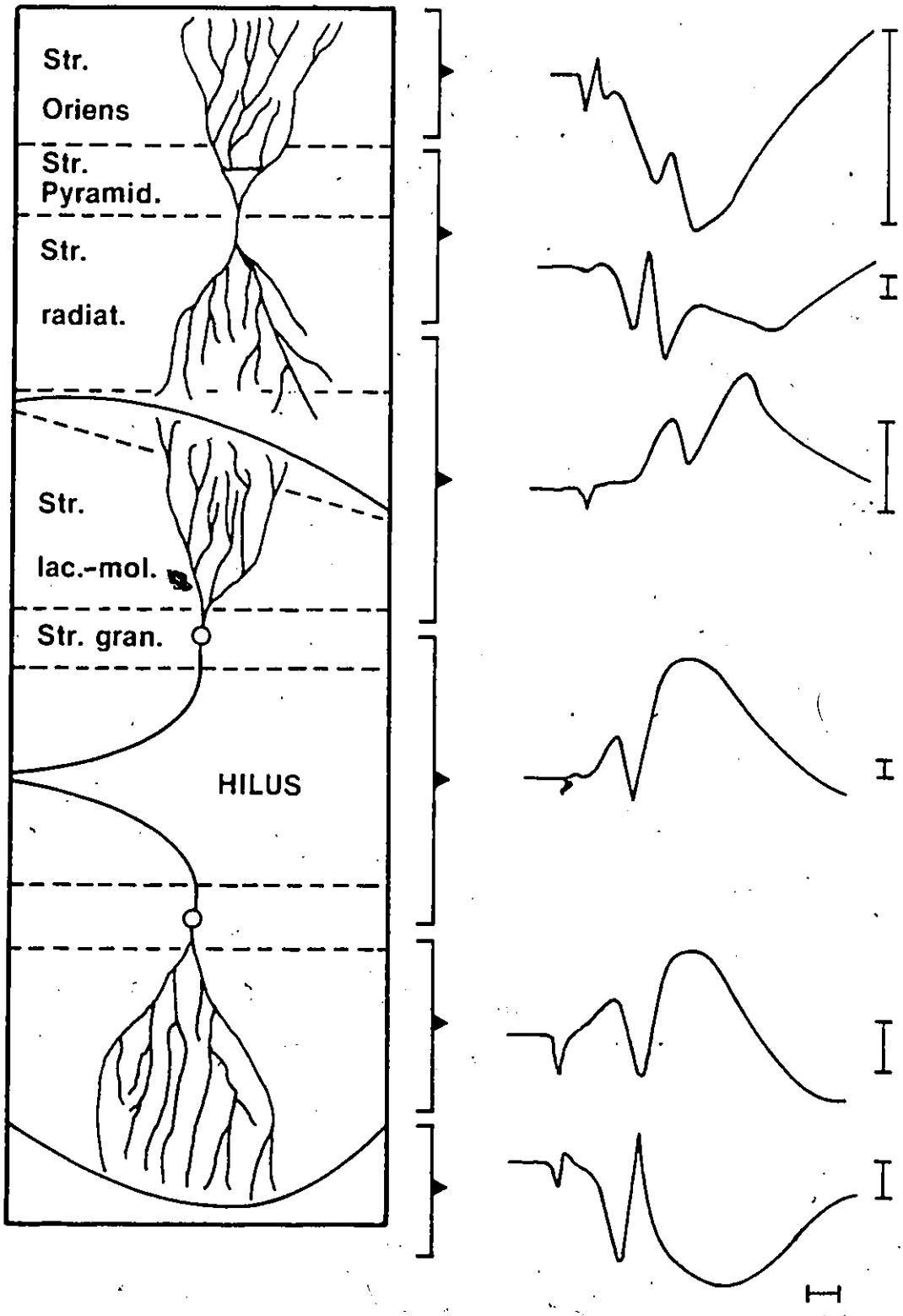
3.2 The Excitatory System

3.2.1 Anatomy and Pharmacology

Andersen et al. (1971a) described the intralamellar circuitry. Axons from granule cells in the dentate gyrus, the mossy fibres, synapse onto CA3 pyramidal cells. These CA3 neurons send their axons

Figure 5.

A depth profile of responses evoked by perforant path stimulation. The recording electrode is lowered through the region indicated in the rectangular box of dashed lines in figure 2 of which a close-up is presented on the left in this figure. The different layers are termed stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum-moleculare, stratum granulosum, and the hilus. Vertical calibration: 1 mV; Horizontal calibration: 1 ms. Adapted from Douglas and Goddard, 1975.


Str.
Oriens
Str.
Pyramid.
Str.
radiat.
Str.
lac.-mol.
Str. gran.
HILUS

as the Schaffer collaterals to the apical dendrites of CA1 pyramidal cells (The hippocampus proper, the part that contains the pyramid shaped cells, is also known as "Cornus Amonis"; hence the abbreviation "CA"; Fig. 2, p. 7). Both granule and pyramidal cells have collateral axons that innervate inhibitory basket cells (Andersen et al., 1964; Fig. 4, p. 20).

Hippocampal afferents come from at least six different areas (Segal and Landis, 1974). The major input into the hippocampus is formed by fibres of the perforant path. The site of origin and termination of these fibres were described in more detail by Hjorth-Simonsen (1972), Hjorth-Simonsen and Jeune (1972) and Steward (1976). They found evidence for the existence of a medial and lateral component of the perforant path. The medial perforant path consists of axons from stellate neurons in layers II and III of the dorsomedial entorhinal cortex which contact granule cell dendrites in the middle third of the molecular layer. The lateral perforant path originates from cells in the ventrolateral entorhinal cortex and synapse onto the dendrites in the outer third of the molecular layer. The perforant path fibres, mossy fibres and the Schaffer collaterals appear to contain the neurotransmitter glutamate (White et al., 1977; Sandoval et al., 1977; Storm-Mathisen and Iversen, 1979). A second source of hippocampal input is formed by axons of septal neurons. Cholinergic fibres arising from septal nuclei ascend via the fornix to the hippocampus where they contact CA3 and granule cells (Segal and Landis, 1974). Third, axons from CA4 cells in the contralateral

hippocampus project via the anterior commissure to the dentate gyrus (Swanson and Cowan, 1977; Hjorth-Simonsen and Laurberg, 1977; Laurberg, 1979). These fibres are believed to terminate on inhibitory interneurons as well as on the granule cells. The main effect of this pathway is inhibitory however (Douglas et al., 1983). Fourth, fibres from the nucleus coeruleus terminate in the ipsilateral hippocampus where they have a long-lasting (up to 2 min) inhibitory effect, presumably mediated by norepinephrine (Segal and Bloom, 1976). Fifth, the raphe nuclei provide the hippocampus with serotonin containing fibres. A final source of hippocampal input has been traced back to the supramammilary area (Segal and Landis, 1974; Segal, 1979). These fibres terminate in the dentate gyrus where they inhibit granule cell activity with a short latency (3-5 ms) for a long period of time (about 30 ms; Segal, 1979). The transmitter used by this pathway remains unknown.

Hippocampal efferents are formed by CA3 and CA1 neurons. CA3 neurons send their axons via the fimbria to the fornix system (Andersen et al., 1973). Fornix fibres project to the lateral septum, to the contralateral hippocampus, to the lateral preoptic, thalamic and hypothalamic areas or the mammillary complex (Isaacson, 1974; Swanson and Cowan, 1977). Finch and Babb (1981) found that some CA1 axons also project to the lateral septum via the fimbria and fornix but the majority of these fibres terminate in the subiculum.

3.2.2 Electrophysiology

When the perforant path is electrically stimulated, a characteristic evoked response can be recorded in the hippocampus. The form of the evoked response depends on the position of the recording electrode in the hippocampus. Figure 5 (p. 34) shows the shape of the evoked response recorded at various depths. Note that when the recording electrode is positioned in hilus of the dentate gyrus the evoked response is largest.

Focusing on the evoked response recorded in the hilus, three components become apparent (Top of Fig. 3, p. 8). The first component represents the stimulus artifact and is largely caused by current flowing directly from the stimulating electrode to the recording electrode via low resistance cytoplasm. The second component consists of a positive change in potential which is caused by the depolarization of the granule cell dendrites. The slope of this component is believed to be proportional to the amplitude of the cellular EPSP and is often called the population EPSP (Lomo, 1971; Bliss, 1979; Dunwiddie and Lynch, 1979). If the intensity of the stimulation is large enough, a third component can be observed. It consists of a negative inflexion, the population spike, superimposed on the population EPSP and reflects the near synchronous discharge of granule cells (Lomo, 1971; Andersen et al., 1971b). The population spike height is proportional to the number of granule cells firing.

3.3 The Inhibitory System

3.3.1 Anatomy

Buzsaki (1984) recently summarized a substantial body of information about hippocampal interneurons. Although interneurons account for only 2-4% of hippocampal neurons, they do not constitute a uniform class of cells. The most common hippocampal interneuron is the basket cell of which there are believed to be five types. Basket cells are responsible for the GABA-mediated inhibition in the hippocampus. Investigations using electron microscopy and studies using antisera to GAD (Glutamate Acid Decarboxylase is an enzyme that catalyzes the formation of GABA and is restricted to GABA-ergic neurons) show that basket cells can be found within the principal cell layers of the hippocampus (the pyramidal and granule cell layers) or within 10-50 um of these layers. Occasionally they are found in the molecular layer as well. Basket cell axons form complex networks around many principal cell somata (hence the name basket cell). They may also terminate on the proximal dendrites or on the distal apical dendrites of these principal cells. Since the perforant path also innervates the distal apical dendrites of the granule cells, it is possible that the inhibition in this area is of the axo-axonic type. Buzsaki and Eidelberg (1982) proposed the existence of axo-axonic contacts between commissural and perforant path fibres. Evidence for axo-axonic synapses of GABA containing cells in cat hippocampus (as well as in the cat visual cortex) was reported by Somogyi et al. (1985). Valentino and Dingledine (1981) described a presynaptic inhibitory

correspondence exists between type of basket cell and the area projected to by that type of cell.

It is known that hippocampal basket cells play a key role in recurrent inhibition. Thus, collateral axons of principal cells activate basket cells which in turn inhibit those same principal cells (Andersen et al., 1964; Fig. 4, p. 20). Gradually, evidence is accumulating showing that basket cells are activated in a feedforward manner as well. Buzsaki (1984) argues that basket cells receive substantial amounts of feedforward input from the contralateral hippocampus via the commissure, from the perforant path, the septal afferents, hypothalamic afferents, afferents from the raphe nuclei, from the locus coeruleus and from other subcortical regions. Again, the possibility arises that the type of basket cell determines whether it receives feedback and/or feedforward input. No evidence is available on that matter.

3.3.2 Pharmacology

Although interneurons have been found to secrete a number of substances, the basket cells are GABA-ergic (see Buzsaki, 1984). The membrane sites to which GABA molecules bind are receptor complexes rather than simple receptor sites (Olsen, 1982). There are two kinds of GABA receptors, GABAa and GABAb receptors. The GABAa receptor, affecting Cl-channels (Olsen, 1982; Haefely, 1984), is associated with regulatory sites. These sites, when occupied by certain drugs and by particular internally produced substances, either potentiate or

inhibit the effects of GABA (Simmonds, 1983; Meldrum, 1980). Some of these regulatory sites bind barbiturates and picrotoxin-like substances (Olsen, 1982). Other regulatory sites correspond to the benzodiazepine (Bz) receptors discovered by Squires and Braestrup (1977). When Bz receptors are activated, the probability of GABA activation of the Cl- channels is increased (Haefely, 1984). In agreement with this is the evidence showing that Bzs (e.g. valium) increase recurrent inhibition in the hippocampal dentate gyrus (Adamec et al., 1981a; Tuff et al., 1983a). Interestingly, Bzs are also potent anticonvulsants (Eadie and Tyrer, 1980). Some evidence indicates that Bzs may exert their action via the Ca++/calomodulin protein kinase system in brain membranes (Delorenzo et al., 1981). GABAb receptors, at least in the PNS, are not associated with Bz receptors (Doble and Turnbull, 1981; Muhyaddin et al., 1982). The GABAb receptor, when activated, is believed to modulate voltage-dependent Ca++ channels (Dunlap, 1981; Dunlap and Fischbach, 1981).

3.3.3 Electrophysiology

Intracellular recordings of basket cell activity has established the following characteristics. Action potentials of basket cells have a very short duration, less than 1.2 ms (Knowles and Schwartzkroin, 1981). These cells have a low threshold and fire repetitively at a high frequency (up to 700 Hz) in response to afferent stimulation. Basket cells in the hippocampal dentate gyrus appear to support LTP in the form of an increase in probability of

afferent stimulation. Basket cells in the hippocampal dentate gyrus appear to support LTP in the form of an increase in probability of firing when activated by commissural or perforant path stimulation (Buzsaki and Eidelberg, 1982). Basket cell activity is maximal at about 10-15 ms after stimulating axons that innervate these cells and subsides after about 100 ms (Thalmann and Ayala, 1982). This first inhibitory phase is Cl- mediated (Eccles et al., 1977; Thalmann and Ayala, 1982). Electrophysiological records often reveal a second inhibitory component, believed to be mediated by a K+ current, which peaks at about 150 ms and may last for several hundreds of ms. This late hyperpolarization of principal cells may be voltage-dependent and/or Ca++ dependent (Nicoll and Alger, 1981; Thalmann and Ayala, 1982). This raises the interesting possibility that activation of GABAb receptors (see previous section) is responsible for the late inhibitory component. In support of this hypothesis, Newberry and Nicoll (1984) found that baclofen, which activates GABAb receptors, strongly hyperpolarized hippocampal CA1 cells, possibly due to an increase in K+ conductance.

The characteristics of hippocampal inhibition have also been investigated by means of the paired-pulse paradigm (Adamec et al., 1981a; Tuff et al., 1983a; Oliver and Miller, 1985). In a typical paired-pulse experiment the first pulse delivered to an excitatory pathway will activate target cells which, via collaterals, activate inhibitory basket cells. The recorded evoked response of target cells to the first pulse (the conditioning pulse) is not affected by

depressed if the time between the two pulses (the interpulse interval or IPI) falls within the period of peak recurrent inhibition (about 10-30 ms). At IPIs longer than 30 ms, the test response is often increased in amplitude relative to the conditioning response. Presumably this facilitation is due to an increase in neurotransmitter output to the test pulse (Creager et al., 1980). The net effect during the first 100 ms will depend upon the balance between the inhibition and facilitation effects. A second inhibitory component is apparent in many cells. It is difficult to determine when this late inhibition begins because of the presence of the facilitatory component. The late inhibitory component lasts longer than 500 ms, however, and is believed to correspond to the late component of inhibition discussed earlier. Thus, the magnitude of the test evoked response is determined by the net contribution of each of the three phases just described (early inhibition, facilitation, and late inhibition).

Figure 6 shows a typical example of the effects of the application of paired-pulse stimulation to the perforant path while recording from the dentate granule cells. The left-hand column depicts the first response of each pair. From top to bottom, the IPIs between the two members of each pair are 20, 30, 50, 70, 100, 150, 200, 300, 500, and 1000 ms. Pulse-pairs are separated by 10 s. The amplitude of the population spike (reflecting the amount of cells firing more or less synchronously; Lomo, 1971; Andersen et al., 1971b) is about the same for all first members of the pairs. The spike amplitude of the test evoked response, however, is depressed at short IPIs (recurrent

inhibition), then facilitated and, finally, shows a second phase of slowly recovering inhibition (presumably due to K+ mediated late hyperpolarization). It is common practice to express the test spike amplitude as a percent of the conditioning spike amplitude (i.e. P2/P1x100) such that 100% implies identical conditioning and test spike amplitudes. Anything below 100% reflects a depression of the test spike amplitude while measures above 100% imply a facilitation. Expressed in this manner the data of figure 6 appear as depicted in figure 7 (Top). Paired-pulse measures of the slope of the onset of the evoked responses (reflecting the population EPSP; Lomo, 1971; Dunwiddie and Lynch, 1979) can be expressed in a similar fashion (Bottom of Fig. 7).

Often, input/output relationships are determined during electrophysiological experiments involving the hippocampus. The procedure involves recording responses evoked by stimulation of a nerve pathway at a number of different intensities. Figure 8 provides an example of recordings taken from the dentate gyrus while the perforant path was stimulated at the indicated intensities. Input/output relationships provide information about the tonic effects of an experimental treatment. For example, a small increase in tonic inhibition might result in a smaller population spike at the lower stimulation intensities since the basket cells are believed to have a very low threshold (Buzsaki and Eidelberg, 1982). At higher intensities, however, excitation might overcome any increased inhibition. Input/output relationships also indicate what stimulation intensity must be used to maintain a constant population spike height.

Figure 6.

An example of potentials evoked in the dentate gyrus by paired-pulse stimulation of the perforant path. The left-hand column shows the conditioning response of each pair, the right-hand column shows the test response of each pair. The interstimulus interval increases from top to bottom (see text for actual values). Each evoked potential is 20 ms long and has a magnitude of about 6 mV.

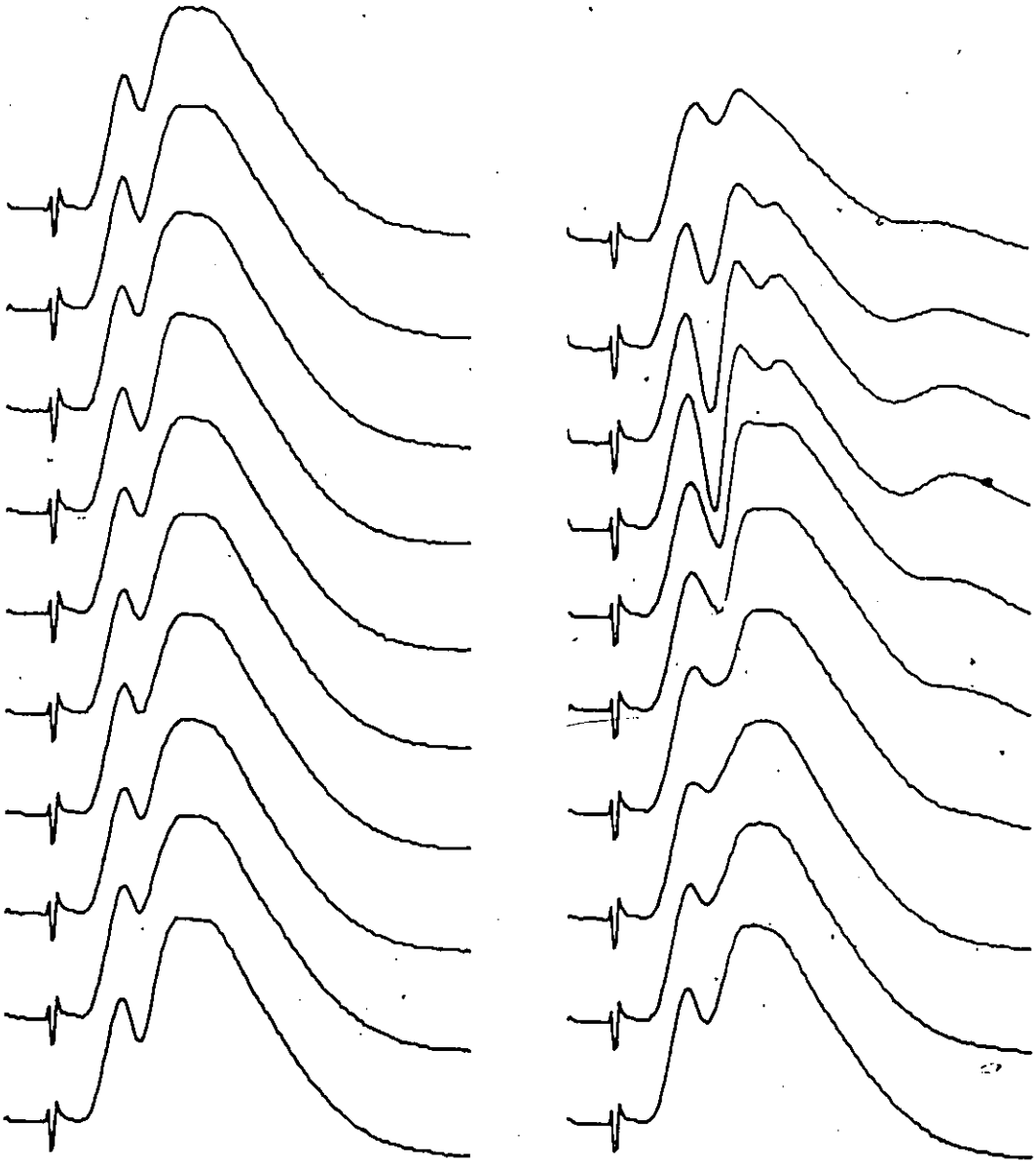


Figure 7.

Top: The test spike heights from figure 6 expressed as a percent from the conditioning spike height (P1= conditioning spike height; P2=test spike height).

Bottom: The same for the population EPSP (E1=slope of the conditioning response; E2=slope of the test response).

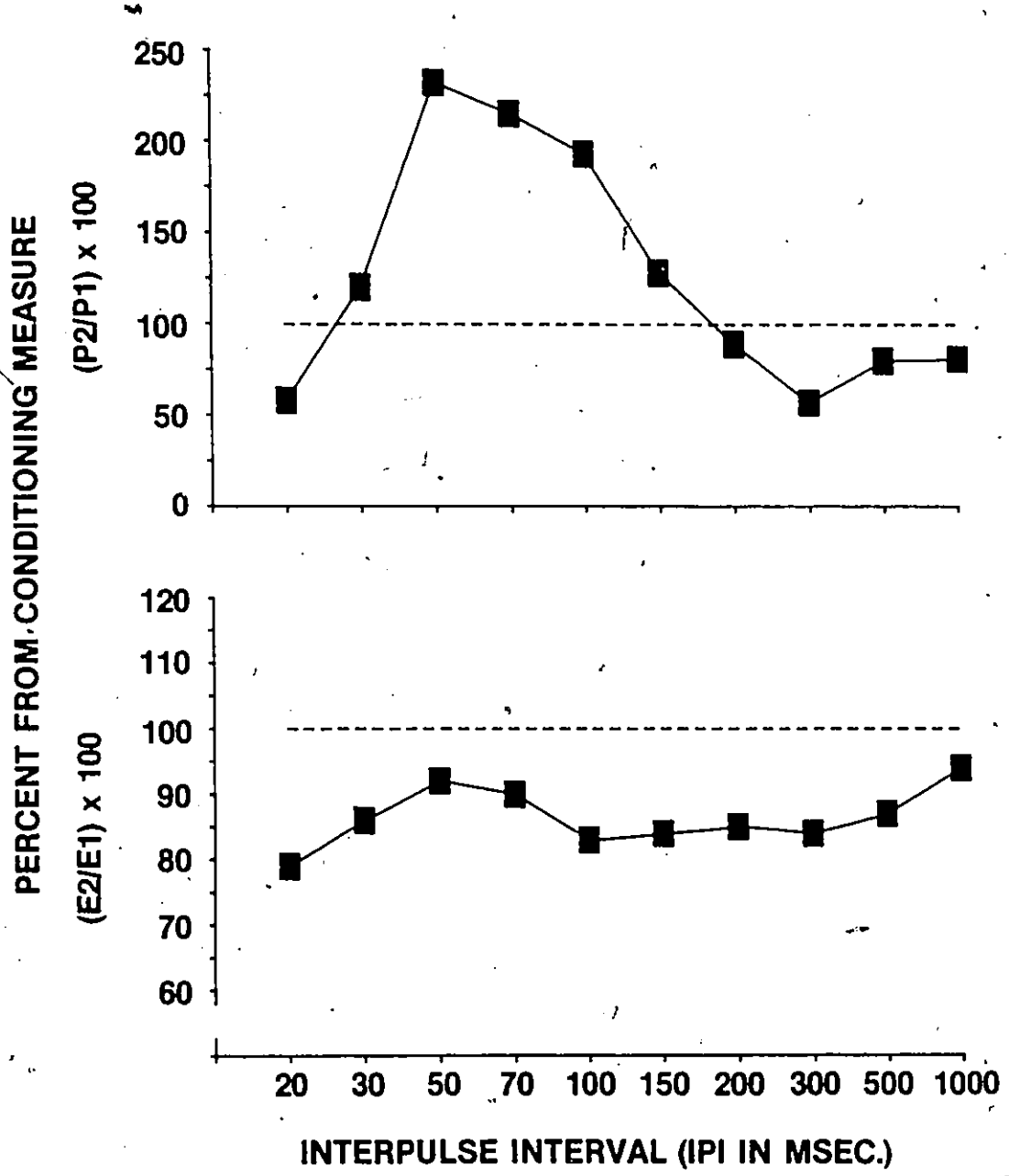
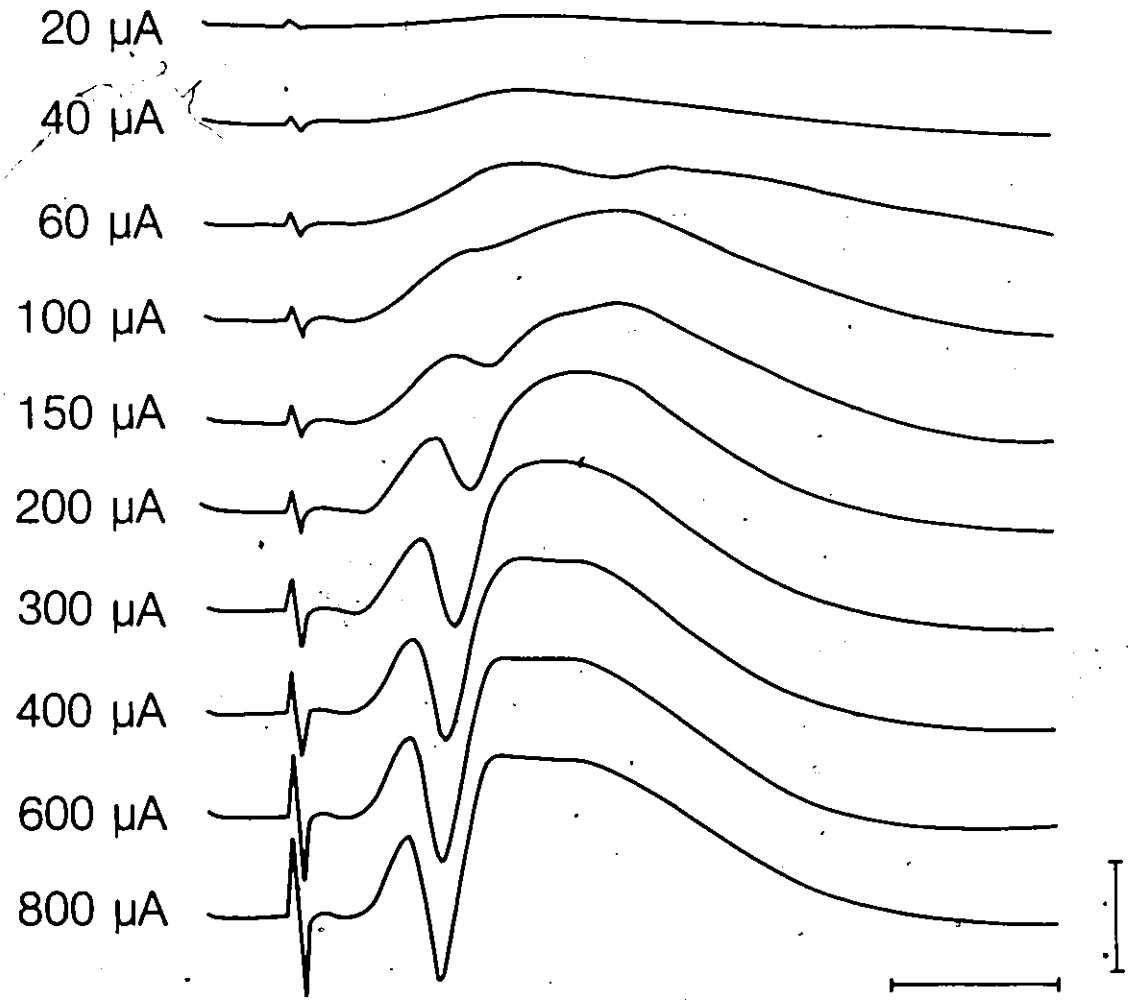

PERCENT FROM CONDITIONING MEASURE
(P2/P1) x 100
250
200
150
100
50
0
(E2/E1) x 100
120
110
100
90
80
70
60
20
30
50
70
100
150
200
300
500
1000
INTERPULSE INTERVAL (IPI IN MSEC.)

Figure 8.

An example of responses evoked in the dentate gyrus by pulses applied to the perforant path during determination of input/output relationships. Each evoked response is an average of 10, evoked at 1 of the 10 indicated intensities. Vertical calibration: 6 mv; Horizontal calibration: 4 ms.

INPUT/OUTPUT MEASURES



3.4 Objectives

In chapter 2 (Kindling) it was mentioned that kindling increases paired-pulse depression. This increase was deduced from the observed downward shift of the whole curve in figure 7 (Top) after kindling (Tuff et al., 1983a). Kindling-induced changes in responses evoked by single pulses can also be observed (Racine, 1978; Goddard, 1982; Giacchino et al., 1984). However, all these kindling-induced changes were measured after the animals were fully kindled (i.e. after several Stage 5 seizures). Furthermore, evoked responses were only looked at once after kindling. Therefore no information is available on when, during the kindling process, these changes occur and how long they last after kindling is completed.

The kindling-induced increase in paired-pulse depression is believed to be caused by an increase in the number of Bz receptors (Tuff et al., 1983a,b). If this is true, then the time course of this increase in the number of receptors after kindling should parallel the time course of the increase in paired-pulse depression.

The following experiments were designed to answer these questions.

CHAPTER 4

GENERAL METHOD

4.1 Surgery

Male Long Evans hooded rats, weighing between 350 and 450 grams, were used in all experiments. Bipolar electrodes were lowered to the hippocampal dentate gyrus and to the perforant path of the animals participating in the electrophysiological experiment, while rats used in the binding study received electrodes in the perforant path only. All animals received bilateral implants under sodium pentobarbital anaesthesia (50 mg/kg) while fixed in a stereotaxic apparatus (David Kopf Instruments) with bregma and lambda in the same horizontal plane.

All electrodes consisted of two teflon coated stainless steel wires (130 um in diameter) twisted together. The coordinates of the recording electrodes, positioned in the hilus of the hippocampal dentate gyrus, were 3.8 mm posterior and 2.3 mm lateral relative to bregma. Stimulating electrodes were placed 8 mm posterior and 4.6 mm lateral to bregma. Optimal placement of the electrodes was achieved by monitoring the evoked response to electrical stimulation of the perforant path while slowly lowering recording and stimulating electrodes. Only one pole of the recording electrode was used for recording. As mentioned earlier (p. 37), the rate of rise (slope) of the population EPSP provides a relatively uncontaminated measure of

response amplitude. This component was monitored, as the electrodes were lowered, until no further increase in magnitude of the evoked response could be obtained. Depth of the electrodes in animals receiving perforant path implants only, was based on the depth of the electrodes recorded in animals that received implants under electrophysiological control (3.0 mm from brain surface).

Electrodes were fixed in place with dental acrylic. Subsequently, the connecting pins of each recording, stimulating and ground electrode were led into a plastic headcap which was also cemented in place. Finally, all animals received penicillin (Derapen C, 0.2 cc intra muscular) in order to prevent infections. All animals were allowed to recover from surgery for at least two weeks.

4.2 Apparatus

Electrical stimulation was delivered by means of a Grass S88 stimulator. Constant current output was provided by photoelectric stimulus isolation units (Grass, model PS106). During the experimental phase a Grass Wide Band A.C. EEG Pre-Amplifier (model 7P5B) and a Polygraph D.C. Driver Amplifier (model 7DAE) were used when recording evoked responses or EEG. During surgery evoked responses were preamplified by a Grass P15 A.C. pre-amplifier before being fed into the Wide Band A.C. EEG pre-amplifier. Cutoff frequencies were set at 1 Hz and 3 kHz. Data were stored and analyzed by means of a computer program written by R. Adamec and R. Douglas (1976) which was run on an on-line LSI-11 microcomputer :

4.3 Stimulation Parameters

4.3.1 Kindling

Rats were kindled (1 s train of 1 ms biphasic pulses, at a frequency of 60 Hz and an intensity of 400 uA) in either the dentate gyrus or in the perforant path. All animals participating in the binding study were kindled in the perforant path. On the day kindling was started only 1 AD was triggered. Afterwards three ADs were triggered each day, 3 hours apart, until a Stage 5 seizure occurred (a tonic/clonic rearing and falling generalized convulsion; Racine, 1972b). Only one Stage 5 seizure was then triggered a day. EEG was always monitored during kindling. Control animals received no kindling stimulation but were treated identically otherwise.

4.3.2 Electrophysiology

4.3.2.1 Evoked Response Measures

Measures were derived from evoked responses recorded from the dentate gyrus in response to perforant path stimulation. Evoked response measures consisted of the height of the population spike and the slope of the population EPSP (Top of Fig. 3, p. 8). The population spike was measured from a tangent drawn between the first and third turnover points of the evoked response. This measure of population spike height was preferred to the one obtained from the first turnover point only. This preference is based on observations from paired-pulse experiments indicating that the latter method often yields similar

spike heights for conditioning and test evoked potentials (not unlike some of those in Fig. 13, p. 68) even though unit activity in response to the test pulse is depressed (R. Racine, unpublished observations).

4.3.2.2 Input/Output

In order to determine input/output relationships, 100 pulses were delivered to the perforant path at a rate of 0.1 Hz. After every 10 pulses the intensity of the stimulation was increased. The lower and upper limits for stimulation intensity were 10 uA to 1200 uA. Different ranges of intensities were used for individual animals, however, in order to obtain similar increases in evoked potentials with increasing intensities for all animals.

4.3.2.3 Paired-Pulse Stimulation

Interpulse intervals (IPIs) used during paired-pulse stimulation were 20, 30, 50, 70, 100, 150, 200, 300, 500, and 1000 ms. Pulse pairs were separated by 10 s (0.1 Hz). At the beginning of the experiment the intensity of the paired-pulse stimulation was set so that the test spike height was about 70% of the conditioning spike height at an IPI of 20 ms. During the rest of the experiment the intensity was always adjusted so as to maintain similar amplitudes for all conditioning response-population spikes (i.e. the population spikes evoked by the first member of each stimulus pair).

In what follows, the term "baseline" refers to the average of those measures collected over the 4 days before the 1st AD was

triggered. Data obtained during input/output sessions were standardized to 1000 as follows: All input/output measures from any one animal were divided by its maximum baseline value and subsequently multiplied by 1000. This was done to facilitate individual and group comparisons.

CHAPTER 5

EXPERIMENT I. Electrophysiology: Development and Decay of the Kindling-Induced Increase in Paired-Pulse Depression.

5.1 Introduction

At present, the only reliable way to induce long-term changes at (hippocampal) inhibitory synapses is to kindle an animal. (The reader will remember that in order to kindle an animal, epileptogenic stimulation, which elicits AD, must be applied repeatedly to brain sites with a minimum of two hours elapsing between successive stimulation sessions; Racine, 1978). A first indication that hippocampal inhibition might be increased two weeks following kindling in the hilus of the hippocampal dentate gyrus was reported by Tuff et al. (1981) and later by Goddard (1982). In the Goddard study, responses were evoked in the dentate gyrus by pulses applied to the perforant path, the contralateral hilus, or both. In the latter case, the contralateral pulse preceded the ipsilateral pulse, providing a measure of inhibition. The responses were recorded before and two weeks after kindling. The results, although not significant, suggested an increase, rather than a decrease, in inhibition.

Using paired-pulse stimulation of the perforant path to assess the amount of inhibition in the rat hippocampal dentate gyrus, Tuff et al. (1981; 1983a) were able to show that paired-pulse

inhibition was increased two weeks after the last kindling stimulation was applied. Similar results were reported by Oliver and Miller (1985) who used hippocampal slices from kindled and non-kindled animals, and recorded from granule cells.

It is not clear whether this kindling-induced increase in inhibition is a form of LTP. It is not known, for example, whether the increased inhibition decays over time as LTP invariably does (Racine et al., 1983; de Jonge and Racine, 1985). The kindled state itself appears to be based upon a permanent change in cell function, as revealed by the permanently increased responsivity to epileptogenic stimulation (Wada et al., 1974).

There are some similarities between the increased inhibition due to kindling and LTP in excitatory systems. For example, there is an increase in benzodiazepine (Bz) receptors, which might underly the increased inhibition (Tuff et al., 1983b; McNamara et al., 1981; Shin et al., 1983), and an increase in the number of active glutamate receptors is believed by some to be the mechanism underlying LTP of excitatory synapses in the hippocampus (Baudry and Lynch, 1982). Furthermore, the increase in dentate Bz receptors, due to amygdala kindling, seems inversely related to the amount of time elapsed between last kindling treatment and time of sacrifice of the animal. Thus, McNamara et al. (1981) found a 35% increase in Bz receptors 24 hours after the last kindling stimulation. Tuff et al. (1983b) found only a 14% increase in Bz receptors in kindled animals 2 weeks after the last kindling treatment. Shin et al. (1985) were unable to detect

an increase in Bz receptors 28 days after the last kindling stimulation. If the increase in inhibition is an LTP-like phenomenon, and if the increase in Bz receptors underlies the increase in inhibition, then electrophysiological measures of inhibition should also show a decrease over time.

It is not known whether generalized convulsions need to be elicited in order to obtain the increase in inhibition or whether the occurrence of AD is sufficient. The data regarding the increase in Bz receptors after kindling indicate that triggering AD might be adequate. These receptors appear to decline in number after kindling during a period when convulsions, if triggered, would still be fully generalized, suggesting receptor number is independent of the kindled state. In further support of this argument is the positive correlation between the number of Bz receptors and the number of ADs administered (McNamara et al., 1981). The assumption, of course, is that the increase in Bz receptors underlies the increase in inhibition induced by kindling.

It is now evident that the inhibitory action in the hippocampus has at least two components. An early inhibitory phase is Cl-mediated and peaks at 10-15 ms. A second inhibitory phase, believed to be mediated by K-current, reaches its maximal effect at about 150 ms and may last for several hundreds of ms (p. 41). Both inhibitory components, as revealed during paired-pulse stimulation, potentiate as a result of kindling (Tuff et al., 1983a; Oliver and Miller, 1985). Possibly, the two components potentiate through independent

mechanisms. If so, then the rate of development as well as the rate of decay of the increase in inhibition might differ for the 2 inhibitory components. Oliver and Miller (1985) suggested that a decrease in latency to onset of the second inhibitory component is responsible for the increase in early inhibition. Consequently, in their view only one component is affected.

The following study investigated development and decay of the increase in paired-pulse depression in the rat dentate gyrus due to kindling.

5.2 Procedures

On the first 4 days after recovery from surgery, baseline measures were taken for each animal. These measures consisted of evoked responses recorded during input/output and paired-pulse stimulation. Half of the animals in each group received input/output stimulation first, immediately followed by the paired-pulse stimulation. The other animals received each type of stimulation in the reversed order. The order of stimulation for each animal was maintained throughout the experiment. On the 5th day, animals in the dentate gyrus-kindled group (n=8) received 1 kindling train in the hilus of the dentate gyrus. A second group of animals, the perforant path-kindled group (n=8), received an identical train of pulses in the perforant path. Animals in the third group, the control group (n=8), were treated in the same manner as animals in the two preceding groups except that they never received any kindling stimulation. The kindling

train of pulses always elicited at least 1 AD, lasting a minimum of 10 s, as verified by EEG recordings. On Day 6, 24 hours after the first AD was elicited, or after a stimulation-free period treatment for control animals, input/output and paired-pulse measures were taken again. Immediately afterwards, 3 kindling trains separated by 3 hours were given to the animals. On Days 7 and 8, the animals again received 3 kindling trains. On Day 9, after the animals had accumulated a total of 10 ADs, input/output and paired-pulse tests were reapplied. This was followed by another 9 evoked ADs, 3 per day, followed by a repeat of the input/output measures and paired-pulse measures. This was continued until the animals showed Stage 5 seizures, 24 hours after which input/output and paired-pulse measures were again taken. Three additional Stage 5 seizures were elicited on subsequent days after which input/output and paired-pulse measures were again collected. This phase is termed "development". These same measures were collected 3, 7, 14, and 28 days after the last Stage 5 seizure was evoked. This phase is termed " decay ". Finally, animals received 1 more kindling train, after the stimulation-free decay period, followed by input/output and paired-pulse measurements 24 hours later. This final AD was evoked to test for possible residual effects. If kindling-induced increases in inhibition decay back to pre-kindling baseline levels during the 4-week decay period, a final AD might still reinstate these effects to their full extent. If so, it would be inappropriate to speak about a simple "decay" of the kindling-induced effects. The final AD was induced to investigate that possibility.

Input/output and paired-pulse data for control animals were taken on Days 1-4 (Baseline), Days 6, 9, 12, 15, 18, and 21 (Equivalent to the development phase in the experimental groups), Days 24, 28, 35, and 49 (Equivalent to the decay phase in the experimental groups), and on Day 50 (Equivalent to the day after the final AD in the experimental groups). This choice was based on the minimum amount of time required by dentate-kindled animals to show a Stage 5 seizure). Table 1 summarizes the procedures.

In what follows, baseline data implies the average of those measures collected over the first 4 days (see General Method, p. 48).

5.3 Results

5.3.1 Kindling

Animals kindled in the perforant path required an average of 20.75 (sd=5.26; n=8) ADs to reach criterion, the 4th Stage 5 seizure. This was significantly fewer ($t=3.31$; $0.001<P<0.01$) than the average of 72.50 (sd=40.42; n=6) ADs required by the dentate-kindled animals to reach criterion. Two animals in the latter group never showed a Stage 5 seizure. The kindling-induced electrophysiological effects in these two animals, however, were similar to those of the other animals in the dentate-kindled group. In fact, the evoked potentials displayed in Figure 13 (DENTATE, p. 68) are from an animal that did not fully kindle. Consequently, their results were included in the data-analysis. No other differences between the two groups could be observed, with respect to the kindling effect.

Table 1.

This table summarizes the sequence of treatments to which experimental animals were subjected. The upper row indicates when animals received kindling stimulation in the perforant path or in the hilus of the dentate gyrus. The lower row indicates when measures (I/O: Input/Output; PP: Paired-Pulse) were taken. These measures were derived from responses evoked by perforant path stimulation while recording in the hilus of the dentate gyrus.

PROCEDURE

KINDLING: 1. Perf. Path 2. Dentate		1 AD	3 ADs a day until 1st Stage 5 seizure	1 Stage 5 a day until 4th Stage 5 seizure					Final AD
MEASURES: I/O and PP Stim.: Perf.Path Rec.: Dentate	Baseline	1 day after 1st AD	1 day after every 9 ADs	1 day after 1st and 4th Stage 5 seizure	3 days after 4th Stage 5 seizure	1 week	2 weeks	4 weeks	1 day after final AD

TIME $\longrightarrow$

5.3.2 Input/Output Measures- Spike Height

After the first AD, the spike height of animals in both experimental groups was potentiated at the higher stimulation intensities. This effect was relatively small in the dentate-kindled group. Control animals showed a small decrease in spike height (Fig. 9). Subsequent stimulation did not appear to have any additional effect on the spike height of perforant path-kindled animals although a small decrease in spike height at low stimulation intensities was apparent when kindling was completed. Spike height of dentate-kindled animals became progressively depressed with subsequent stimulation. This depression was first obvious at lower intensities and gradually spread to the higher stimulation intensities (Fig. 9). Four weeks after kindling was completed, the increased spike height of the perforant path-kindled group had returned to pre-kindling baseline levels. A recovery of the depressed spike height of the dentate-kindled animals was also apparent 4 weeks post-kindling. This recovery began at the higher stimulation intensities. The spike height of control animals remained stable, slightly below baseline, over a comparable period of time (Fig. 10).

5.3.3 Input/Output Measures- Slope

After the first AD, the slope was increased for both experimental groups at the upper half of the input/output curve. When kindling was completed the slope magnitude of the perforant path-kindled animals was similar to that observed after the first AD. The

slope of the dentate-kindled animals however, increased progressively after the 10th AD. The increase was largest, about a 100% increase from baseline when kindling was terminated, at the higher stimulation intensities. The slope of control animals remained very stable over a comparable period of time, deviating only slightly from baseline levels (Fig. 11). Interestingly, the slope remained increased over the 4 week period post-kindling. In fact, the slope of both experimental groups increased even more during this period. However, a similar small increase in slope was observed in control animals (Fig. 12).

5.3.4. Paired-Pulse Measures- Spike Height

Figure 13 shows an example of paired-pulse depression before, during and after kindling for one animal in each group (Interpulse interval=20 ms). Baseline paired-pulse measures of the spike height were typical for all three groups (Fig. 14; squares). An initial, early depression of the test spike height, presumably due to recurrent inhibition, was apparent at an interpulse interval (IPI) of 20 ms. A net facilitation of the test spike height was observed when IPIs ranged from 30-100 ms. Finally, a secondary, late depression of the test spike height began at an IPI of 150 ms, peaked at an IPI of 300 ms, and decreased with longer IPIs.

After the first AD, the early depression was increased and prolonged (to IPI=30 ms) in both experimental groups. This effect was strongest in the dentate-kindled group where all animals showed this increased and prolonged early inhibition as opposed to 5 out of 8

animals in the perforant path-kindled group. Most of the control animals (6 out of 8) showed a slight increase in depression, but to a smaller degree than the experimental animals (Fig. 14). The dentate-kindled animals showed what appeared to be a decrease in inhibition at longer IPIs (70-200 ms) whereas inhibition at these IPIs in the other two groups did not change.

After AD 10, an increase in inhibition was apparent at all IPIs in both experimental groups, although there was some variability in the dentate-kindled animals at the longer IPIs. The increase in inhibition appeared to peak after about 19 ADs. Subsequent stimulation did not alter the levels of inhibition at any IPI (Fig. 14).

The early inhibitory component remained potentiated for at least 2 weeks after kindling and some potentiation was still evident 4 weeks after the last stimulation was applied. It was quite clear in the perforant path-kindled animals, however, that the increase in early inhibition was returning towards pre-kindling levels (Fig. 15). The decay process appeared to be considerably slower for the dentate-kindled group.

One week post-kindling, the late inhibitory component in the perforant path-kindled group had returned to pre-kindling levels. This return to baseline of the late inhibitory component was already well underway 3 days after kindling. The late inhibitory component of the dentate-kindled group returned to baseline within 2 weeks post-kindling (Fig. 15). The paired-pulse measures of the spike height in control animals remained close to baseline levels with the exception

of the one aberrant measure at IPI=70 ms four weeks after kindling (Fig. 15).

5.3.5 Paired-Pulse Measures- Slope

Both experimental groups showed an increase in paired-pulse depression of the population EPSP. This increase was first obvious at short IPIs and, with subsequent stimulation, spread gradually to longer IPIs. This effect was particularly clear in the dentate-kindled animals. Control animals did not show any change in paired-pulse depression of the EPSP over time (Fig. 16). Although this depression was still potentiated four weeks after kindling, a movement towards baseline levels could be observed (Fig. 17).

5.3.6 Re-Kindling Stimulation

Four weeks after kindling, at the end of the post-kindling decay period, the final kindling stimulation was given to the experimental groups. All animals in the perforant path-kindled group responded with a Stage 5 seizure to the stimulation, while the animals in the dentate-kindled group showed nothing stronger than a Stage 1 seizure (mouth movements; Racine, 1972b).

The effects of the post-decay stimulation on the input/output relationships of both experimental groups were similar in magnitude and direction to those observed after AD 1 (Top of Fig. 18), although paired-pulse measures of the dentate-kindled animals and paired-pulse slope measures of the perforant path-kindled animals were not affected by the stimulation (Bottom of Fig. 18).

Figure 9.

This figure shows the effects of kindling the perforant path (PERFORANT PATH: SPIKE DEVELOPMENT) or the hilus of the dentate gyrus (DENTATE: SPIKE DEVELOPMENT) on the population spike amplitude vs stimulus intensity. Squares: pre-kindling baseline measures; Triangles: after afterdischarges 1, 10, 19, 28, and after the 1st and 4th Stage 5 seizure. Since perforant path-kindled animals kindle faster, there are fewer data points for this group until the 1st Stage 5 seizure (as indicated by the arrow between the AD10 data and the 1st Stage 5 data). Both group averages and standard error bars are shown. Standard error bars were used as a descriptive statistic in all figures. The rational behind the use of standard error bars in this manner, is that the interest is in the time course of the kindling-induced changes, not in testing a hypothesis.

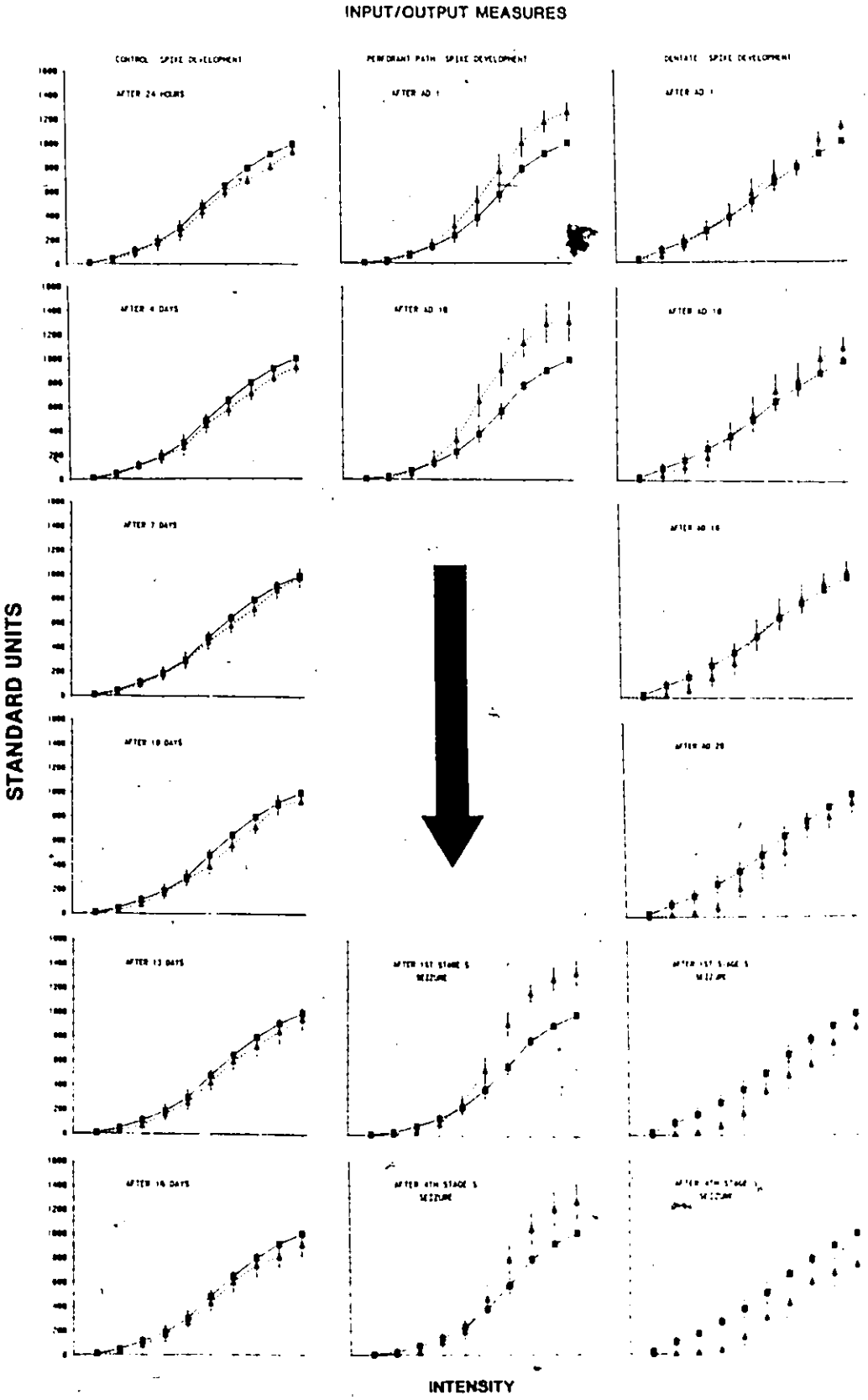

INPUT/OUTPUT MEASURES
CONTROL SPIKE DEVELOPMENT
PERFORANT PATH SPIKE DEVELOPMENT
DENTATE SPIKE DEVELOPMENT
AFTER 24 HOURS
AFTER AD 1
AFTER AD 1
AFTER 4 DAYS
AFTER AD 10
AFTER AD 10
AFTER 7 DAYS
AFTER AD 15
AFTER 10 DAYS
AFTER AD 20
AFTER 13 DAYS
AFTER 1ST STAGE 5 SEIZURE
AFTER 1ST STAGE 5 SEIZURE
AFTER 16 DAYS
AFTER 4TH STAGE 5 SEIZURE
AFTER 4TH STAGE 5 SEIZURE
STANDARD UNITS
INTENSITY

Figure 10.

This figure shows the decay of kindling-induced effects on the population spike 3 days, 1 week, 2 weeks, and 4 weeks post-kindling in the perforant path-kindled group (PERFORANT PATH: SPIKE DECAY) or in the dentate gyrus-kindled group (DENTATE: SPIKE DECAY). Solid lines and squares: pre-kindling baseline measures. Solid lines and triangles: population spike height after the 4th Stage 5 seizure. (post-kindling measures). Dashed lines and diamonds: population spike height at various times post-kindling. Group averages and standard error bars are shown.

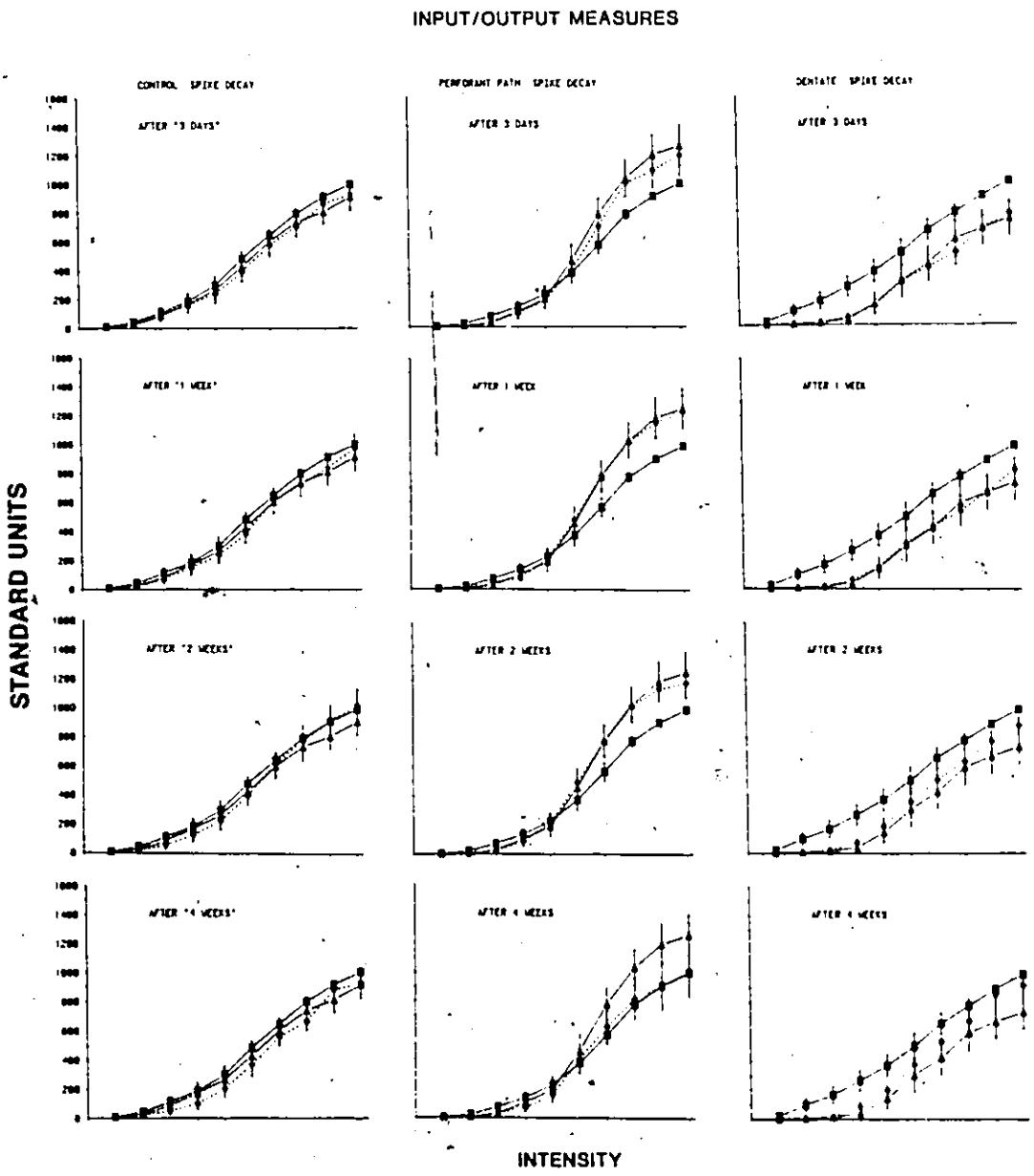

INPUT/OUTPUT MEASURES
CONTROL SPIKE DECAY
PERFORANT PATH SPIKE DECAY
DENTATE SPIKE DECAY
AFTER "3 DAYS"
AFTER 3 DAYS
AFTER 3 DAYS
AFTER "1 WEEK"
AFTER 1 WEEK
AFTER 1 WEEK
AFTER "2 WEEKS"
AFTER 2 WEEKS
AFTER 2 WEEKS
AFTER "4 WEEKS"
AFTER 4 WEEKS
AFTER 4 WEEKS
STANDARD UNITS
INTENSITY

Figure 11.

This figure shows the effects of kindling the perforant path (PERFORANT PATH: SLOPE DEVELOPMENT) or the hilus of the dentate gyrus (DENTATE: SLOPE DEVELOPMENT) on the slope amplitude vs stimulus intensity. Squares: pre-kindling baseline measures; Triangles: after afterdischarges 1, 10, 19, 28, and after the 1st and 4th Stage 5 seizure. Both group averages and standard error bars are shown.

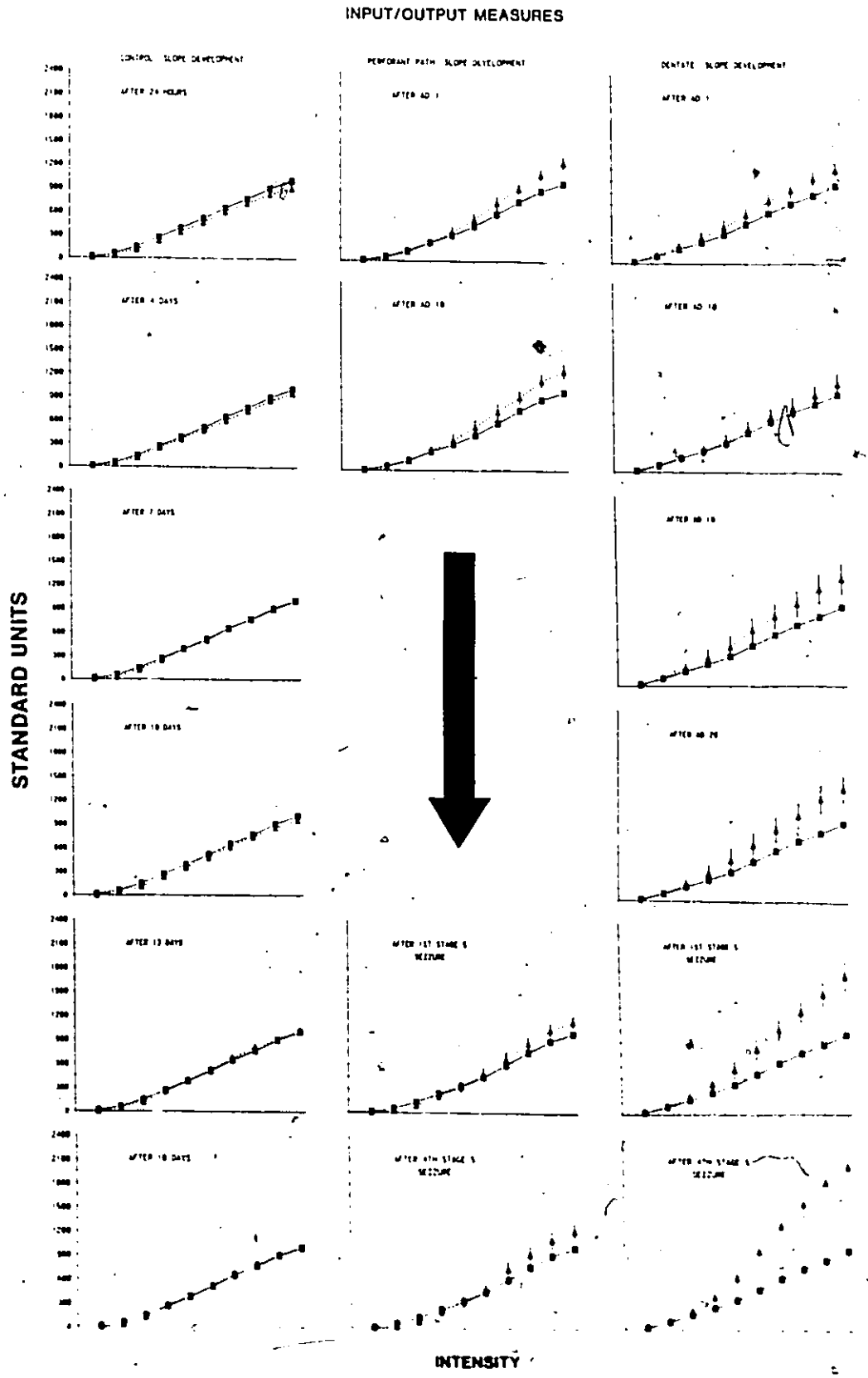

INPUT/OUTPUT MEASURES
STANDARD UNITS
INTENSITY

Figure 12.

This figure shows the "decay" of kindling-induced effects on the slope 3 days, 1 week, 2 weeks, and 4 weeks post-kindling in the perforant path-kindled group (PERFORANT PATH: SLOPE DECAY) or in the dentate gyrus-kindled group (DENTATE: SLOPE DECAY). Solid lines and squares: pre-kindling baseline measures. Solid lines and triangles: slope amplitude after the 4th Stage 5 seizure. (post-kindling measures). Dashed lines and diamonds: slope amplitude at various times post-kindling. Group averages and standard error bars are shown.

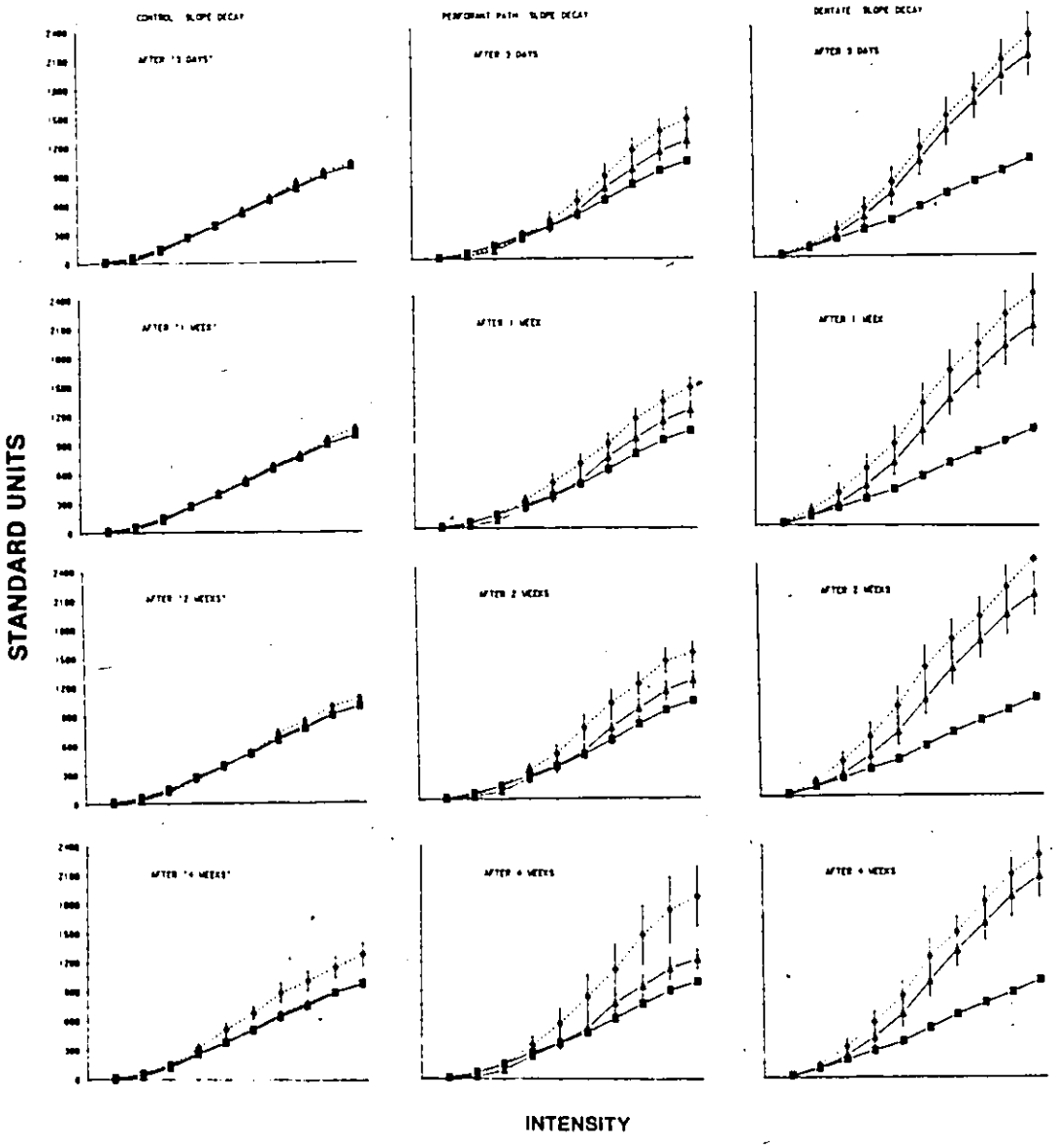

INPUT/OUTPUT MEASURES
STANDARD UNITS
INTENSITY

Figure 13.

An example of the effects of kindling on paired-pulse depression. The evoked potentials were taken from one randomly chosen animal in each group (the dentate gyrus-kindled animal was not randomly chosen; see p. 58): the control group (CONTROL), the perforant path-kindled group (PERFORANT PATH), and the dentate gyrus-kindled group (DENTATE). Solid line: an average of 10 conditioning evoked potentials. Dashed line: an average of 10 test evoked potentials. Interpulse interval=20 ms; Vertical calibration: 3 mv; Horizontal calibration: 4 ms.

PAIRED-PULSE MEASURES

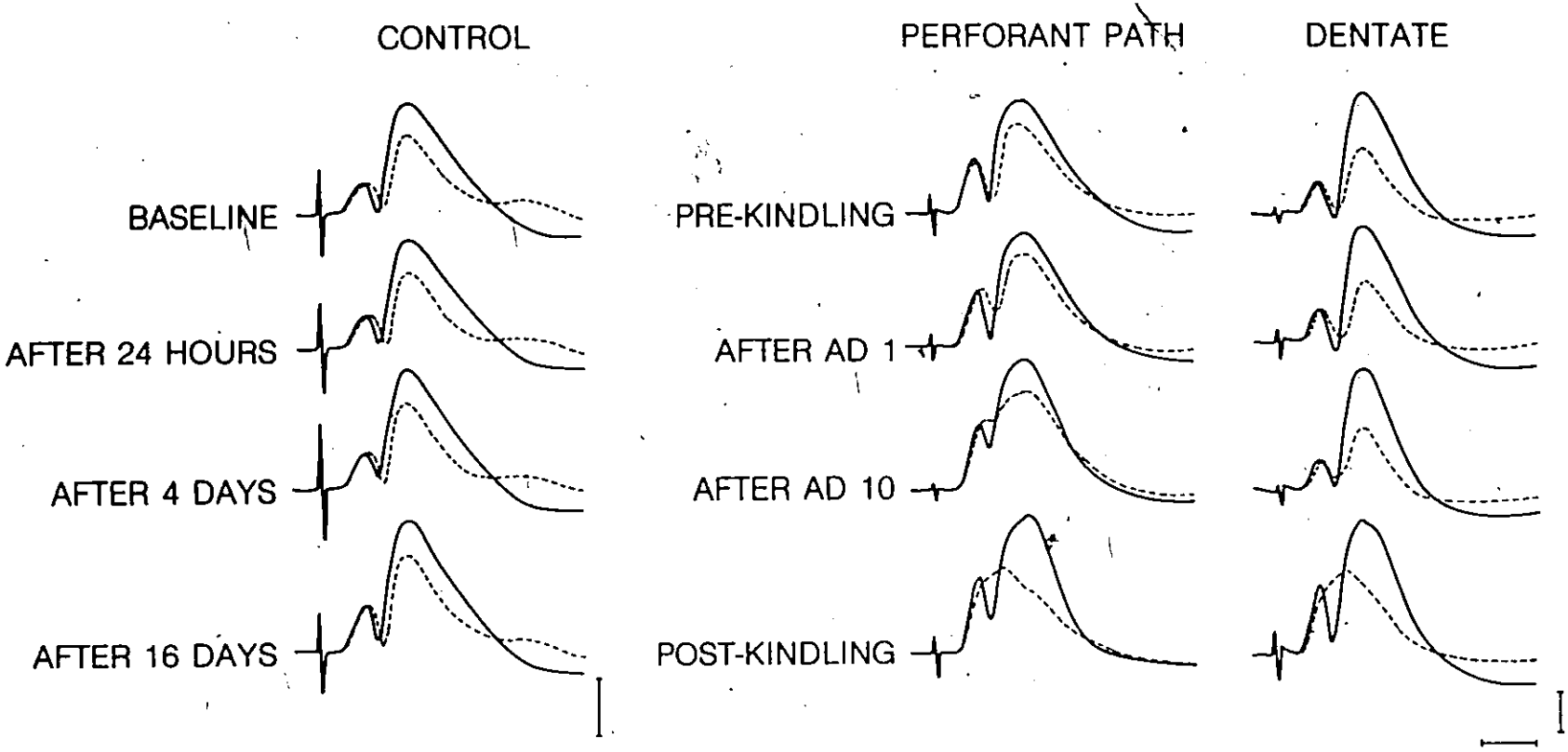


Figure 14.

The effects of kindling the perforant path (PERFORANT PATH: SPIKE DEVELOPMENT) or the hilus of the dentate gyrus (DENTATE: SPIKE DEVELOPMENT) on the population spike paired-pulse measures are shown above. Squares: pre-kindling baseline measures; Triangles: after afterdischarges 1, 10, 19, 28, and after the 1st and the 4th Stage 5 seizure. (P1=conditioning spike height; P2=test spike height).

PAIRED-PULSE MEASURES

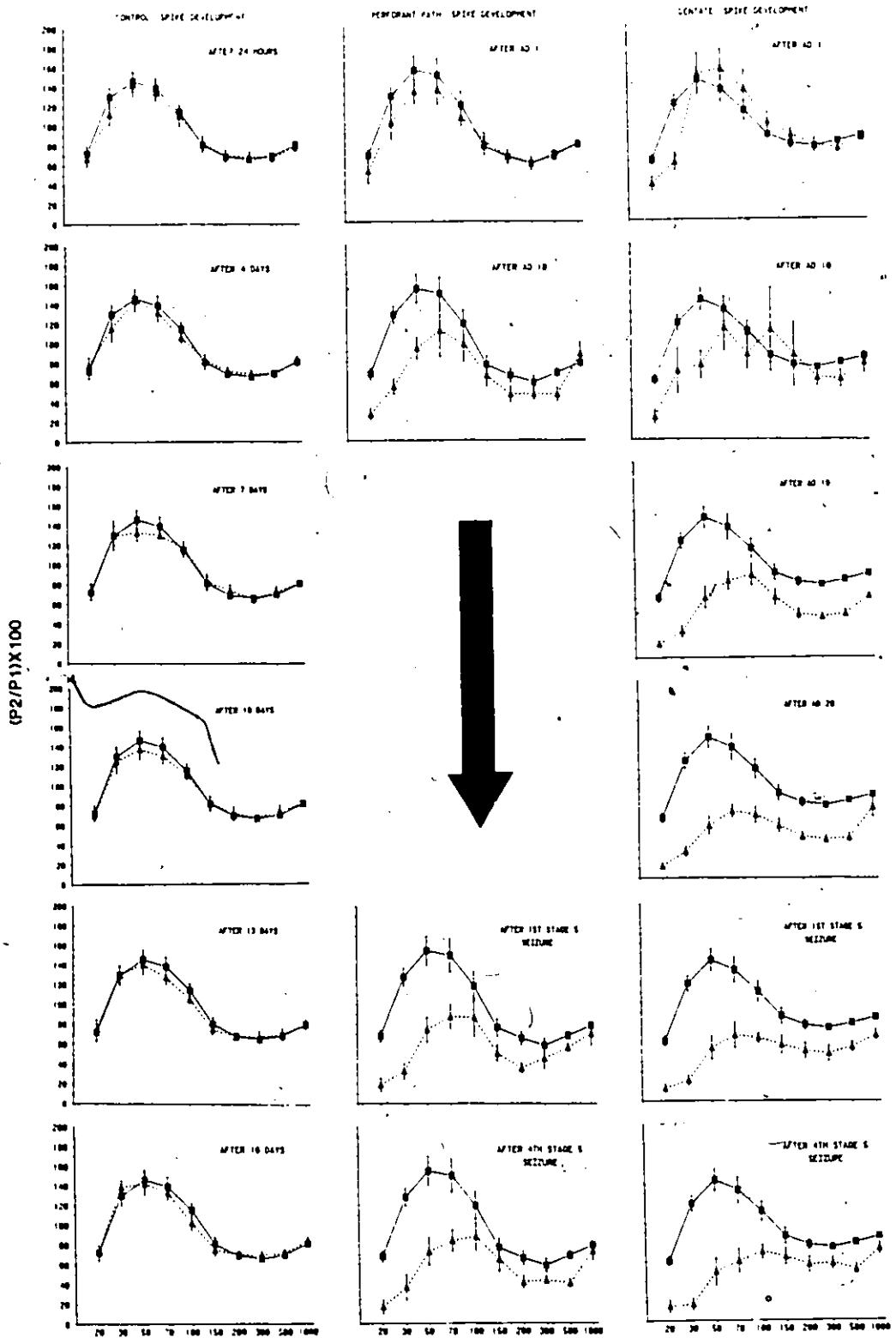


Figure 15.

This figure shows the decay of kindling-induced effects on the population spike paired-pulse measures 3 days, 1 week, 3 weeks, and 4 weeks post-kindling in the perforant path-kindled group (PERFORANT PATH: SPIKE DECAY) or in the dentate gyrus-kindled group (DENTATE: SPIKE DECAY). Solid lines and squares: pre-kindling baseline measures. Solid lines and triangles: paired-pulse measures after the 4th Stage 5 seizure (post-kindling). Dashed lines and diamonds: paired-pulse measures at various times post-kindling.

PAIRED-PULSE MEASURES

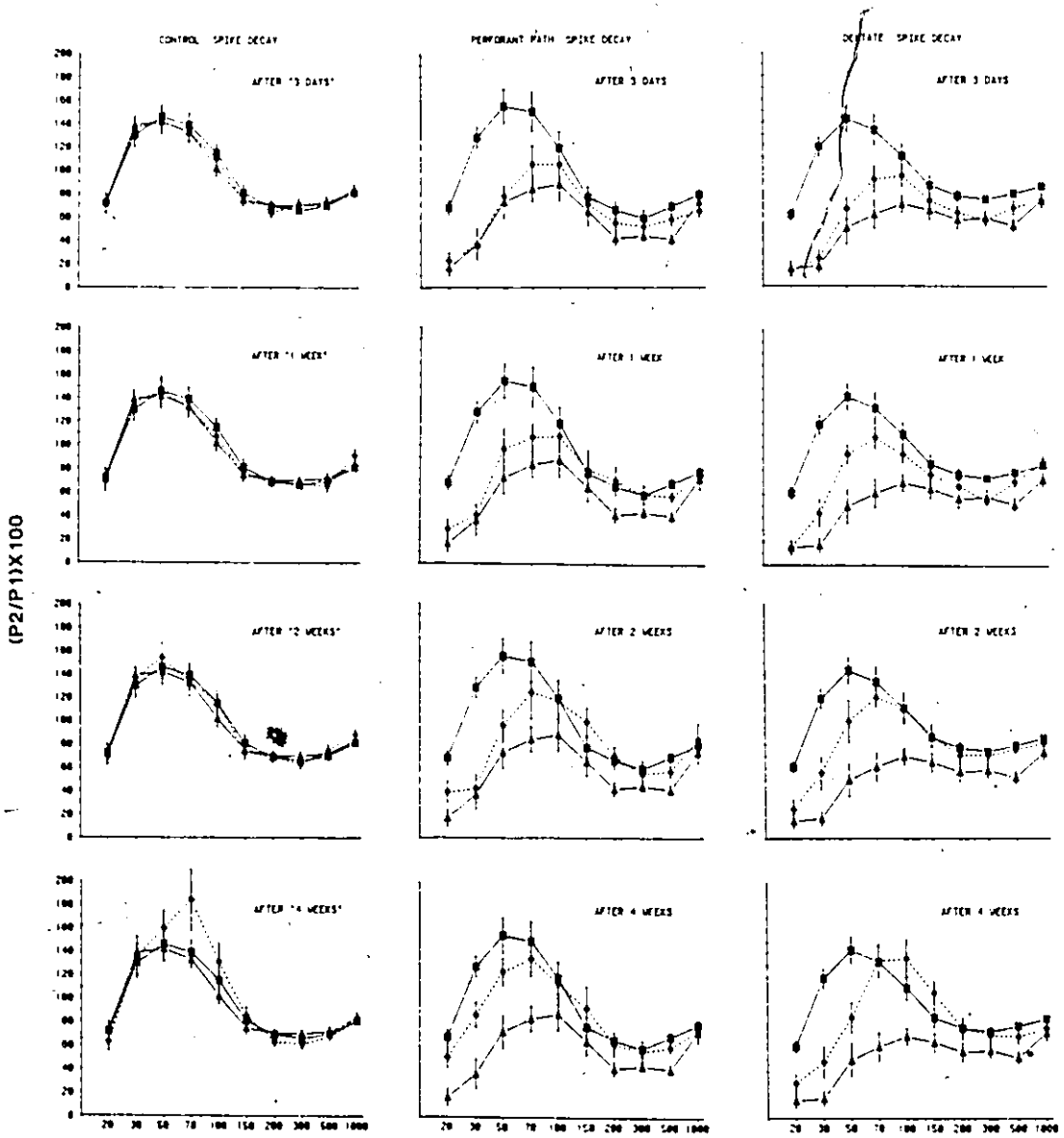


Figure 16.

The effects of kindling the perforant path (PERFORANT PATH: SLOPE DEVELOPMENT) or the hilus of the dentate gyrus (DENTATE: SLOPE DEVELOPMENT) on the slope paired-pulse measures are shown above. Squares: pre-kindling baseline measures; Triangles: after afterdischarges 1, 10, 19, 28, and after the 1st and the 4th Stage 5 seizure. (P1=conditioning spike height; P2=test spike height).

PAIRED-PULSE MEASURES

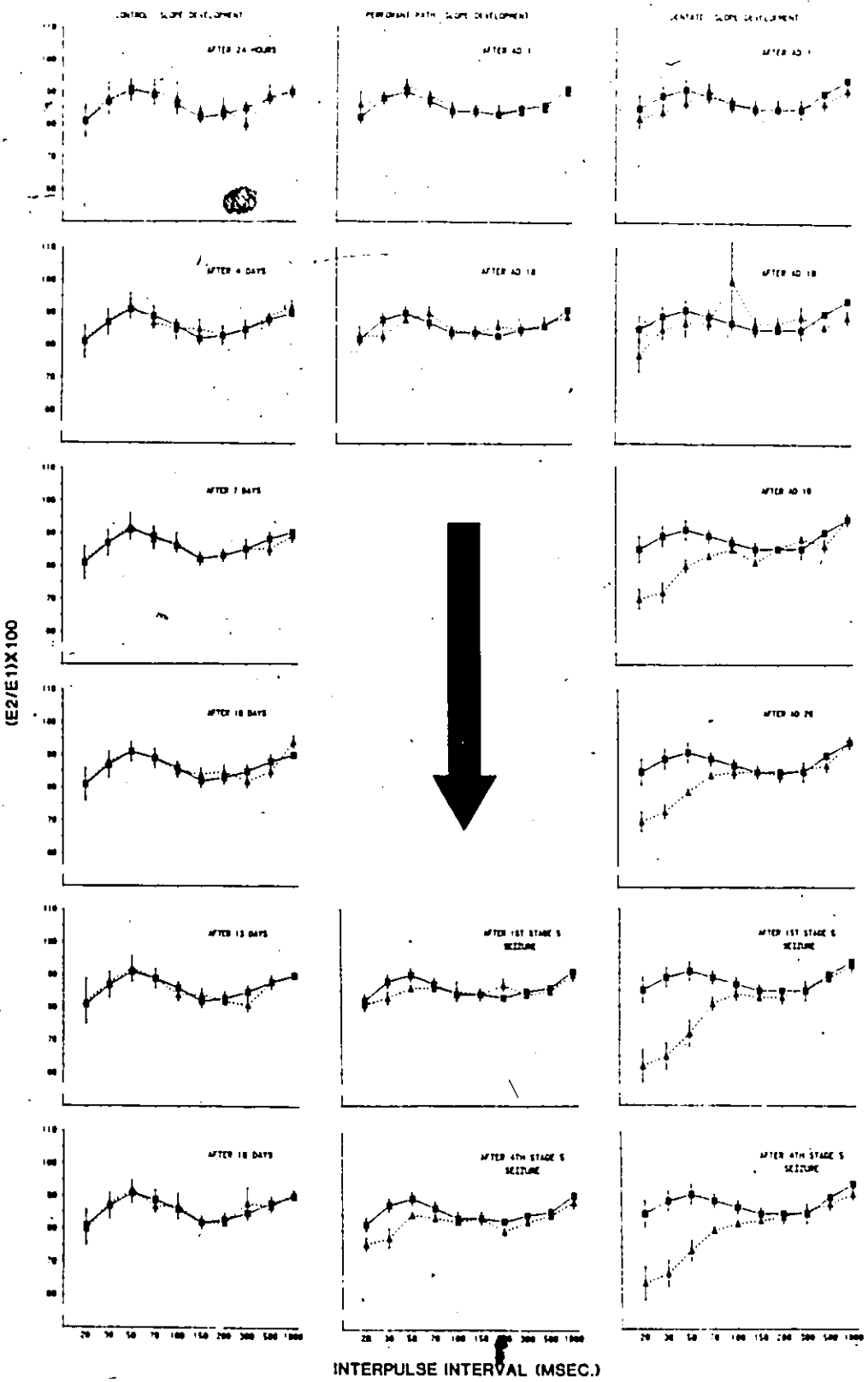


Figure 17.

This figure shows the decay of kindling-induced effects on the slope paired-pulse measures 3 days, 1 week, 2 weeks, and 4 weeks post-kindling in the perforant path-kindled group (PERFORANT PATH: SLOPE DECAY) or in the dentate gyrus-kindled group (DENTATE: SLOPE DECAY). Solid lines and squares: pre-kindling baseline measures. Solid lines and triangles: paired-pulse measures after the 4th Stage 5 seizure (post-kindling). Dashed lines and diamonds: paired-pulse measures at various times post-kindling.

PAIRED-PULSE MEASURES

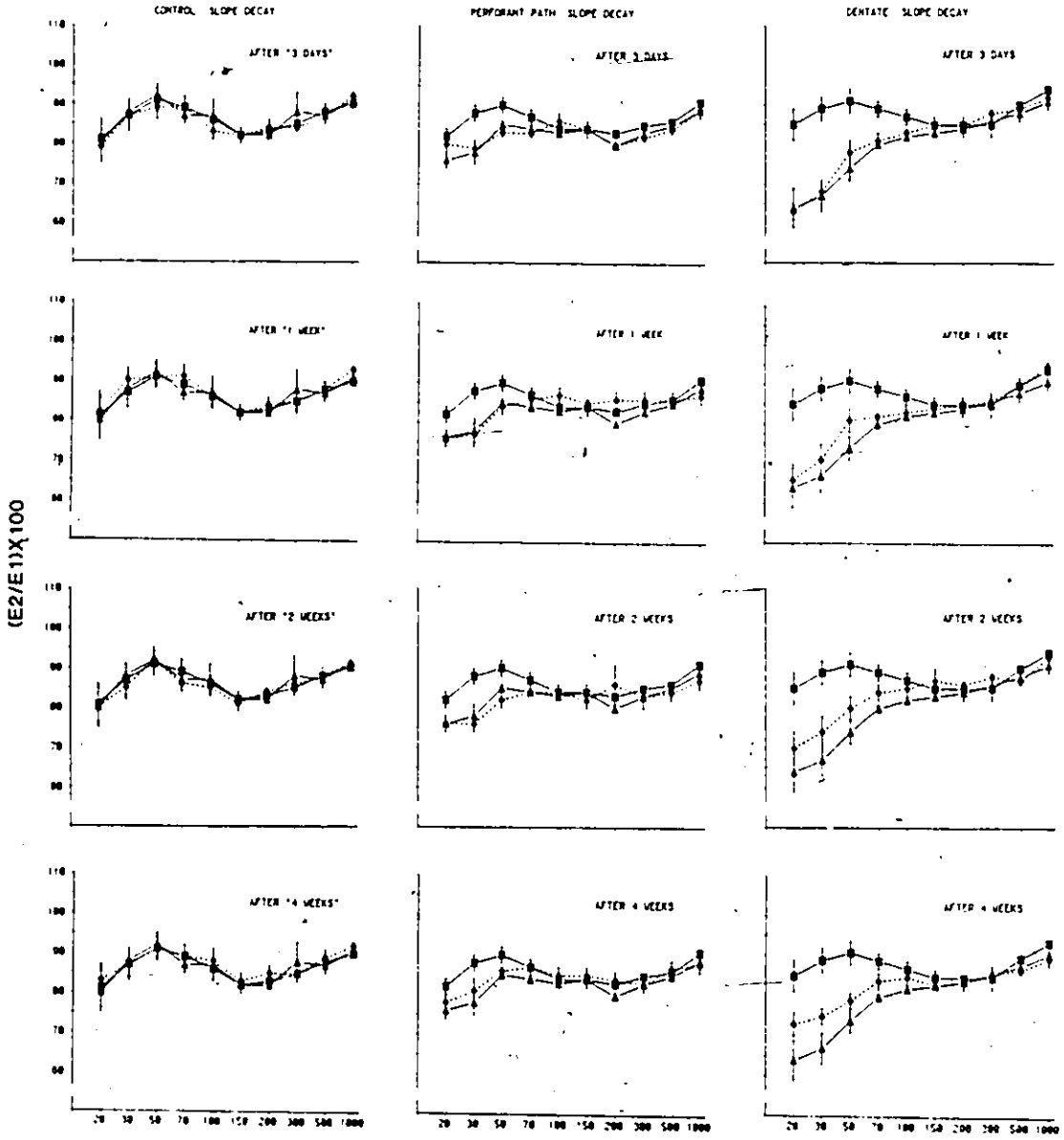


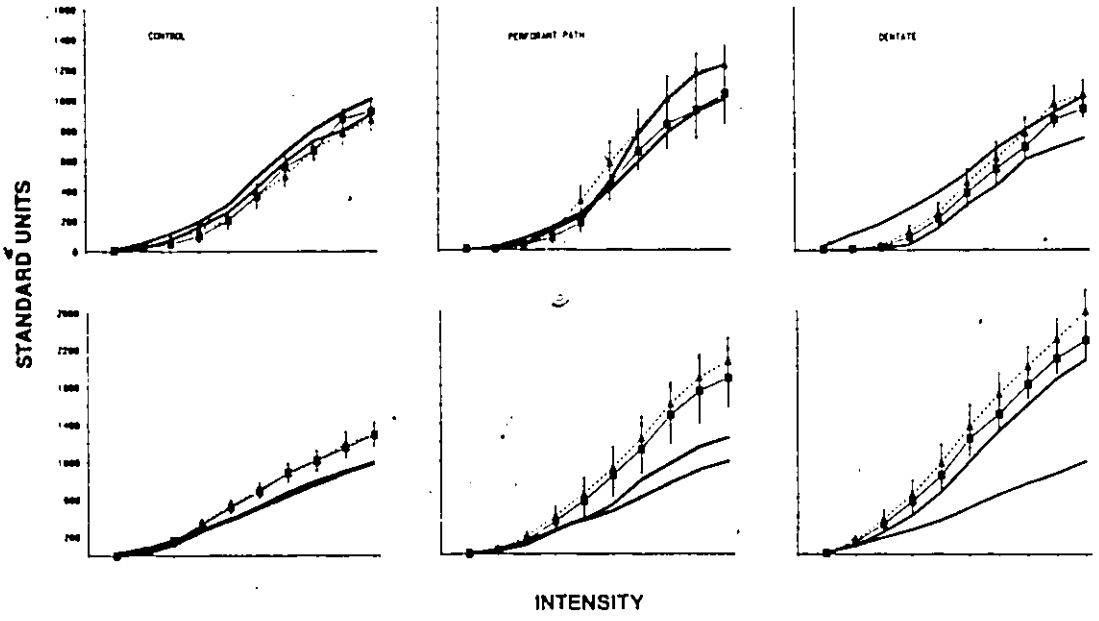
Figure 18.

This figure shows the effect of the final afterdischarge (AD). The solid lines without symbols represent pre-kindling baseline measures and measures taken one day after the completion of kindling. The solid line with squares shows measures after the 4 week decay period. The dashed line with triangles shows the effect of the final AD.

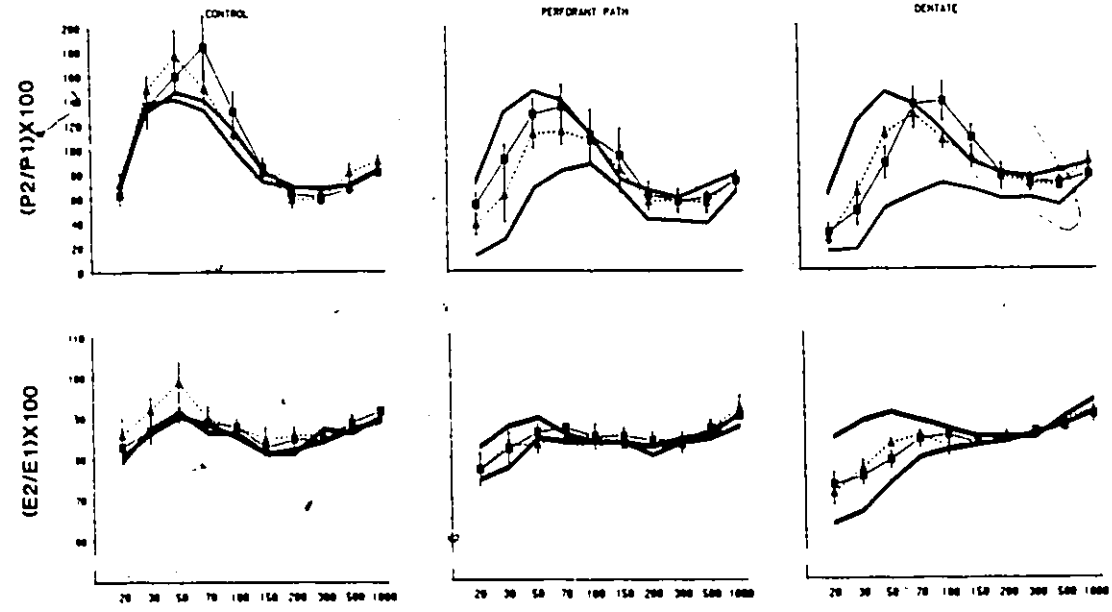
Top: INPUT/OUTPUT MEASURES of the population spike for the control group (CONTROL), the perforant path-kindled group (PERFORANT PATH), and for the dentate gyrus-kindled group (DENTATE) are shown in the upper graphs while those for the slope are shown in the lower graphs.

Bottom: PAIRED-PULSE MEASURES of the population spike for the control group (CONTROL), the perforant path-kindled group (PERFORANT PATH), and for the dentate gyrus-kindled group (DENTATE) are shown in the upper graphs while those for the slope are in the lower graphs.

INPUT/OUTPUT MEASURES



PAIRED-PULSE MEASURES



5.4 Discussion

It is known that animals kindle faster when stimulated in the entorhinal cortex than when stimulated in the dorsal hippocampus (Goddard et al., 1969). In the above experiment, kindling stimulation was applied to the perforant path or directly to the dentate gyrus. Cells in the entorhinal cortex are driven antidromically, and possibly orthodromically as well, if kindling stimulation is applied to the perforant path. Since stimulation of these different locations leads to the recruitment of different populations of neurons, this is most likely the cause of the difference in kindling rate between the two experimental groups. The number of ADs required to induce kindling when stimulating the entorhinal cortex corresponds well to the number of ADs required when stimulating the perforant path. In the above experiment the perforant path-kindled animals required an average of 20.75 ADs until the 4th Stage 5 seizure occurred. Giacchino et al. (1984) reported that animals kindled in the lateral entorhinal cortex required an average of 15 ADs until the 3rd Stage 5 seizure (their criterion) was observed. In the Goddard et al. (1969) study, animals stimulated in the entorhinal cortex required 37 ADs until a behavioral seizure was seen. The stimulation intensity used in the latter study, however, was very low (50 uA for 60 s at 60 Hz), and EEG recordings were not taken.

The input/output curves for the perforant path-kindled animals revealed a kindling-induced increase in spike height as well as a small increase in slope. However, the increase in spike height

from baseline was never more than 60%, much less than is typically observed during LTP experiments (300-700%). Furthermore, a decrease in spike height at lower stimulation intensities was apparent at the end of kindling. The increase in slope was small (maximum 26% from baseline) and only visible at the higher stimulation intensities. This result is consistent with the findings of Giacchino et al. (1984). They were unable to detect any consistent changes in population EPSP at the perforant path-dentate granule cell synapse during or after kindling the lateral entorhinal cortex. The smaller potentiating effects observed after kindling compared to those observed after LTP, could be due to the almost simultaneous, intense activation of hippocampal excitatory and inhibitory systems. Douglas et al. (1982) have shown that simultaneous activation of these two systems interferes with the development of LTP. Possibly, the increased activity in the inhibitory system leads to an increase in Bz-activity which has been found to interfere with the Ca++/calmodulin protein kinase system (Delorenzo et al., 1981). As was mentioned in the introduction (p. 11), the calmodulin system appears to play an important role in producing LTP (Finn et al., 1980; Mody et al., 1983). Wasterlain and Farber (1984) found that septal kindling leads to a decrease in Ca++/calmodulin stimulated phosphorylation of certain proteins. Non-kindled control animals showed a large Ca++/calmodulin stimulated phosphorylation of those proteins. Apparently, the results were most pronounced in the hippocampus and the amygdaloid-entorhinal area. One of these proteins had a molecular weight of 50 kD which

indicates that it could be the same protein as the one implicated in the mechanism underlying LTP (p. 12). Thus, a kindling-induced decrease in the phosphorylation of this protein might interfere with the development of LTP which requires an increase in phosphorylation of that protein.

The spike height of the dentate-kindled animals was depressed after kindling was completed. This depression was particularly clear at lower stimulation intensities. Since kindling stimulation was applied to the molecular layer, it is unlikely that a lot of perforant path-dentate granule cell synapses were strongly activated. It is not surprising, therefore, that there was little evidence of LTP. In addition, the inhibitory system should be more strongly activated if kindling takes place in the dentate than if it takes place in the perforant path, particularly if the feedforward activation of inhibitory neurons by the perforant path is weak. If the stimulation is applied directly to the dentate gyrus the two systems should be activated virtually simultaneously. The inhibitory system might in fact be activated first since hippocampal basket cells have been shown to have lower thresholds than their excitatory counterparts, the granule and pyramidal cells (Buzsaki and Eidelberg, 1982). It has been argued above that simultaneous activation of hippocampal excitatory and inhibitory systems interferes with the development of LTP in excitatory synapses. If the inhibitory synapses are capable of potentiation, the result would be a net increase in inhibition.

The slope measures for the dentate-kindled animals were greatly increased after kindling. Such a dissociation between the

spike and the slope after kindling the dentate gyrus has been reported before (Goddard, 1982). However, the mechanism underlying this dissociation remains unclear. An increase in feedforward inhibition was initially thought to be responsible for the dissociation. The hypothesis was that an increase in Cl-conductance at the cell body layer must add to the positive change in potential due to the virtually simultaneous depolarization of the distal dendrites. At the same time it would shunt the depolarization at the cell body layer leading to a smaller population spike. However, the paired-pulse data were not in agreement with the hypothesis. At an IPI=20 ms, recurrent inhibition is clearly present. This is reflected by the smaller test spike height compared to the conditioning spike height (Fig. 14). But the test slope is <u>also</u> depressed relative to the conditioning slope (Fig. 16) at this IPI.

A decrease in Na+ conductance might account for the dissociation data. This would shift the resting potential away from threshold, leading to a smaller population spike while increasing EPSP magnitudes.

The increase in paired-pulse depression developed and decayed gradually. This indicates that the increase in paired-pulse depression due to kindling might very well be an LTP-like effect. The same can be said of the changes in input/output relationships of the spike height observed after kindling. Since all animals in the perforant path-kindled group responded with a Stage 5 seizure to kindling stimulation after the 4 week decay period, the kindling-induced effects on the measures studied here are independent of the kindled state.

The differences between the two experimental groups in development and decay rates of the kindling-induced effects might be due to the different amounts of stimulation they received as a result of the difference in kindling rate. Towards the completion of kindling in the perforant path group, a decrease in spike height emerged at lower stimulation intensities supporting this argument. On the other hand, it is possible that direct stimulation of the dentate gyrus produced a much stronger activation of the inhibitory basket cells. The stronger effect of the first AD on paired-pulse depression of the spike height for the dentate-kindled animals is consistent with this argument (Fig. 14).

The late inhibitory component potentiated last and decayed first. This indicates that either the duration of the early inhibitory component increases such as to overlap with the late inhibitory component, and/or both components potentiate and decay through independent mechanisms. The results do not agree with a decrease in latency of the late inhibitory component as proposed by Oliver and Miller 1985).

CHAPTER 6

EXPERIMENT II. Pharmacology: The Number of Bz Receptors at Various Times Post-Kindling.

6.1 Introduction

The increase in Bz receptors in the hippocampus due to kindling appears to be inversely related to the amount of time elapsing between the completion of kindling and sacrifice of the animal. McNamara et al. (1981) found a 35% increase in the number of Bz receptors 24 hours after kindling the right amygdala. A similar increase (38%) was found by Shin et al. (1985) 24 hours after kindling the right amygdala. The latter were unable to detect an increase in the number of Bz receptors 28 days after kindling was completed. Tuff et al. (1983b), however, detected a 14% increase in the number of Bz receptors 2 weeks post-kindling in the amygdala.

From the above it appears that the kindling-induced increase in Bz receptors decays over time after completion of amygdala kindling. The results of Experiment 1 showed that the increase in paired-pulse depression due to kindling the perforant path decays over time as well. Since the increase in Bz receptors is believed to underlie the increase in paired-pulse inhibition, it is important to confirm that the increase in Bz receptors also occurs in perforant path-kindled animals, and that the increase in number of Bz receptors

shows a decay similar to that found for the increase in paired-pulse depression (Experiment 1). The cited literature is consistent with a decay over the appropriate period, but the techniques varied and the issue was not directly addressed. The time course must be established with the appropriate control procedures. The following experiment addressed this issue.

6.2 Procedures

Three groups of ten animals participated in this experiment. All animals received bipolar stimulating electrodes in the perforant path as described in Chapter 4. Half of the animals in each group were kindled in the perforant path as described in Experiment 1. The other half served as controls. Each control animal was paired with an experimental animal. Control animals never received any stimulation but spent an equal amount of time in the same stimulation chamber. Animals in the first group were sacrificed one day after completion of kindling (the occurrence of the 4th Stage 5 seizure). Both hippocampi were removed from the kindled animal as well as from the paired control animal. Hippocampi from animals in the second and third group were removed 2 weeks and 4 weeks post-kindling, respectively. All hippocampi were subsequently assayed for the number of Bz receptors.

6.2.1 Tissue Preparation

6.2.1.1 Removing the Hippocampi

Animals were sacrificed by decapitation. Immediately afterwards, the brain was removed from the skull and dissected on ice.

While the brain rested on its ventral surface, the cerebellum and about 0.5 cm of the frontal brain were removed by cutting in a plane at right angles to the longitudinal axis of the brain. Subsequently, left and right brain were separated by cutting the midline, along the longitudinal axis of the brain. The cortex of each half was removed and the hippocampi exposed. The hippocampi were pealed back from underlying structures and placed into plastic Eppendorf tubes which were then submerged in dry ice. After completion of the dissections the tubes were placed in a freezer kept at a temperature of -70 C.

6.2.1.2 Preparing the Membrane Homogenates

The washed membrane procedure, described by Niznik et al. (1984), was used for binding assays. Washing membranes removes endogenous substances which interfere with the binding of the ligand to the membrane receptors.

The frozen hippocampi were homogenized in the presence of 50 volumes ice-cold 50 mM Tris-HCl buffer (pH 7.4). Homogenates were subsequently centrifuged at 48,000 x g for 15 minutes at a temperature of 0-4 C. The resulting pellets were resuspended (Brinkmann polytron, setting 7,2 x 15 s) in 50 volumes of ice-cold deionized water (osmotic shock) and incubated for 30 minutes at a temperature of 0-4 C. These suspensions were centrifuged, and the resulting pellets were homogenized in an original volume of water. These homogenates were then incubated on ice for an additional 30 minutes. Membrane pellets were resuspended in 50 volumes of ice-cold 50 mM Tris-HCl buffer, at a

pH of 7.4, and subsequently frozen at -80 C. After a minimum of 16 hours, the samples were thawed and centrifuged. The resulting pellets were washed 3 times in an original volume of 50 mM Tris-HCl buffer, and were then frozen at a temperature of -80 C. On the day that binding assays took place, the frozen suspensions were thawed, centrifuged, and finally washed twice in 50 volumes of Tris-HCl buffer at a pH of 7.4.

6.2.2 Binding Assay

Total (specific + non-specific) binding was determined by incubating washed membranes (100 ul containing 40-70 ug protein) with six different concentrations of [3H]flunitrazepam (0.3-6.0 nM, final concentration for saturation analysis) in 100 mM Tris-HCl buffer (pH 7.4). The blank (non-specific binding) was determined by the same incubation procedure but in the presence of 1 uM unlabeled clonazepam. Three samples for each concentration under each of two incubation situations (with or without clonazepam) were incubated for 90 minutes at 0-4 C in a final volume of 200 ul. Binding was measured by means of filter assay technique. After the incubation period, samples were rapidly filtered under reduced pressure through Whatman GF/B glass fibre filters and rinsed three times with ice-cold Tris-HCl buffer. The filters were subsequently placed in 10 ml Aquasol (scintillation fluid, New England Nuclear). Radioactivity was extracted overnight at 4 C and counted on a Searle Analytic Mark III liquid scintillation counter (efficiency > 51%). The amount of displaceable binding

(specific) is given by the difference between total binding and the blank. From these data Scatchard plots were derived. The intercept on the abscissa gives Bmax, the concentration of receptors. The affinity of the receptor for the ligand, Kd, is a function of the slope (slope= -1/Kd). Protein content of membrane homogenate was determined according to the method of Lowry et al. (1951) using bovine serum albumin (Sigma Chemical) as a standard.

6.3 Results

6.3.1 Kindling

The 15 animals that were stimulated had 4 Stage 5 seizures after an average of 25.07 (Sd=13.29) stimulations. The 24-hour group, the 2-week group, and the 4-week group required an average 18.40 (Sd=5.22), 26.80 (Sd=15.74), and 30.00 (Sd=15.91) stimulations, respectively. None of these averages differed significantly from each other, nor did they differ significantly from the 20.75 (Sd=5.26; n=8) stimulations required by the perforant path-kindled animals in Experiment 1 (p. 58; t-tests were used).

6.3.2 Binding

The data (Table 2) were analysed by means of an analysis of variance. Decay time was entered as a between group variable with 3 levels (24 hours, 2 weeks, or 4 weeks). The group factor was treated as a within group variable with two levels, the experimental and the control group, since animals were treated in pairs. Each member of a

Table 2.

This table shows the data obtained from the binding experiment. Bmax (fmol/mg protein) and Kd (nM) values are shown for each perforant path-kindled animal and its non-kindled paired control in each of the three decay groups (24 hours, 2 weeks, and 4 weeks).

DECAY GROUP	KINDLED		PAIRED CONTROL	
	Bmax (fmol/mg protein)	Kd (nM)	Bmax (fmol/mg protein)	Kd (nM)
	962	1.23	903	1.00
	1090	0.89	897	0.94
24 HOURS	891	0.99	732	1.05
	1138	1.09	1085	1.14
	992	0.88	1074	0.93
	1010	0.98	972	0.99
	1136	0.93	1057	0.93
2 WEEKS	942	0.96	879	0.90
	937	1.18	896	1.01
	886	1.04	894	1.06
	1022	0.87	929	0.85
	845	1.06	809	0.97
4 WEEKS	1027	1.00	989	1.06
	885	1.12	817	1.20
	892	1.00	960	1.00

pair received identical treatment except for the kindling stimulation which only the experimental animals received. The dependent variable was the number of Bz receptors (in fmol/mg protein). The results in Table 3 (Top) show that only the main group effect reached significance ($F(1,12)=7.07$; $P=0.0209$). The average kindling-induced increase in number of Bz receptors was 5.92% from control levels. The number of receptors was increased by an average of 9.40% twenty four hours after kindling. Two weeks and four weeks post-kindling the number of receptors was increased by an average of 4.45% and 3.91%, respectively.

At no time did the affinity (Kd) of the Bz receptor for the ligand differ significantly between kindled and control animals (Bottom of Table 3).

6.4 Discussion

These data show that kindling the perforant path leads to an increase in the number of Bz receptors.

There is no simple explanation for the difference in magnitude of the increase in number of Bz receptors described in the literature and those found in the above experiment. The site of stimulation, treatment of control animals, and type of binding assay differed from previous studies. Twenty four hours after kindling the amygdala ,an increase of about 36% was found by McNamara et al. (1981) and by Shin et al. (1985). The increase described above was in the order of 10% at the 24-hour interval. Two weeks after completion of

Table 3.

This table shows the results of the analysis of variance. The source of variance, the sum of squares, the degrees of freedom (df), the mean squares, the F-ratios (F), and the probability of the F-ratios (P) are shown. The DECAY factor refers to the 24 hour, 2 week, and 4 week decay groups. The GROUP factor refers to the number of Bz receptors (Bmax in fmol/mg protein; Top) or to the affinity of the receptor for the ligand (Kd in nM; Bottom) in kindled and control animals. The interaction between The DECAY and GROUP factor is designated GD.

ANOVA RESULTS

Number of Receptors (Bmax)

Source	Sum of Squares	df	Mean Square	F	P
MEAN	27166276.80	1	27166276.80	1568.22	
DECAY	18643.40	2	9321.70	.54	.60
ERROR 1	207875.80	12	17322.98		
GROUP	19354.80	1	19354.80	7.07	.02*
GD	2563.40	2	1281.70	.47	.64
ERROR 2	32871.80	12	2739.32		

AFFINITY (Kd)

Source	Sum of Squares	df	Mean Square	F	P
MEAN	30.5021	1	30.5021	1677.01	
DECAY	.0016	2	.0008	.04	.96
ERROR 1	.2183	12	.0182		
GROUP	.0012	1	.0012	.27	.61
GD	.0029	2	.0015	.33	.73
ERROR 2	.0536	12	.0045		

*: 0.02 < P < 0.05

amygdala kindling, Tuff et al. (1983b) described a 14% increase in the number of Bz receptors. The above results showed an increase of about 4.5% two weeks post-kindling. The paired-pulse data from the Tuff et al. (1983a) study indicated that amygdala kindling might have had a stronger and longer-lasting effect on paired-pulse depression than perforant path kindling. The increase in paired-pulse depression observed by Tuff et al. (1983a) 2 weeks post-kindling, was similar in magnitude to the increase which was observed in the above study 24 hours after completion of perforant path kindling. If the kindling site accounts for the observed binding differences, and if an increase in Bz receptors underlies the increase in paired-pulse depression, then the similar 10-14% increase in the receptor number in the 24 hour perforant path-kindled group and the 2 week amygdala-kindled group could account for the similar increases in paired-pulse depression. The kindling-induced increase in the number of Bz receptors was larger 24 hours after kindling than it was 2 weeks or 4 weeks post-kindling (9.40% vs 4.45% and 3.91%). Although this result was in the predicted direction, it was not statistically significant, presumably due to the small number of observations. Furthermore, there was no difference between the 2-week and 4-week decay groups. If the increase in paired-pulse depression 24 hours after kindling is indeed due to a 10-15% increase in Bz receptors, the range over which decay can take place is small. Although the evidence as to whether the number of Bz receptors decreased over time after kindling remains inconclusive, the results of the present time-course study, together with the separate studies

in the literature, suggest rather strongly that such a decline actually occurs. Furthermore, the decay values obtained are consistent with those obtained in the electrophysiological experiments.

CHAPTER 7

GENERAL DISCUSSION

7.1 Kindling-Induced Plasticity: Electrophysiology

The results of Experiment 1 confirmed that kindling the perforant path or the hippocampal dentate gyrus leads to an increase in paired-pulse depression. Presumably, this increase in paired-pulse depression reflects the way in which the CNS defends itself against an excess of stimulation resulting in AD. On the other hand, plasticity of inhibitory systems may serve other functions as well. For example, this plasticity may have evolved to improve information storage mechanisms (see section 7.3).

The results of the dentate-kindled group showed that the increase in paired-pulse depression developed long before an animal could be said to be kindled (after about 10-19 ADs). The occurrence of AD appeared to be the main cause of the increase in paired-pulse depression. The perforant path-kindled group showed that the increase in paired-pulse depression returned to pre-kindling levels if AD was not elicited. This leads to the interesting possibility that, if the kindled state has been established but no seizures are induced for a period of time, AD thresholds may be reduced compared to those at the beginning of the kindling. This in turn indicates that it would be a good policy to keep those who had AD, because of some traumatic blow to the head for example, on medication for a long period of time after

seizures have subsided. The length of this period would depend on how far the abnormality underlying the kindled state had progressed.

All kindling-induced effects, except for the increase in slope, appeared to return towards pre-kindling levels after kindling was terminated. They do not appear to be associated with the kindled state. Therefore, none of these effects can provide information about the nature of the kindled state. It is unlikely that the increase in slope is associated with the kindled state, since the increase was not very large in the perforant path-kindled group, and it was this group that showed the least disruption of kindling after a 4 week delay. Furthermore, it would be difficult to account for the kindled state by means of an increase in slope only. Cell discharge, which is reflected in the population spike, is required for the propagation of AD.

The late inhibitory component (IPI=300 ms) evoked during paired-pulse tests showed smaller, delayed kindling-induced potentiation, compared to the early component. It also returned to pre-kindling levels more rapidly (within 2 weeks post-kindling). The early inhibitory component (IPI=20 ms) in the perforant path-kindled group had not fully returned to pre-kindling levels by 4 weeks post-kindling. The decay of the early component in the dentate-kindled group was even slower. The decay rates for both components in both groups, however, are within the range of those reported for LTP in excitatory pathways (see Chapter 1). If the postsynaptic mechanism of LTP proposed by Lynch and co-workers is correct, the mechanisms underlying both phenomena would appear to be similar, as well.

7.2 Kindling-Induced Plasticity: Pharmacology

Kindling the perforant path resulted in an increase in the number of Bz receptors. The results from Experiment 2 indicated that this increase in receptor number may decline over a period of time during which no stimulation is applied. Although the differences were not significant, the proportional decreases in mean binding were similar to the decay of paired-pulse depression. That similarity also applies to the binding data in the literature. It must be stressed, however, that the evidence for an increase in receptor number as a mechanism, for either kindling or LTP, is far from conclusive. Problems with the LTP-related evidence have been mentioned before (p. 11). With respect to kindling, it remains to be shown that the kindling-induced increase in Bz receptors actually underlies the increase in paired-pulse depression. This problem might be resolved by making use of a Bz receptor blocker. First, the amount of interference of the drug, at saturating concentrations, with paired-pulse depression would be determined by comparing pre- and post-drug values. Animals would then be kindled in the usual manner. Subsequently, the drug effect would be evaluated again. If the effect of the drug on paired-pulse depression post-kindling were larger than the pre-kindled drug effect, then it could be concluded that the increase in number of Bz receptors was responsible for the kindling-induced increase in paired-pulse inhibition.

7.3 Kindling-Induced Plasticity and Learning

The inhibitory system seems plastic in much the same way as the excitatory system. In both cases the function of this plasticity remains unclear. LTP has been proposed to play an important role in memory processes (see Chapter 1). The most obvious function of plastic inhibitory systems, in the context of these experiments, appears to be to protect the CNS from an excess of excitation. However, it is possible that such inhibitory systems play an important role in memory processes as well. If this were so, it should be possible to induce plastic changes in the inhibitory system by other, less drastic, means than epileptogenic stimulation. At this point, however, no conclusions as to the function of plasticity of inhibitory systems in memory processes can be made.

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