EFFECTS OF EXTENDED KINDLING IN HIPPOCAMPAL AND OLFACTORY CORTICAL SYSTEMS ON MEASURES OF TRANSFER, INHIBITION, CELL LOSS AND SPROUTING

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Abstract

Human epilepsies are a family of disorders of the nervous system, characterized by transient, recurring episodes of neuronal hypersynchronous seizure activity. These often begin locally, but they may generalize to produce convulsions. The basic mechanisms responsible for the seizures are unknown, but several animal models of epilepsy are available for the study of the neuronal abnormalities associated with these events. One of these is the kindling model in which repeated application of electrical stimulation to certain brain regions results in the progressive development of electroencephalographic and behavioural seizures.

In this thesis, we compared two excitatory monosynaptic systems for rates of transfer kindling effects, levels and alterations in inhibition, kindling-induced cell loss and mossy fiber sprouting. Animals were kindled for 30 or more stage 5 seizures. The two monosynaptic systems studied were: 1) entorhinal cortex-perforant path-dentate gyrus system and 2) olfactory bulb-lateral olfactory tract-piriform cortex.

Our major findings may be summarized as follows: a) there was immediate transfer in the olfactory bulb-lateral olfactory tract-piriform cortex system but not in the entorhinal cortex-perforant path-dentate gyrus system. We hypothesized that the difference in transfer rates was due to differences in the spatio-temporal patterns of discharge at the network level. b) In both systems, the levels of inhibition were increased

following kindling, remained increased throughout administration of kindling stimulations and then returned towards baseline levels after discontinuation of the kindling stimulations. This suggests that a disturbance in the inhibitory system of the dentate gyrus or the piriform cortex can not explain the epileptogenesis. c) We found a decrease in the number of hilar cells of the dentate gyrus following kindling in the entorhinal cortex-perforant path-dentate gyrus system but not following kindling of the olfactory bulb-lateral olfactory tract-piriform cortex system. d) Sprouting of mossy fibers was found into the IML of the dentate gyrus following kindling of the lateral olfactory tract but only after at least 14 stage 5 seizures had been elicited. There was no evidence of sprouting of these fibers into the stratum oriens of area CA3.

These findings suggest that kindling results in a remarkable specificity in the seizure circuitry dependent on the site of stimulation and does not require cell loss in the hilus or mossy fiber sprouting into either the CA3 or the IML for it to occur. Since we found enhanced inhibition in both systems with kindling, a failure of inhibition, at least in these systems, can not explain epileptogenesis.

Dedicated to my father,

Aidan E. Spiller, Ed. D.

who has inspired me to follow my dreams.

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AD	afterdischarge
B	basket cell
Bz	benzodiazepine
CCK	cholecystokinin
DG	dentate gyrus
EPSP	excitatory postsynaptic potential
EC	entorhinal cortex
ECS	electroconvulsive shock
EEG	electroencephalogram
G	granule cell
IML	inner molecular layer
IPI	interpulse interval
KIP	kindling induced potentiation
LEC	lateral entorhinal cortex
LOT	lateral olfactory tract
LTP	long term potentiation
MEC	medial entorhinal cortex
MML	medial molecular layer
NGF	nerve growth factor
OB	olfactory bulb
OML	outer molecular layer
PC	piriform cortex
PP	perforant path
TLE	temporal lobe epilepsy
VIP	vasoactive interstitial polypeptide

CHAPTER 1

General Introduction

The term neuroplasticity when applied to the adult nervous system, refers to the modifiability of the structure and the physiology of neurons, usually in response to altered activation patterns, i.e. experiences. There may be numerous plasticity mechanisms, but at least some subset of these might be expected to underly the information storage that leads to long-lasting changes in behaviour. These plasticity mechanisms, in turn, might be based upon an extension of developmental plasticity mechanisms into adulthood. After the normal developmental growth and differentiation of neuronal systems have ceased, neuroplastic events still entail changes in cellular structure including dendrites, dendritic spines, and synaptic terminals, or at the level of responsiveness, including discharge threshold or amplitude. In the adult, these plastic changes are primarily activation-induced, and can be brought under experimental control with chemical and electrical as well as environmental stimulation.

One well studied example of neuroplasticity in the adult preparation is the phenomenon of long term potentiation (LTP). LTP refers to to an increase in amplitude of the responses evoked in a neural pathway following activation of that pathway (Bliss and Lomo, 1973). Since this modification can last for several weeks, and occurs readily

in the hippocampus, a structure known to be important for memory processing. LTP has been suggested as a possible basis for memory. The exact nature of the plastic changes involved in LTP is still a mystery. For example, it is not yet known whether the cellular changes that support the expression of LTP are located pre- or post-synaptically, or both (Collingridge and Bliss, 1995).

Another example of neuroplastic change, although more clearly pathological, occurs in epilepsy. Human epilepsies are a family of disorders of the nervous system, characterized by transient, recurring episodes of neuronal hypersynchronous seizure activity. This activity can appear with local or widespread onset. If local, it can generalize to produce convulsions. The annual incidence of epilepsy ranges from 28.9 to 53.1 per 100,000. It is highest in children under the age of 10 (80:100,000) and adults over the age of 60 (82:100,000). The mean prevalence of epilepsy, defined as patients taking antiepileptic drugs or who have had a seizure in the last 5 years, is generally accepted to be about 6.5:1,000 (Hauser and Hersdorffer, 1990).

In 1981, the International League Against Epilepsy proposed a classification system of epileptic seizures, based on clinical and electroencephalographic (EEG) criteria which is now widely accepted. Of the classifiable seizures, it defines 2 major categories: partial and generalized. Partial seizures are defined by their focal origin (one or more cerebral foci) and are classified into 3 groups: simple, complex and secondarily generalized. With simple partial seizures, the patient retains consciousness whereas with complex partial seizures, the patient shows impaired consciousness. Seizures with a focal origin that

progressively spread to the whole brain are referred to as secondarily generalized tonic-clonic seizures. Generalized seizures cannot be localized and show widespread electrical activity throughout the brain from the onset. The simplest form of generalized seizure is the absence seizure (petit mal) which is characterized by a sudden, momentary impairment of consciousness. The tonic-clonic seizure (grand mal) is the classic example of a primary generalized epileptic seizure (Commission on Classification and Terminology of the Internationsal League Against Epilepsy,1981).

The basic mechanisms responsible for the seizures and the means by which seizures propagate to involve other brain areas, are not well understood. The amount of information that can be obtained from human epilepsy research is limited by the ethical and technical considerations placed on researchers. That is, there are a limited number of experimental manipulations that can be ethically performed on human subjects. Also, it is unlikely that brain tissue from chronic epileptic patients will provide the answers to the basic mechanism of seizure genesis because these patients usually have had many seizures and drug regimens which may confound the original source of epileptogenesis (Ribak, 1991). Elucidating the basic cellular mechanisms of epilepsy may be easier using a model system approach. The goal of this approach is to understand specific principles in the context of a simpler system and then relate the findings back to more complex systems.

It is important, when using a model system, not to lose site of the "big picture" questions, but one must also resist the temptation to overgeneralize results. The use of

the LTP phenomenon as a model of learning and memory provides a case in point. As yet, there is no strongly compelling evidence to link LTP and memory mechanisms. Nevertheless, a perusal of the literature shows that many investigators have already assumed that such a link exists. Researchers working in this field have tended to ignore or minimize obvious problems with LTP (e.g. its abnormal synchronous activation levels), focusing instead on the features which are common to LTP and normal learning. Overgeneralization may occur when nontrivial jumps are required to move from invertebrate to vertebrae, from one kind of learning to another, or from the cellular level to the intact network or organism (Milgram, 1987). To look at the basic mechanisms underlying epilepsy, a number of animal models have been developed. One experimental model of epilepsy that allows good control over the nature and site of the epileptogenic lesion and the duration and severity of the seizures is kindling.

Kindling

The process whereby repeated, temporally separated stimulations of certain brain structures leads to the progressive development of electroencephalographic and generalized behavioural seizures was called "kindling" by Graham Goddard and his colleagues (1969). Kindling is one of the few models of epilepsy that allows precise control over the epileptogenic agent and one of the few models of neural plasticity that involves a *permanent* change in brain function in the adult mammalian brain (Racine, 1978). The use of kindling as a model for human epilepsy rests on the assumption that

these changes may be analogous to those involved in certain types of clinical seizures, especially to complex partial seizures of temporal origin which secondarily generalize (Adamec, McNaughton, Racine and Livingston, 1981). In the subsequent sections, the kindling phenomenon will be described and this assumption will be addressed.

Kindling as a model of memory

Goddard and Douglas (1975) argued that kindling satisfies many of the criteria for a model system of memory. It is robust and reliable and results in a relatively permanent change as a consequence of neural activation. It can be demonstrated in several vertebrate species and occurs in brain regions thought to be important for learning, memory and motivation. The gradual development of kindled seizures over repeated kindling trials is predictable and has been compared with a learning curve. This suggests that the process of kindling involves some form of growth process initiated after each kindling trial .

One of the changes in neural function produced by kindling, is kindling induced potentiation (KIP). KIP refers to an increase in amplitude of evoked responses seen following kindling of forebrain pathways (Douglas and Goddard, 1975; Racine, 1972a,b; Racine, Milgram and Hafner, 1983; Racine, Newberry and Burnham, 1975; Russell and Stripling, 1985). KIP has been found following amygdala (Racine et al, 1975), hippocampus (Douglas and Goddard, 1975), lateral olfactory tract and piriform cortex kindling (Racine et al, 1983). The characteristics of KIP show similarities to and differences from those of LTP, another model of memory which is produced by non-

epileptogenic stimulation. First of all, KIP and LTP are both produced by neural activation. The stimulation parameters most effective at producing LTP are repetitions of brief, high frequency trains. It is probable that these trains result in brief bursts of action potentials in the stimulated axons. The epileptiform discharge triggered by the KIP-inducing stimulation is itself characterized by a series of brief, high frequency bursts of action potentials in the affected cells (Racine and Zaide, 1978; Racine et al, 1991). Several differences exist between KIP and LTP. For example, the population spike (S), a field measure of cell firing, becomes relatively more potentiated than does the population EPSP (E), a field measure of synaptic responses, with LTP in the dentate gyrus. This results in the E/S potentiation effect, in which a larger population spike is evoked at any given level of EPSP (Abraham, Bliss and Goddard, 1985; Bliss and Lomo, 1973). Kindling, on the other hand, produces a greater effect on the EPSP than on the population spike, reversing the E/S relationship (de Jonge and Racine, 1987). Although, LTP has been demonstrated in many structures outside the hippocampus, it is difficult to produce it in the piriform cortex and neocortex (Racine et al, 1983; Stripling, Patneau and Gramlich, 1988). Kindling induces potentiation in both of these sites (Racine et al, 1983; Racine et al, 1975; Russell and Stripling, 1985). On the other hand, recent results indicate that both LTP and KIP develop relatively slowly in the neocortex (Racine, Teskey, Wilson, Seidlitz and Milgram, 1994). One of the most striking differences between the two phenomena may be in the decay rates of the potentiation. LTP decays within days or weeks (Racine et al, 1983), whereas KIP of the population EPSP in the dentate gyrus did not decay over a

period of 30 days and may be as permanent as the seizure susceptibility (deJonge and Racine, 1987). Clearly, some of the features of KIP make it an attractive alternative to LTP as a memory model.

There are, however, problems with kindling as a model of learning and memory. The most difficult to reconcile are the pathological characteristics of kindling. Seizures, themselves, are due to excessive and synchronous discharge of neurons. As kindling progresses, the epileptiform discharge, initially localized to the site of stimulation, propagates to other sites, recruiting those sites into the discharge. Other pathological processes that have been implicated in the kindling process include 1) activation of astrocytes in response to hyperactivity (Khurgel, Switzer, Teskey, Spiller, Racine and Ivy, 1995) 2) increases in the density of Timm granules in the dentate gyrus and CA3 of kindled animals which is thought to reflect the induction of mossy fiber sprouting (Cavazos, Golarai and Sutula, 1991; Represa and Ben-Ari, 1992) and 3) cellular damage to hilar neurons in the dentate gyrus (Cavazos and Sutula, 1990).

Kindling as a model of epilepsy

There are a number of similarities between the features of kindling and the features of human epilepsy which support the validity of kindling as a model of human epilepsy. Kindling is one of the few models of complex partial seizures with secondary generalization which shows a long-lasting seizure susceptibility sufficient to produce recurrent spontaneous epileptic seizures (Sato, Racine and McIntyre, 1990). The arrested

activity, oral automatisms and reduced responsiveness to external stimuli are common features of both human complex partial seizures with presumed temporal origin and kindled seizures at stages 1 and 2. Likewise, the clonic motor movements with secondarily generalized limbic seizures are similar in kindled rats and human epileptics. Also, seizures in rats can occur spontaneously after 300-400 kindling stimulations (mean of 348) in the amygdala, hippocampus or entorhinal cortex. These spontaneous seizures endure after discontinuation of stimulations, suggesting that the seizure susceptibility is permanent (Pinel and Rovner, 1978; Milgram et al, 1995)).

The electroencephalograghic (EEG) patterns recorded during amygdala kindled seizures from electrodes in the hippocampus and amygdala are similar to the rhythmic polyspikes which evolve into polyspike and wave patterns in human complex seizures (Sato et al, 1990). Also, transient interictal spikes are found on the EEG recordings in the amygdala and hippocampus in both the kindling model and in human complex partial epilepsy. The variability in the frequency of these interictal spikes is another commonality. In humans with seizures of equivalent severity, the frequency of interictal spikes can vary up to 100 fold. Likewise, rats kindled to equivalent behavioural stages of seizure activity, show a similar variability in the frequency of these interictal spikes (Sato et al, 1990). Also, the frequency of interictal spikes increases following a seizure (Fitz and McNamara, 1979) or during slow wave sleep (Sato and Nakashima, 1976) in both the kindling model and in human complex partial epilepsy.

The kindling model and human temporal lobe epilepsy also show similar drug

effects. For example, the anticonvulsant drugs used to treat human complex partial seizures, including phenobarbital, carbamazepine, valproate and diazepam are all effective in suppressing amygdaloid kindled seizures at doses with minimal or no behavioural side effects (Wada and Osawa, 1976, Albright and Burnham, 1980). Conversely, ethosuximide, an anticonvulsant that is only effective against human absence seizures and not complex partial seizures is similarly ineffective in suppressing kindled seizures.

The question of whether kindling occurs in humans is partially answered by some experimental and clinical observations. First of all, kindling has been demonstrated in many species including frogs (Morrell and Tsuru, 1976), mice (Leech and McIntyre, 1976), rabbits (Whieldon and Van Harreveld, 1950), dogs (Watanabe, 1936), cats (Alonso-De Florida and Delgado, 1958, Wada and Sato, 1974), monkeys (Wada, Mizoguchi and Osawa, 1978) and baboons (Wada and Osawa, 1976) and thus it is highly likely that it also occurs in humans. Clinically, Perrin and Hoffman (1979), in a retrospective study of surgically treated patients with temporal lobe epilepsy (TLE), found that the longer the interval from first seizure to removal of the temporal lobe focus, the less favourable the result. This suggests an evolving seizure disorder as seen with kindling. Similarly, Hughlings Jackson (1931) described a kindling-like progression of untreated human epilepsy whereby each seizure was progressively more intense. Thus, it seems reasonable that an understanding of the kindling phenomenon could help elucidate the mechanisms of epilepsy and the effects of seizures on the brain.

Seizure circuitry

One of main questions to be answered in epilepsy research is, what is the nature of the 'seizure circuitry'? This rather general question opens up many different avenues of research. One can look at the anatomical substrate of epileptic discharge, the changes in connectivity or the changes in cellular reactivity. Research into the anatomical substrate has focused on the seizure susceptibility of different brain sites and on the propagation patterns of seizure discharge. It has been found that different sites kindle at different rates. The olfactory bulb and its major target site, the piriform cortex (Cain, 1977; Cain, Corcoran, Desborough and McKitrick, 1988), require very few kindling stimulations to develop fully generalized seizures whereas the hippocampus kindles relatively slowly. Perhaps the most reactive structure is the perirhinal cortex, which can kindle in as few as 3 stimulations (McIntyre, Kelly and Armstrong, 1993). Sato et al (1990) suggested that the differences in kindling rates between these sites could be due to: 1) differences in their connections to the motor systems responsible for driving the convulsive response, 2) differences in their connections to other forebrain areas responsible for augmenting the seizure discharge, or 3) differences in the reactivity of the stimulation sites themselves. Evidence suggests that all 3 theories may be correct. For example, the amygdala/piriform area, a fast kindling area, has relatively strong connections with motor systems (Ben-Ari, 1985). The rapid growth and propagation of the forebrain discharge, even before the onset of motor seizures, indicates that these systems have inherently reactive cells.

It is difficult to determine whether there is a common seizure circuit by examining

propagation patterns. This is because the epileptiform discharge propagates so widely within the forebrain that it is impossible to determine the relative importance of each structure. Within the brainstem, at least, there is a good positive correlation between the amplitude of the epileptiform spikes recorded there and the appearance of convulsive responses (Wada and Sato, 1974; Racine, 1978).

The piriform cortex and/or perirhinal cortex appear to be important structures in epileptogenesis. Tseng and Haberly (1988) found cells with regenerative depolarizing potentials in layer III of the piriform cortex that could serve as pacemakers for spontaneous discharge. Similar burst-mode cells have been found in perirhinal cortex (McIntyre, Kelly and Armstrong, 1993). Also, the piriform cortex begins to generate spontaneous interictal spikes, an index of increasing epileptogenesis, early in the kindling process, even when it is not the site being kindled (Kairiss, Racine and Smith, 1984). Other structures are also capable of generating these spikes and their order of reactivity after the piriform and perirhinal cortices is the amygdala, entorhinal cortex, ventral hippocampus, septal area and dorsal hippocampus (Racine, Mosher and Kairiss, 1988). Wada and his colleagues distinguished between dependent and independent interical spikes according to their dependence on the spikes recorded in the kindled brain site itself. The independent spikes, a marker of secondary functional change due to primary site kindling, appeared in all limbic structures and in the midbrain reticular formation after the development of generalized seizures (Wada and Sato, 1975; Wada, Mizoguchi and Osawa, 1978; Sato, 1976). A similar result was found using the 2-deoxyglucose

technique which allowed monitoring of metabolic activity of kindled brains during seizures. The amygdala/piriform region appears to be quite active during the early stages of kindling. After seizure generalization, additional brain sites, including the substantia nigra, become active (Engel, Wolfson and Brown, 1978; Ackermann, Chungani, Handforth, Moshe, Caldecott-Hazard and Engel, 1986).

Kairiss, Racine and Smith (1984), using a tissue slice preparation tested hippocampal slices to determine whether kindled slices were inherently more reactive than control slices. They found no difference between kindled animals and controls except for a slight increased tendency for some slices from kindled animals to show burst responses in high potassium medium. McIntyre and Wong (1986), on the other hand, found burst responses generated by stimulation were much larger in kindled animals as compared to control animals when they tested amygdala/piriform slices. This suggests that the inherent excitability of the hippocampus is not as dramatically altered as a result of kindling as it is in the amygdala/piriform area.

The effects of lesions and knife cuts on kindling rates is controversial. Racine, Paxinos, Mosher and Kairiss (1988) were not able to block kindling with any of several targeted forebrain site lesions. Knife cuts which disrupted communication between the amygdala and hippocampus actually facilitated kindling (Racine, Paxinos, Mosher and Kairiss, 1988). Savage, Rigsbee and McNamara (1985), on the other hand, found that similar but more posterior knife cuts resulted in a large increase in the number of stimulations required to kindle the amygdala .

An antagonistic interaction between the amygdala and hippocampus has been found consistently. For example, the first appearance of motor convulsions during hippocampal kindling often occurs after an obvious depression in the hippocampal EEG and a maximal enhancement of amygdala spiking (Racine, 1972a,b; Racine, Rose and Burnham, 1977). Similarly, McIntyre and Kelly (1993) have demonstrated that stimulation of the hippocampus was capable of generating an extended AD and first-trial motor convulsions if the basolateral amygdala was first suppressed by massed stimulation. As the amygdala recovered, the hippocampus lost its ability to generate extended ADs and motor convulsions. Burdette and Dichter (1988) reported that simultaneous dorsal hippocampal stimulation depressed both the convulsive and electrographic response triggered by amygdala stimulation in amygdala kindled animals. If, however, the hippocampal stimulation was delayed by a few seconds, they found an augmented amygdala response.

Altered connectivity such as an increase in excitatory connections or a decrease in inhibitory connections could also contribute to the organization of seizure circuitry. An example of an increase in excitatory connections was eluded to earlier in this chapter when we discussed kindling induced potentiation. KIP was described as a relatively permanent enhancement of the evoked response which resulted from kindling (de Jonge and Racine, 1987) of many sites including the amygdala (Racine, Newberry and Burnham, 1975), hippocampus (Douglas and Goddard, 1975) and piriform cortex (Racine, Milgram and Hafner., 1983). KIP, however, is an unlikely primary kindling mechanism because kindling can, apparently, occur without producing a potentation

effect (Giacchino, Somjen, Frush and McNamara, 1984). Examples of altered inhibition will be discussed in more detail later in this chapter. Briefly, in the CA1 area of the hippocampus inhibition is consistently decreased following kindling (Kapur, Michelson, Buterbaugh and Lothman, 1989; Kapur and Lothman, 1989; Kamphuis and Lopes da Silva, 1990; Kamphuis, Huisman, Veerman and Lopes da Silva, 1991) but in the dentate gyrus, inhibition is increased (King, Dingledine, Giacchino and McNamara, 1985; Oliver and Miller, 1985; Tuff, Racine and Adamec, 1983).

Kindling agents

Animals can be kindled using a variety of epileptogenic agents including electrical stimulation and certain convulsant drugs. The latter may be applied either directly to the brain or given systemically. Electrical stimulation typically consists of a 1 second train of biphasic pulses at a frequency of 60 hertz at an intensity large enough to elicit an afterdischarge. Many of the chemical agents used to induce seizures do so by mimicking neurotransmitters in the brain. There are a number of neurotransmitters that have been implicated in epileptogenesis. Among the inhibitory neurotransmitters, GABA-ergic systems are the most widely studied. The most important excitatory neurotransmitters, in this respect, are glutamic acid and to a lesser extent aspartic acid (Sherwin, Robinbaille, Quesney, Olivier, Villemure, Leblanc, Feindel, Andermann, Gotman, Andermann, Ethier and Kish, 1988). Therefore, the application of any agonist of the excitatory neurotransmitters or any antagonist of the inhibitory neurotransmitter system has the

potential to cause seizures. For example, the cholinomimetics carbachol (Goddard, McIntyre and Leech, 1969) and pilocarpine (Turski, Cavalheiro, Schwartz, Czuczwar, Kleinrok and Turski, 1983), and the excitatory amino acids glutamate, kainate (Bradford & Dodd, 1977; Croucher & Bradford, 1989), and aspartate (Bradford & Dodd, 1977; Croucher & Bradford, 1989), cause convulsions by affecting excitatory systems. On the other hand, the proconvulsive effects of bicuculline, picrotoxin, and pentylenetetrazol (Masson & Cooper, 1972; Pinel & Cheung, 1977; Corda, Orlandi, Leeca, Carboni, Frau and Giorgi, 1991; Barkai, Grossman and Gutnick, 1994) , are mediated via a persistent reduction of the GABAa receptor function. Similarly, it has been thought that the proconvulsive effects of lindane and endosulfan, two pesticides, act by decreasing binding of GABA to receptors (Gilbert and Mack, 1995; Gilbert, 1992). N-methyl-beta carboline-3-carboxamide (FG 7142) has also been found to affect the inhibitory system by decreasing the functioning of GABA regulated chloride channels (Lewin, Peris, Bleck, Zahniser and Harris, 1989). For heavy metals such as alumina gel and cobalt (Van Gelder and Courtois, 1972), anatomical evidence indicates that GABAergic neurons and terminals degenerate in these chronic models of epilepsy. Other convulsive agents that have been used in kindling experiments include electroconvulsive shock (Ramer and Pinel, 1976), chlordimeform (Pfister, Noland, Lowy, Nichols and Yim, 1978), cocaine (Kilbey, Ellinwood and Easler, 1979; Post and Kopanda, 1976), fluorothyl (Pritchard, Gallagher and Glaser, 1969), and lidocaine (Post, Kopanda and Lee, 1975).

Transfer kindling

Earlier in this chapter, we discussed seizure circuitry in terms of propagation patterns of epileptiform discharge to other sites and the eventual culmination of fully generalized seizures. We know that the neural reorganization that occurs during kindling likely extends far beyond the actual site of stimulation. The most compelling evidence for this conclusion is the "transfer kindling" phenomenon. After generalized convulsions have been evoked from kindling one site, other brain sites also show a greatly enhanced potential for triggering seizures. This effect was first described by Goddard, McIntyre and Leech (1969) when they demonstrated that kindling the amygdala leads to facilitation of subsequent rekindling of the contralateral amygdala or ipsilateral septum (Goddard et al, 1969). The facilitated kindling of secondary sites is found even after the primary kindled site has been destroyed (Racine, 1972a,b).

Transfer appears to be a general phenomenon, found throughout the limbic system and not restricted to specific subsystems, structures or pathways (Burnham, 1976). Numerous studies have demonstrated positive transfer in a variety of limbic and cortical sites (Racine, 1972b, Racine, 1975; Cain, 1985) using a variety of species including primates (Wada and Osawa, 1976; Wada, Mizoguchi and Osawa, 1978). Burnham (1976) carried out a preliminary mapping study of transfer effects and found that transfer occurred between all limbic sites tested including the amygdala, septal area, dorsal hippocampus and ventral hippocampus regardless of which structure served as primary or secondary site. There was a tendency for more secondary site stimulations to be required

if the secondary site normally kindled more slowly than did the primary site. Consequently, the dorsal hippocampus showed only a 35.7% savings in kindling rate after amygdala kindling, but the savings were significant.

From a clinical perspective, understanding the transfer phenomenon may help us understand the mechanism underlying the development of secondary epileptogenesis and routes of preferred propagation. The transfer effect also raises the possibility that, in humans, increased epileptogenic susceptibility to one kindling agent or treatment might imply increased epileptogenic susceptibility to other agents or treatments (Cain, 1979). For example, a person who had previously been treated with electroconvulsive shock (ECS) for depression may have an increased susceptibility to seizures upon withdrawl from alcohol (Cain, 1985). Pinel (1980) tested this hypothesis in rats and found that indeed rats that were repeatedly given ECS gradually kindled and were significantly more susceptible to seizures upon withdrawl from alcohol exposure. The clinical relevance of kindling and the transfer phenomenon is also supported by the fact that all species tested to date, from reptile to baboon, have been successfully kindled, and all species tested to date, from rat to baboon, have demonstrated transfer (Cain, 1985).

The transfer phenomenon may also help elucidate seizure circuitry in terms of whether unique temporo-spatial patterns of activation are specific to the primary site generators and whether these patterns are important for determining the subsequent propagation of the seizure discharge. The current view of seizure discharge propagation is that it follows 'preferred' pathways. This general notion leaves a broad range of

possibilities. At one extreme, this could mean that there is complete activation of whole structures with propagation between structures occurring over fixed pathways. At the other extreme, this could mean that there is partial and more distributed and modifiable activation within structures with propagation occurring between structures over modifiable pathways.

Transfer mechanisms

Three different mechanisms have been proposed to explain the transfer effect. The first is that AD's triggered in secondary sites activate the primary site via afferent bombardment. There are two pieces of evidence that make this mechanism unlikely. Racine (1972b) included lesion control groups in the transfer experiments. After amygdaloid kindling, the stimulated site was destroyed by an electrolytic lesion. Subsequent tests of secondary site kindling showed no disruption of the transfer effect. Similarly, researchers studying transhemispheric transfer effects in rats with lesions of the interhemispheric commissure demonstrated normal or even accelerated transfer of forebrain kindling (McCaughran, Corcoran and Wada, 1976, 1977, 1978; McIntyre, 1976). Both these findings clearly indicate that transfer does not depend on activation of primary site by the transfer site.

The second mechanism proposed is that transfer sites eventually become activated by propagated discharge during primary kindling and then proceed to kindle as if they were stimulated directly. Burnham (1976) found that the number of apparently reactive

discharges in secondary sites was inversely proportional to their transfer kindling rates. This is consistent with the idea of independent kindling of the transfer site during primary kindling.

The third proposed mechanism states that transfer depends on neural changes within a common midbrain or brainstem target site during primary kindling (Cain, 1985). There are 3 observations which are consistent with this mechanism. The first observation was made by Wada and his colleagues who demonstrated that strong independent spike discharge occurs in the midbrain and brainstem reticular areas concommitantly with the development of generalized convulsive activity during kindling in cats and baboons (Wada and Osawa, 1976; Wada and Sato, 1974,1975; Wada, Sato and McCaughran, 1975). The second observation is that kindling different sites results in convulsions with similar form which suggests a common brainstem generalization mechanism (Racine and Burnham, 1984). The final observation was that the duration and strength of transfer convulsions were often mature from their first appearance (Burnham, 1976).

Based on these and similar data, Racine and Burnham (1984) proposed a model of kindled seizure generalization which also provided a basis for a model of transfer. Their model proposed that the occurrence of major motor seizures depended on the recruitment of and neuroanatomical convergence of, sufficient forebrain epileptiform discharge into midbrain and brainstem reticular areas to drive a generalized convulsion. With the addition of epileptogenic changes within either or both forebrain and brainstem sites, a mechanism is provided for transfer kindling.

Changes within forebrain sites would ensure critical mass in forebrain recruitment, while change in brainstem targets could ensure enhanced activation regardless of the forebrain source. Whether the brainstem is passively driven by forebrain afferents, or actively participates in the amplification of the seizure response remains to be determined. The transfer phenomenon may involve some combination of the mechanisms described above. For example, the epileptiform discharge that emanates from the secondary site might utilize forebrain seizure circuits established by primary site kindling, even though the primary site itself is no longer critical (Racine, 1972a,b).

Kindling Antagonism

Applegate and Burchfiel's kindling antagonism model, in which alternating stimulation between 2 sites results in a blockade of the progression of kindling from one of the two sites, suggests that there are complex interactions involved in seizure propagation (Applegate, Burchfiel and Konkol, 1986; Applegate, Konkol and Burchfiel, 1987; Burchfiel and Applegate, 1990). They proposed that the architecture of kindling is a discontinuous process and involves the sequential expression of qualitatively distinct mechanisms. Seizure development in animals stimulated in the suppressed site is arrested either at stage 1-2 or at stage 3. Each animal subjected to the alternating, two-site stimulation paradigm will exhibit one of these two classes of behaviour or no antagonism at all. They interpreted this to mean that the kindling antagonism phenomenon defines two critical transitions in the process of kindled seizure development. The first is the

transition from stage 1 and 2 behaviour to stage 3, and the second is from stage 3 to stage 4 and 5. This suggests that these transition points represent major steps in the kindling progression at which some discrete process of neural reorganization must take place before seizure development can proceed to the next phase of generalization.

Kirkby, Gilbert, Westcott and Corcoran (1995) examined the assumption that kindling antagonism reflects an arrest of kindling at an intermediate stage at the suppressed site rather than a transient inhibition of seizures. They found that a 30 day stimulation free period imposed after the completion of kindling antagonism, failed to result in immediate generalization of seizures in the suppressed site. They also found, however, some savings following the rest period. The animals only required half the number of stimulations in the suppressed site for the expression of generalized seizures as compared to rats receiving stimulation immediately after the establishment of antagonism. This suggests both an arrested kindling as well as a superimposed transient component to the antagonism effect.

The development of generalized seizures in a secondary kindling site was virtually identical in rats previously stimulated in a primary site and in rats expressing antagonism between two stimulated sites. However, the number of ADs required to kindle the secondary site was less than kindling the primary site in both groups. That is, the transfer effect was equivalent in the single site and dual site rats (Kirkby et al, 1995).

In this thesis, the transfer phenomenon was studied in two monosynaptic systems, the entorhinal cortex-perforant path-dentate gyrus system (EC-PP-DG) and the olfactory

bulb-lateral olfactory tract-piriform cortex system (OB-LOT-PC). It was thought that if the transfer site was monosynaptically and strongly connected to the primary site, then it should propagate a similar pattern of discharge and fully kindle in response to the original primary kindling. Consequently, transfer kindling should be rapid or immediate. Some pilot studies that have looked at this question have suggested that the mechanism is much more complex. For example, L. Tuff (unpublished data) looked at transfer kindling in the dentate gyrus immediately following primary kindling of the perforant path in a small group of animals and found no significant transfer effect. M. Pepkowski, in another pilot study waited 2 weeks before he transfer kindled in the dentate gyrus following primary kindling of the perforant path. He found a barely significant transfer effect after this 2 week delay.

Inhibition

We may assume that seizure circuitry depends upon changing the balance of excitation and inhibition, resulting in an increase in excitatory drive. Failure of inhibition is one mechanism widely proposed by epilepsy researchers to explain most of the features of epileptogenesis (Cornish and Wheal, 1989; Morimoto, 1989; Sloviter, 1987). Recurrent and feed forward inhibition can be measured in the intact preparation using the paired-pulse method. With this technique, the first, or conditioning pulse, triggers cell discharge if the intensity is above threshold. The second, or test pulse, will then trigger a depressed response, providing it is applied during the period of recurrent or feed forward

inhibition. Recurrent inhibition is produced when the discharging neurons activate local inhibitory interneurons. Feedforward inhibition results from the direct activation of inhibitory interneurons.

One site that has consistently shown a kindling-induced decrease in paired-pulse depression is area CA1 of the hippocampus (Kapur, Michelson, Buterbaugh and Lothman, 1989; Kapur and Lothman, 1989; Kamphuis and Lopes da Silva, 1990; Kamphuis, Huisman, Veerman and Lopes da Silva, 1991). Kamphuis et al (1991) found increased GABA exocytosis in CA1 following kindling even though paired-pulse inhibition was decreased. Therefore, they suggested that the chronic enhanced seizure susceptibility must depend upon changes occuring at the level of the GABA receptor complex.

Sloviter (1983,1987, 1991), using an electrically induced *status epilepticus model*, found a persistent loss of recurrent inhibition in the dentate gyrus following an episode of status epilepticus. Milgram, Yearwood, Khurgel, Ivy and Racine (1991), controlling the duration of time the animal was allowed to remain in *status epilepticus*, found a transient loss of inhibition but this was generally replaced by an enhanced inhibition within 24 hours. The enhanced levels of inhibition lasted for at least 1 month. Sloviter (1992) also used a kainic acid induced status epilepticus model and found decreased granule cell inhibition which was restored when he tested the animals 2 months later.

With *kindling*, an increase in paired-pulse depression of the population spike was found in the dentate gyrus (Tuff, Racine and Adamec, 1983; de Jonge and Racine, 1987; Milgram, Michael, Cammisuli, Head, Ferbinteanu, Reid, Murphy and Racine, 1995). The

increases in the recurrent and feedforward phases of inhibition were apparent after the first AD (de Jonge and Racine, 1987) while the increase in the late component of the depression probably reflecting late afterhyperpolarizations, did not develop until about 10 ADs had been elicited (de Jonge and Racine, 1987). The paired-pulse inhibition remained increased until the kindling stimulations were stopped even in animals given 300 kindling stimulations or in animals that developed spontaneous seizures (Milgram et al, 1995). The inhibition then gradually returned to baseline levels, with the late component decaying more rapidly than the early component. It has been suggested that the increased inhibition in the dentate gyrus (DG) reflects a compensatory mechanism to suppress excessive input to the CA3 and thus slow down the kindling process (Kamphuis & Lopes da Silva, 1990). It seems more likely that it reflects an inherent activation-dependent plasticity in inhibitory systems that subserves more normal processes. Epileptiform events are relatively rare in most species.

In the piriform cortex, the paired-pulse procedure normally produces a net facilitation and this facilitation decreases following kindling but returns to baseline levels about 3 months after the discontinuation of kindling stimulations (Racine, Moore and Evans, 1991). A decrease in paired-pulse facilitation is interpreted to reflect an increase in inhibition rather than a decrease in excitation. While it is conceivable that the decrease in paired-pulse facilitation could be due to a depression of facilitation itself, rather than an increase in inhibition, Racine (unpublished observations) has found that GABA agonists produced a decrease in paired-pulse facilitation that is almost identical to that produced

by kindling. In the dentate gyrus, where a clear net depression effect is seen in paired-pulse tests, GABA agonists produce an enhancement of inhibition, that is also similar to that seen with kindling. Figure 1.1 shows the alterations in the paired pulse curves when recording in the piriform cortex and the dentate gyrus following the administration of GABA agonists.

Another electrophysiological measure appeared to confirm a failure of the inhibitory system in the amygdala and hippocampus. Morimoto (1989) investigated seizure triggering mechanisms using the kindling model and found three major components of response during and following high frequency stimulation: an initial single-evoked potential, a subsequent EEG suppression and an eventual rhythmic synchronous discharge. The EEG suppression was sensitive to pharmacological manipulations of the GABAa/benzodiazepine system such that muscimol, a selective GABA receptor agonist, prolonged the duration of the suppression phase. They hypothesized that the essential seizure triggering events included the collapse of

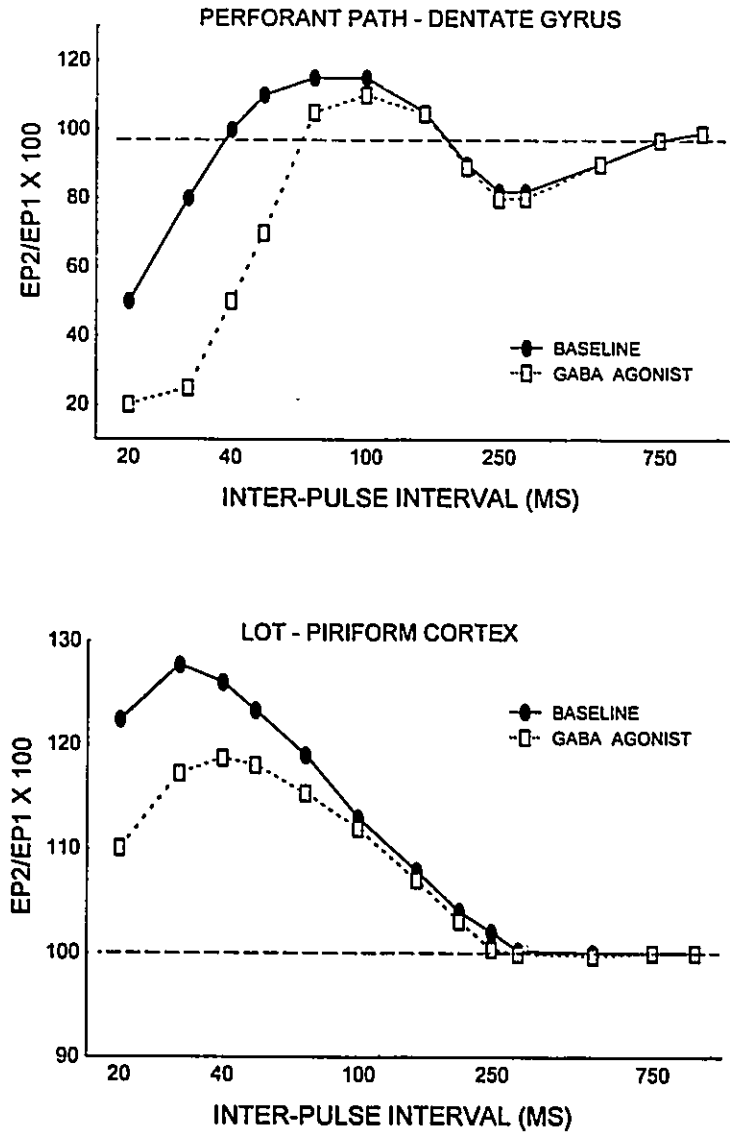


Fig. 1.1. This figure demonstrates the effect of GABA agonists on the paired-pulse curves in both the dentate gyrus (top panel) and the piriform cortex. In the dentate gyrus, GABA agonists increase the inhibition to the same extent as is seen with kindling. In the piriform cortex, GABA agonists decrease the normal paired-pulse facilitation, which is similar to the effects seen during kindling (Racine, unpublished data).

GABAa- mediated inhibition and the concomitant activation of the NMDA receptor complex.

Cell Loss

One variable that could be contributing to the determination of seizure circuitry is cell loss. An increase in excitation would be expected, for example, if inhibitory cells were damaged or if the damage induced sprouting in excitatory pathways. Early research on the brains of kindled animals reported no cell loss (Goddard, McIntyre and Leech, 1969), no gliosis (Brotchi, Tanaka and Leviel, 1978) and no changes in dendritic architecture (Crandall, Bernstein, Boast and Zornetzer, 1979). However, more recently, the picture is not quite so clear. For models of status epilepticus, it is clear that one episode of status can result in hippocampal cell loss (Milgram, Yearwood, Khurgel, Ivy and Racine, 1991; Sloviter, 1992; Bertram & Lothman, 1993), but there are conflicting reports about whether cell loss occurs during kindling.

On the one hand, numerous studies have reported cell loss in the hilus of the dentate gyrus (Cavazos and Sutula, 1990; Cavazos, Das and Sutula, 1994), CA1 and CA3 (Cavazos and Sutula,1990; Cavazos, Das and Sutula, 1994; Kamphuis, Wadman, Buijs and Lopes da Silva, 1986) following kindling. The hilar cells and the CA1 pyramidal cells seem to be the most sensitive to damage as cell loss was reported in these areas after only 3 stage 5 seizures (Cavazos, Das and Sutula, 1994). Cell loss in the CA3, entorhinal cortex and rostral endopiriform nucleus was evident only after 30 stage 5 seizures. After

150 stage 5 seizures, cell loss was reported in the granule cell layer and CA2.

Several studies examining the morphological changes associated with human temporal lobe epilepsy have demonstrated selective cell loss of pyramidal cells in the CA1 and CA3 fields and the polymorphic cells of the dentate gyrus, with relative sparing of the dentate granule cells and pyramidal cells of CA2 (Houser, 1992; de Lanerolle, Kim, Robbins and Spencer, 1989). de Lanerolle et al (1989) determined that there was a selective loss of somatostatin and neuropeptide Y immunoreactive interneurons. Sloviter (1991) found a similar loss of somatostatin/neuropeptide Y immunoreactive interneurons in an experimental model of status.

On the other hand, Bertram and Lothman (1993) found a decrease in the absolute hilar cell counts following an episode of status epilepticus but no cell loss following kindling. They did find, however, an increase in the area of the dentate gyrus following kindling which was the result of an expansion of the molecular layer and the hilus. Thus, they suggested that the reports of cell loss in the literature may be due to decreased cell densities rather than to actual loss of neurons. Khurgel, Switzer, Teskey, Spiller, Racine and Ivy (1995), using a cupric silver stain which is sensitive to neuronal degeneration, found no degenerative changes specific to the kindling process. Thus, they suggested that neuronal loss was not a prerequisite for epileptogenesis. Adams et al (personal communication) found that animals given nerve growth factor (NGF) during amygdala kindling had hilar cell counts similar to the implanted controls while the amygdala kindled group without NGF showed a decrease in hilar cell density. Both the NGF and

the nonNGF groups still kindled to fully generalized seizures but the NGF group kindled faster than the non NGF group. Thus, NGF appears to provide a protective factor to the hilar cells (or whatever is leading to the decreased density measures) without interfering with the kindling process.

Sprouting

Recently, there has been much interest in the phenomenon of sprouting of the mossy fibers of the dentate gyrus granule cells and its role in the determination of seizure circuitry. In kainic acid-induced status models, it is thought that destruction of pyramidal cells in CA3/CA4 (Ben-Ari, 1985; Nadler, Perry and Cotman, 1978) and of dentate hilar neurons (Sloviter, 1992) induces sprouting of the mossy fibers of the dentate granule cells. These fibers cross the granule cell layer into the inner molecular layer of the granule cell dendrites (Sundstrom, Mitchell and Wheal, 1993; Cronin and Dudek, 1988). Similarly in human temporal lobe epilepsy, there have been reports of moderate to severe loss of neurons in the hilus, CA1 and CA3 (Bruton, 1988) with associated sprouting of the mossy fibers into the supragranular layer of the dentate gyrus (Sutula, Cascino, Cavazos, Parada and Ramirez, 1989; Masukawa, Uruno, Sperling, O'Connor and Burdette, 1992). In kindling models, researchers have found that the sprouting mossy fibers appear to form novel synapses with the basilar dendrites of the CA3 pyramidal neurons (Represa and Ben-Ari, 1992) and to form a plexus in the inner molecular layer of the granule cell dendrites (Cavazos, Golarai and Sutula, 1991; Sutula, Xiao-Xian,

Cavazos and Scott, 1988). Noebels and his colleagues, using a mutant mouse with inherited spike-wave seizures, found that mossy fiber axonal sprouting into the supragranular layer of the dentate gyrus followed the onset of hypersynchronous discharge but occurred prior to any observable cell loss (Qiao and Noebels, 1993; Chafetz, Nahm and Noebels, 1995). As is evident from the literature, the issue of what constitutes a necessary and sufficient stimulus to induce mossy fiber sprouting has yet to be resolved. It is unclear whether a hippocampal lesion is a necessary stimulus for mossy fiber axonal sprouting or whether the hypersynchronous activity associated with seizures is sufficient to induce the sprouting.

The functional significance of mossy fiber sprouting and its role in epileptogenesis has yet to be defined. Sutula and his colleagues (Sutula, Xiao-Xian, Cavazos and Scott,1988; Cavazos, Golarai and Sutula, 1991) found alterations in the circuitry early in the course of kindling, before the development of generalized seizures. He suggested that the abnormal synchronous activity induces the structural reorganization that promotes epileptogenesis. This hypothesis assumes that the fibers form the majority of their synapses with other excitatory granule cells, thus enhancing recurrent excitation and producing a permanent hyperexcitability.

However, another possibility in keeping with recent studies which have demonstrated an *increase* in recurrent inhibition in the dentate gyrus of kindled rats (Tuff, Racine and Adamec, 1983) is that the sprouted mossy fibers may form synapses mainly with the aspinous dendrites of inhibitory, GABAergic basket cells (Seress and Ribak, 1983; Ribak

and Seress, 1983). Sloviter (1992) also suggested that the effect of mossy fiber sprouting was primarily to increase inhibition. In kainic acid treated animals, he found a decreased hilar cell count, a decreased granule cell inhibition and an increased granule cell excitability which preceded synaptic reorganization. Using immunocytochemical staining techniques, Sloviter (1987) determined that the GABA containing interneurons thought to mediate inhibition in the dentate gyrus were not damaged in this status epilepticus model but that there was almost a complete loss of somatostatin-containing interneurons and mossy cells. The mossy cells which are monosynaptically activated by both granule cells and perforant path stimulation (Scharfman and Schwartzkroin, 1988, 1990) excite inhibitory cells (Scharfman, 1995) and thus may initiate or amplify recurrent and feedforward inhibition. Thus, the loss of the mossy cells which normally excite GABA-containing basket cells of the dentate could explain the seizure-associated loss of inhibition that he had found. Sloviter (1992) also found that granule cell recurrent inhibition was restored or even increased within 2 months of kainic acid treatment. Sloviter suggested that the cell loss was the stimulus for mossy fiber sprouting and that granule cell sprouting resulted in innervation of inhibitory neurons concomitant with the restoration of inhibition and normal excitability. However, evidence against an inhibitory explanation of the functional significance of sprouting is the fact that the increased inhibition found in the dentate gyrus returns to baseline levels following discontinuation of kindling stimulations (Chapter 4: Spiller and Racine, 1994) even though evidence of sprouting has been observed as long as 8 months after the last evoked

seizure (Cavazos, Golarai & Sutula, 1991).

Experimental Rationale

A number of variables contributing to the determination of seizure circuitry have been described. In this thesis, we will focus on a selection of these. We looked at the transfer phenomenon in two monosynaptic pathways because it was thought that if the transfer site was monosynaptically, and strongly connected to the primary site, then it should propagate a similar pattern of discharge and partially or fully kindle in response to primary kindling. However, pilot data from our lab indicated that the entorhinal cortex/dentate gyrus monosynaptic system did not respond as expected. The role of inhibition in this system remains somewhat controversial, as well. In particular, there was reason to predict that extended kindling might compromise inhibitory systems, because it had been reported to produce a more massive loss of hilar neurons. Altered connectivity such as a decrease in inhibitory influences resulting in excess excitation have been implicated in the development of seizure circuitry. Therefore, we also looked at paired-pulse inhibition in the dentate gyrus and piriform cortex throughout the extended kindling process and for weeks after the discontinuation of kindling stimulations to monitor the effects of kindling on inhibition. Structural changes such as cell loss or mossy fiber sprouting could also contribute to development of seizure circuitry. Therefore, we looked at cell loss in the hilus of the dentate gyrus, using Cresyl Violet stain and sprouting of mossy fibers into the IML of the granule cells and into the CA3, using Timm sulphide-

silver method. Cell counts and Timm granule measures were taken after the induction of 4 stage 5 seizures or after extended kindling. The monosynaptic pathways that we studied were the EC-PP-DG pathway and the OB-LOT-PC pathway. The final section of this introduction will describe the anatomy and physiology relevant to these two pathways.

Anatomy and Physiology

Anatomy of the hippocampus/parahippocampus

The hippocampal formation can be divided into the Ammon's horn and the dentate gyrus. The Ammon's horn or 'hippocampus proper' refers to the area containing the pyramidal cells whereas the dentate gyrus refers to the area containing the granule cells. The parahippocampus will refer to a group of structures that are tightly coupled functionally with the hippocampal formation. These structures include the subiculum, presubiculum, parasubiculum and entorhinal cortex. Only the entorhinal cortex will be described in detail.

Dentate gyrus

In a coronal section, the dentate gyrus can be recognized by a relatively narrow band of small to medium sized, densely packed granule cells. The dentate gyrus consists of three zones of which the granule cell layer is the middle zone. The molecular layer contains the granule cell dendrites and in this thesis will be further broken down into the inner molecular layer (IML), the middle molecular layer (MML) and the outer molecular

layer (OML). The third zone, the area between the upper and lower blades of the granule cell layer is the called the polymorph layer in the hilus. It contains the granule cell axons (the mossy fibers) which project to Ammon's horn and a variety of neurons. The polymorphic cells vary from medium sized stellate and pyramidal shaped basket cells to very large mossy cells (Amaral, 1978). The basket cells lie just inside the granule cell layer and project into it (Amaral, 1978). Immunocytochemistry reveals that some cells contain GABA, cholecystokinin (CCK), vasoactive interstitial polypeptide (VIP) or somatostatin alone (Bakst, Morrison and Amaral, 1985) or in combination. Sloviter and Nilaver (1987) also suggested that some groups of cells may contain glutamate as the primary neurotransmitter.

Ammon's horn

Ammon's horn is characterized by a single layer of pyramidal cells that begins at the hilus of the dentate gyrus and subsequently curves out and above the dentate gyrus and ends distally in the subiculum. The primary neuron of Ammon's horn is the pyramidal cell, which has a medium to large cell body, a large apical dendrite, a number of shorter basal dendrites at the opposite pole and a single axon exiting from the basal portion. Subregions of Ammon's horn include areas CA1, CA2 and CA3. Area CA1 is the most distant from the dentate hilus and extends into the subiculum. Area CA2 is found between CA1 and CA3 and can be distinguished from CA3 by the absence of dendritic spines on the proximal apical dendrites of the CA2 pyramidal cells. It can also be

distinguished from CA3 using Timm's stain as the area distal to the termination of the mossy fibers which only terminate in area CA3. Large basket cells (interneurons) are also positioned in the pyramidal cell layer. On either side of the pyramidal cell layer are the regions which contain the basal and apical dendrites of the pyramidal cells. The region with the basal dendrites is known as the stratum oriens, and the region with the apical dendrites is divided into the following layers (from proximal to distal): the stratum lucidum, the stratum radiatum and the stratum moleculare-lacunosum. A number of different types of interneurons are found in these two regions and include triangular, fusiform, and stellate cells which may contain GABA, somatostatin, CCK or VIP (Sloviter and Nilaver, 1987; Bakst, Morrison and Amaral, 1985).

Entorhinal Cortex

The entorhinal cortex has 6 cell layers and can be divided into medial and lateral subfields based on cytoarchitectural differences as well as on differences in efferent and afferent projections (Amaral, 1987). Cell layers I-VI of the entorhinal cortex are as follows: (I) superficial plexiform or molecular layer; (II) superficial cell layer which contains stellate and small pyramidal cells; (III) a wide layer which contains medium sized neurons; (IV) a cell sparse zone, rich in myelinated fibers; (V) a layer of decreasing density and cell size; (VI) a layer of neurons with variable morphology. There are two main cytoarchitectural differences between the medial (MEC) and lateral (LEC) entorhinal subfields. First of all, layer II of the LEC has clusters of densely packed

neurons while layer II of the MEC has larger, less tightly distributed neurons. Secondly, MEC has more distinct deep cell layers than does LEC. Differences in connectivity will be discussed later.

Internal Connections

The hippocampal formation has been described as a series of parallel lamella oriented at right angles to the longitudinal axis of the hippocampus. Each of the lamella contain a very similar neural circuitry known as the 'trisynaptic pathway' (Anderson, Bliss and Skede, 1971). This circuit begins with neurons in the entorhinal cortex. These cells send axons to the molecular layer of the dentate gyrus by means of the perforant path (Ramon y Cajal, 1968; Witter, Groenewegen, Lopes da Silva and Lohman, 1989). Two distinct branches of the perforant path have been identified. One crosses the hippocampal fissure to enter the dentate gyrus (the temporodentate pathway) while the other stays within Ammon's horn, making contact with the pyramidal cells in CA3 and CA1 (the temporoammonic pathway). The temporodentate pathway gives rise to the first synapse of the trisynaptic pathway by exciting granule cells which then potently excite the proximal apical dendrites of the CA3 pyramidal cells via the mossy fibers (the second synapse of the trisynaptic pathway). Subsequently, the CA3 neurons excite the CA1 pyramidal neurons via the Schaeffer's collaterals (the third synapse of the trisynaptic pathway). Fig. 1.2. shows the internal circuitry of the hippocampal lamella.

It was initally thought that CA1 pyramidal neurons form the major output for the

hippocampus, sending axons via the fimbria and fornix. However, more recently, studies have shown this view to be overly simplified. As was discussed earlier, each lamella contains a full trisynaptic circuit but contrary to earlier beliefs, these lamella are not physiologically separate with little interaction between lamellae along the septo-temporal axis. Rather, longitudinal associational connections, originating from the hilar mossy cells or from CA3 are known to pass out of the plane of one lamella and distribute up and down the long axis (Amaral and Witter, 1989; Witter, Groenewegen, Lopes da Silva and Lohman, 1989).

Synopsis of Intrinsic Connections

Neurons in layer II in the medial and lateral entorhinal areas provide the primary source of fibers in the perforant path leading to the temporodentate branch (Ruth, Collier and Routtenberg, 1982, 1988). These fibers branch extensively along the septo-temporal axis, and fibers from the LEC synapse on the OML while fibers from the MEC synapse on the MML of the granule cells. The temporodentate branch of the perforant path also excite basket cells, hilar mossy cells and hilar nonmossy cells (Scharfman and Schwartzkroin, 1988, 1990). Neurons in layer III of the entorhinal cortex provide the primary source of fibers in the perforant path leading to the temporoammonic branch. These fibers synapse in Ammon's horn, predominantly in the molecular layer (Amaral and Witter, 1989; Witter, Groenewegen, Lopes da Silva and Lohman, 1989).

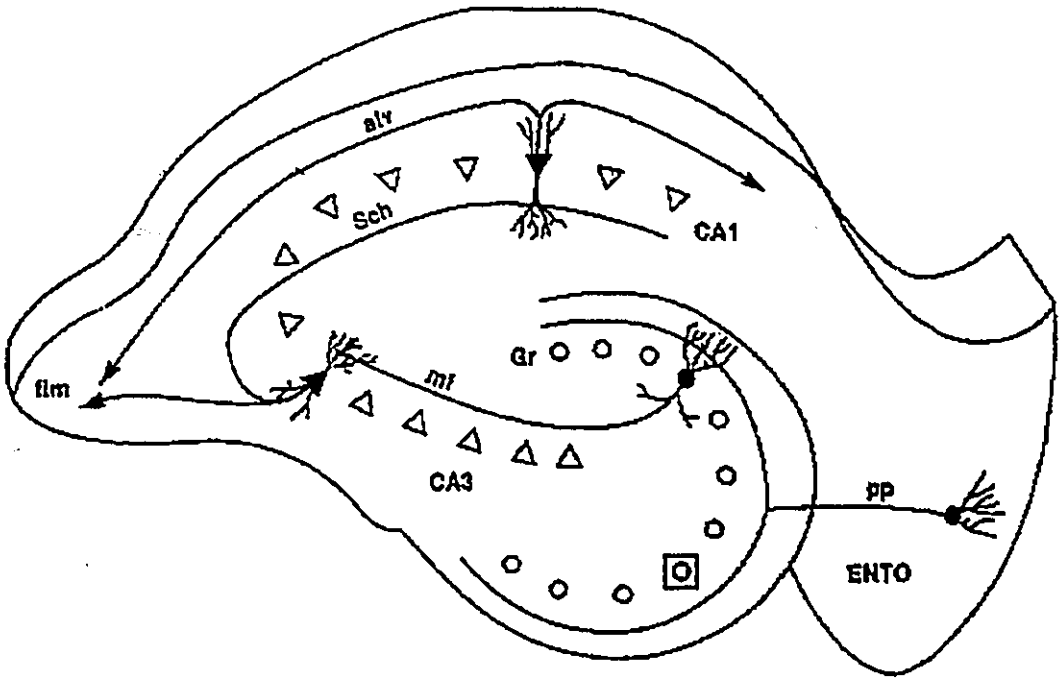


Fig. 1.2. This figure shows the internal circuitry of the hippocampal lamella which is oriented at right angles to the longitudinal axis. Stimulation of the entorhinal cortex (Ento) results in the successive activation of the trisynaptic pathway. The perforant path (pp) makes synaptic contact with the granule cells of the dentate gyrus. Axons from the granule cells, the mossy fibers (mf), innervate CA3 pyramidal cells which via the Schaffer collaterals (Sch) innervate CA1 pyramidal cells. CA1 and CA3 cells send their axons via the alveus (alv) into the fimbria (fim).

Two axonal systems exist in the dentate gyrus. The first axonal system is the mossy fibers which project from granule cells to the hilus and to the stratum lucidum of CA3. The second is the hilar mossy cells which give rise to commissural fibers which branch widely along the septo-temporal axis with little input near the cells of origin (Amaral and Witter, 1989; Witter et al, 1989). Area CA3 receives input from the mossy fibers and gives rise to two major projection pathways: the Schaeffer collaterals and the longitudinal association bundle (Ramon y Cajal, 1968; Swanson, Sawchenko and Cowan, 1981). Area CA1 receives input from the Schaeffer collaterals and projects to the ipsilateral subiculum (Finch and Babb, 1981) and entorhinal cortex (Finch, Wong, Derian and Babb, 1986). The subiculum, in turn, projects to the deep layers of the entorhinal and perirhinal cortex (Finch et al, 1986; Van Groen and Lopes da Silva, 1986) and to the presubiculum and parasubiculum.

Inhibitory system in the hippocampus

The interneurons which make up the inhibitory system, only account for 2-4% of hippocampal neurons. The most common hippocampal interneurons are the basket cells. There are five types of basket cells and they are responsible for the GABA-mediated inhibition in the hippocampus. The basket cells are found mainly within the cell layers of the pyramidal and granule cells, or within 10-50 μm of these layers but occasionally within the molecular layer. They terminate on the proximal dendrites or on the distal apical dendrites of the principal cells (Buzsaki, 1984). Activation of basket cells occurs

via a feedforward activation from the perforant path or via a feedback activation of granule or pyramidal cells (Andersen, Holmquist and Voorhoeve, 1966).

There are two types of GABA receptors: GABAa and GABAb receptors. The GABAa receptor affects Cl^- channels (Olsen, Snowman, Lee, Lomax and Wamsley, 1984; Haefely, 1984) and is associated with regulatory sites which either potentiate or inhibit the effects of GABA (Meldrum, 1975; Simmonds, 1983). Some of these regulatory sites bind barbituates and picrotoxin-like substances while others involve the benzodiazepine (Bz) receptor (Braestrup and Squires, 1977). When Bz receptors are activated, the probability of GABA activation of the Cl^- channels is increased (Haefely, 1984). The GABAb receptors are not associated with Bz receptors (Doble and Turnbull, 1981; Muhyaddin, Roberts and Woodruff, 1982). When activated, the GABAb receptor depresses Ca^{2+} conductance presynaptically (Dunlap, 1981; Dunlap and Fischbach, 1981; Wu and Saggau, 1995) or increases potassium conductance postsynaptically (Newberry and Nicoll, 1984; Premkumar and Gage, 1994).

Extrinsic Connections

The entorhinal cortex projects widely to most of the ipsilateral cortex including the piriform cortex, cingulate gyrus, prefrontal cortex (Sorensen, 1985) and the basolateral and lateral nuclei of the amygdala. The LEC also projects less densely to the contralateral side (Swanson and Kohler, 1986). Areas including the piriform cortex, septal nuclei, amygdala, the cingulate gyrus and the inferior temporal cortex (Alonso and Kohler, 1984;

Haberly and Price, 1978) also reciprocally connect with the entorhinal cortex. The LEC receives input from olfactory sensory areas whereas the MEC is linked to visual and parietal sensory areas through the presubiculum (Witter, Groenewegen, Lopes da Silva and Lohman, 1989).

Although most of the axons originating in Ammon's horn remain within the hippocampal complex, a massive projection exists from the hippocampus to the lateral septal nucleus (Swanson, Sawchenko and Cowan, 1981). This projection forms the first stage of a feedback loop, composed of the circuit of the hippocampus → lateral septum → medial septum → hippocampus (Milner and Amaral, 1984). Axons from the medial septum synapse diffusely throughout the hippocampus including the DG, CA3 and CA1 (Nyakas, Luiten, Spencer and Traber, 1987). Axons from the amygdala and midline thalamic nuclei also provide significant numbers of afferent synapses in Ammon's horn (Yanagihari, Niimi and Ono, 1987).

There are two commissures, one dorsal and one ventral, that connect the hippocampal-parahippocampal regions. The ventral commissure carries fibers primarily from Ammon's horn and the dentate gyrus while the dorsal commissure connects regions of the parahippocampal structures, including the subicular complex and entorhinal cortex. The ventral commissure contains axons from the dentate hilar mossy cells and from CA3 pyramidal neurons (Laurberg and Sorensen, 1981). The axons from the hilar cells project to both the IML of the granule cells and throughout the contralateral hilus of the DG (Hjorth-Simonsen and Laurberg, 1977). The CA3 fibers project to the contralateral CA3

and to CA1 (ipsi- and contralateral) where they synapse with pyramidal cells and interneurons. The dorsal commissure connects the parahippocampal areas. For example, connections have been described between the two entorhinal cortices and reciprocal projections between the pre- and parasubiculum and the contralateral medial entorhinal cortex (Amaral, Insausti and Cowan, 1984).

Fig. 1.3 shows a micrograph of the dentate gyrus/CA3 region which has been double stained with Cresyl Violet and Timm stain. It shows the distribution of the collateral synaptic expansions in the hilus and in the stratum lucidum of the CA3 region that can be seen using the Timm sulfide-silver method, which selectively stains axons and terminals with a high content of heavy metals, especially zinc. The granule and pyramidal cell layers are highlighted by Cresyl Violet.

Physiology

When the perforant path is stimulated, a characteristic evoked response can be recorded in the dentate gyrus of the hippocampus. The form of the evoked response changes depending on the depth of the recording electrode in the hippocampus. The response is the largest when the recording electrode is in the hilus. Figure 1.4 shows a characteristic evoked potential recorded in the dentate gyrus following stimulation of the perforant path. The first component of the response is the stimulus artifact which is largely the result of electrical coupling between the stimulation circuitry and recording circuitry and current flowing directly from the stimulating electrode to the recording

electrode. The first neural component consists of a positive potential which is the result of depolarization of the granule cell dendrites. The slope of this component is believed to be proportional to the amplitude and number of cellular EPSPs and is often called the population EPSP (Dunwiddie and Lynch, 1979; Lomo, 1971). If the intensity of the stimulation is large enough, a population spike can be observed. It consists of a negative inflection superimposed on the population EPSP and reflects the near synchronous discharge of granule cells. The amplitude of the population spike is proportional to the number of granule cells firing. The most common interneuron in the hippocampus which is responsible for GABA-mediated inhibition is the basket cell. Intracellular recordings of basket cell activity has determined that: 1) the basket cell action potentials have a very short duration (less than 1.2 ms), 2) they have a low threshold, and 3) they fire repetitively at a high frequency (up to 700 Hz) in response to perforant path stimulation (Knowles and Schwartzkroin, 1981). Basket cell activity is maximal at about 20 ms after stimulation of the perforant path and subsides after about 100 ms (Thalmann and Ayala, 1982).

Based on anatomical and physiological data, Fig. 1.5 presents a schematic diagram showing the excitatory and inhibitory connections in the dentate gyrus. The major excitatory circuit consists of the perforant path innervating the granule cells (Gr) and these send axons, the mossy fibers, to innervate the CA3 pyramidal cells. Feedforward and feedback inhibition constitutes the major inhibitory circuits. Feedforward inhibition occurs when perforant path stimulation directly excites inhibitory interneurons, the basket

cells (B) which then inhibit the granule cells (Scharfman, 1995) Feedback inhibition occurs when the activated granule cells synapse on inhibitory interneurons which then inhibit the granule cells. According to Sloviter (1991), feedforward and feedback inhibition may also occur by activating excitatory mossy cells in the hilus which subsequently activate inhibitory neurons.

Olfactory Bulb-Lateral Olfactory Tract- Piriform Cortex

Anatomy

All the brain regions receiving monosynaptic input from the olfactory bulb collectively define the primary olfactory cortex (Haberly, 1985), with the largest of these areas being the piriform cortex. Other structures which make up the olfactory cortex include the olfactory tubercle, olfactory peduncle, entorhinal cortex, insular cortex and cortical areas associated with the amygdala (Price, 1987). The piriform cortex is a highly laminar paleocortical structure that consists of 3 layers (Haberly and Price, 1978; Haberly,1990). Layer I, the superficial plexiform layer, can be subdivided into 2 layers, Ia and Ib: Layer Ia, the superficial part, contains the afferent fibers from the olfactory bulb while layer Ib, the deep part contains association fibers from other parts of the piriform cortex and other olfactory cortical areas. Layer II, the superficial soma layer, is tightly packed with the soma of pyramidal cells. Layer III, the deep soma layer, is less densely packed with the soma and basal dendrites of pyramidal and other cells and

Fig. 1.3. This micrograph shows a horizontal section of the hippocampus that has been double stained with Cresyl Violet and Timm sulfide-silver method. The Cresyl Violet highlights the granule and pyramidal cells (purple stain) while the Timm stain highlights the mossy fibers which innervate the CA3 pyramidal cells and hilar cells and to a small extent cells within the supragranular layer.

contains additional association fibers.

Mitral cells in the OB project to the PC via the LOT, where they make monosynaptic contact with the apical dendrites of pyramidal cells in layer Ia. Activation of the LOT produces a monosynaptic EPSP in PC pyramidal cells. If this excitatory input is sufficient to fire the cells, they produce a second, disynaptic EPSP in PC pyramidal cells via local axon collaterals or association fibers from other parts of the piriform cortex and other olfactory cortical areas. The pyramidal cells which are fired by these inputs then produce recurrent and feed-forward inhibition of themselves and other pyramidal cells. For example, feedback inhibition occurs when pyramidal cells excited by LOT stimulation are inhibited by a local recurrent pathway through deep inhibitory interneurons and feedforward inhibition occurs when the inhibitory interneurons are directly excited by LOT stimulation (Haberly, 1985). Association fibers also make direct contact with inhibitory interneurons.

In addition to the input from the olfactory bulb, the olfactory cortex receives inputs from the basal forebrain, brainstem, thalamus and hypothalamus (Haberly and Price, 1978). Many structures of the olfactory cortex are reciprocally connected with each other. For example, the piriform cortex, olfactory peduncle and amygdaloid cortex also send fibers to the olfactory bulb (Luskin and Price, 1983). Efferent pathways from

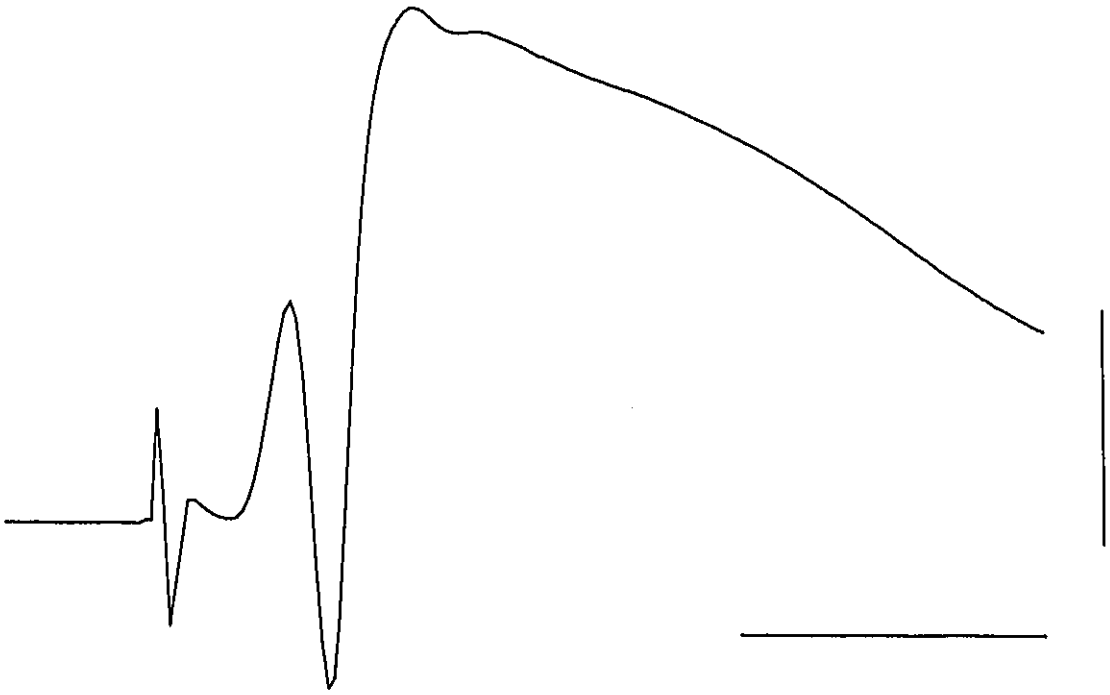


Fig. 1.4. This diagram shows a characteristic evoked potential recorded in the dentate gyrus following stimulation of the perforant path. Three components make up the response: 1) the stimulus artifact which is largely the result of current flowing directly from the stimulating electrode to the recording electrode; 2) A positive potential which is the result of depolarization of the granule cell dendrites (population EPSP); 3) The negative inflection, superimposed on the population EPSP, reflects the near synchronous discharge of granule cells (population spike).

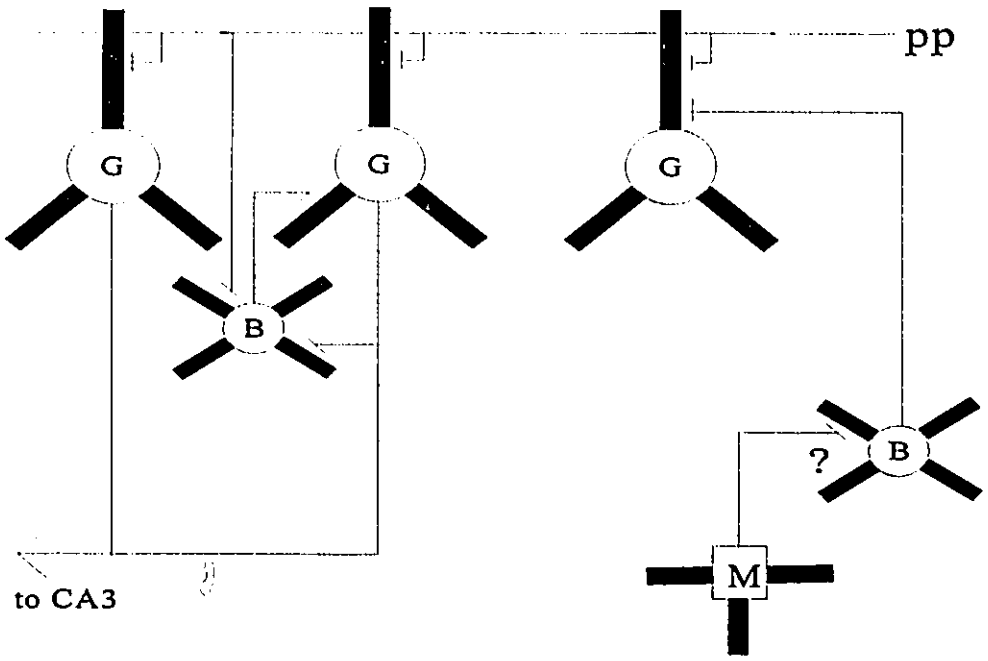


Fig. 1.5. This schematic diagram shows the excitatory and inhibitory connections in the dentate gyrus. The major excitatory circuit consists of the perforant path (pp) innervating the granule cells (G) and these send axons, the mossy fibers, to innervate the CA3 pyramidal cells. Feedforward inhibition occurs when perforant path stimulation directly excites inhibitory interneurons, the basket cells (B) which then inhibit the granule cells. Feedback inhibition occurs when the activated granule cells synapse on inhibitory interneurons which then inhibit the granule cells. Inhibition may also occur by activating mossy cells (M) which then excite inhibitory neurons (Sloviter, 1991).

the olfactory cortex project to the neocortex, thalamus, hypothalamus, hippocampal formation, striatum and limbic system (Price, 1985).

Physiology

The extracellular field potential that is evoked by stimulation of the LOT (Haberly, 1973) or OB (Satou et al, 1983) consists of an initial surface-negative wave followed by a surface-positive wave. The surface-negative wave has two components, A1 and B1, which represent the monosynaptic and disynaptic population EPSPs, respectively, evoked by activation of the LOT (Haberly, 1973; Haberly and Bower, 1984). Population spikes are usually not observed in intact adult rats (Schwob, Haberly and Price, 1984) but they can be seen in potentials evoked in PC slices (Richards and Sercombe, 1968). The origin of the surface-positive wave is less clear, but it is temporally associated with inhibition of PC pyramidal cells following their activation and may therefore be a reflection of an IPSP in pyramidal cells. If so, this wave may well have more than one component. Satou, Mori, Tazawa and Takagi (1983) found evidence for two different types of inhibitory potentials induced in PC pyramidal cells by stimulation of the OB: a rapid IPSP based upon increased choride conductance, and a longer lasting IPSP based upon increased potassium conductance. All components of the PC evoked potential reverse polarity between layers I and III and thus are easily distinguished from potentials generated elsewhere and volume-conducted to the PC (Stripling, Patneau and Gramlich, 1988). Fig. 1.6 shows a characteristic evoked potential recorded in the piriform cortex following

stimulation of the lateral olfactory tract. It consists of an initial surface-negative wave followed by a surface-positive wave. The surface-negative wave has two components, A1 and B1, which represent the monosynaptic and disynaptic population EPSPs, respectively, evoked by activation of the LOT (Haberly, 1973; Haberly and Bower, 1984).

According to Haberly and Bower (1984) 3 different excitatory inputs to the pyramidal cells of the piriform cortex occur at different dendritic segments. The first is a monosynaptic EPSP in distal apical dendrites. The second is a disynaptic EPSP in basal dendrites via local axon collaterals of other pyramidal cells excited by the monosynaptic EPSP. Finally, the third is a disynaptic EPSP in the proximal apical and, to a lesser extent, basal dendrites via long association axons. Inhibitory inputs into the piriform cortex consist of 1) feedback inhibition whereby pyramidal cells excited by LOT stimulation are inhibited by a local recurrent pathway through deep inhibitory interneurons and 2) feedforward inhibition whereby the inhibitory interneurons are directly excited by LOT stimulation. Association fibers also make direct contact with inhibitory interneurons. Based on anatomical and physiological data, Fig. 1.7 demonstrates a schematic diagram of the excitatory and inhibitory connections in the piriform cortex.

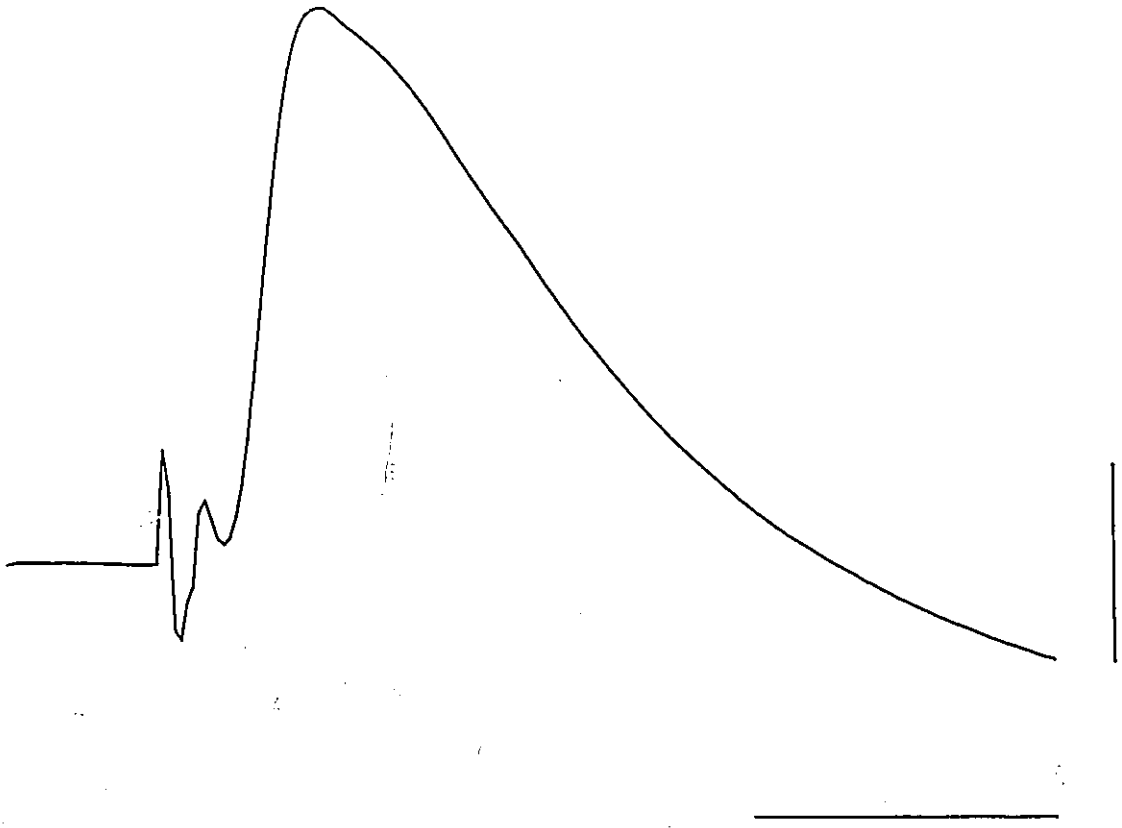


Fig. 1.6. shows a characteristic evoked potential recorded in the piriform cortex following stimulation of the lateral olfactory tract. It consists of an initial surface-negative wave followed by a surface-positive wave. The surface-negative wave has two components, A_1 and B_1, which represent the monosynaptic and disynaptic population EPSPs, respectively, evoked by activation of the LOT. Only the onset of the surface-positive wave is shown in this trace.

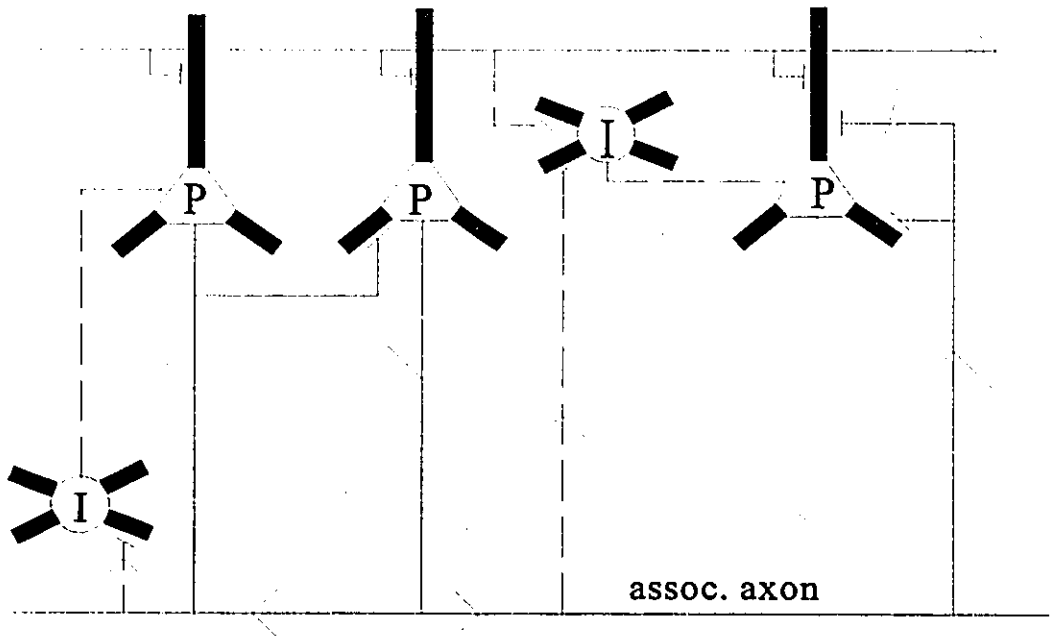


Fig. 1.7. This schematic diagram shows the excitatory and inhibitory connections in the piriform cortex. Excitatory inputs into the piriform cortex are shown: 1) a monosynaptic EPSP in distal apical dendrites, 2) a disynaptic EPSP in basal dendrites via local axon collaterals of other pyramidal cells excited by the monosynaptic EPSP, 3) a disynaptic EPSP in the proximal apical and, to a lesser extent, basal dendrites via long association axons. Inhibitory inputs into the piriform cortex are also shown: 1) feedback inhibition whereby pyramidal cells excited by LOT stimulation are inhibited by a local recurrent pathway through deep inhibitory interneurons and 2) feedforward inhibition whereby the inhibitory interneurons are directly excited by LOT stimulation. Association fibers also make direct contact with inhibitory interneurons.

Chapter 2

TRANSFER KINDLING IN THE ENTORHINAL CORTEX-PERFORANT PATH-DENTATE GYRUS SYSTEM

2.1 Introduction

The neural remodeling that occurs during kindling must extend beyond the stimulation site. Kindling one site, until fully generalized convulsions have been evoked, leads to an increased rate of subsequent kindling in more distant sites (Burnham, 1976; Cain, 1986; Goddard, McIntyre and Leech, 1969; Racine, 1972b). This "transfer effect" is measured in kindled animals as a savings in the number of afterdischarges needed to evoke generalized convulsions in a secondary site (Goddard et al, 1969; Racine, 1972b). Racine (1972b) found that stimulation of the contralateral amygdala led to full strength seizures within a few stimulations even after removal of the kindled amygdala. These AD propagation and transfer kindling effects may provide a paradigm for studying the mechanisms underlying certain aspects of the development of clinical epilepsy (Morrell, 1985; Racine and Burnham, 1984; Sato, Racine and McIntyre, 1990). They should, for example, facilitate the investigation of the emerging seizure circuitry, which as the following experiments will show, can vary substantially depending upon changes in sites of initial activation, even within tightly coupled monosynaptic systems.

Positive transfer could be explained by supposing that any sufficiently intense forebrain discharge, however it is achieved, would be able to kindle convulsions more

rapidly than it would in a naive animal (Cain, 1986). If a transfer site is synaptically close to the primary site (e.g. a monosynaptic target), then it should be partially or fully kindled by the input from the primary site and propagate a similar pattern of discharge. Consequently, transfer kindling should be rapid or immediate.

We have recently collected pilot data, however, that suggests a more complex mechanism. The transfer effect has been tested in the dentate gyrus after kindling its main input pathway, the perforant path, which originates in the entorhinal cortex. In a small group of animals, L. Tuff (unpublished data), found no significant transfer effect when the dentate gyrus was kindled immediately after primary perforant path kindling. In another pilot study (M. Pepkowski), a significant transfer effect was found after a 2 week delay, but the effect was still rather weak.

There have been a number of experiments in which the perforant path has been the site of primary kindling (e.g. de Jonge and Racine, 1987). The implicit assumption is often that the dentate gyrus is the primary kindling site in these experiments because axons cannot directly support epileptic discharge. It is surprising then, that the transfer effects appear to be relatively weak in this system. It would appear that subtle differences in the specific spatio-temporal pattern of discharge may be a more important determinant of kindling-induced neuronal remodeling than we had previously suspected.

To further explore this possibility, we have followed up our pilot data with a transfer kindling experiment in which we test for transfer effects within both the target (dentate gyrus) and the source (entorhinal cortex) after primary kindling of the

monosynaptic pathway (perforant path). We have also included groups in which transfer kindling was delayed for 4 weeks to allow decay of any transient effect which could suppress transfer kindling (McIntyre and Goddard, 1973; see also Burchfiel and Applegate, 1989).

Based upon our pilot experiments, our hypothesis was that the spatio-temporal patterns of discharge during kindling of these sites would be sufficiently different to preclude immediate and possibly even significant transfer kindling.

2.2 Materials and methods

2.2.1. Animals and surgical procedures

A total of 56 male Long Evans hooded rats weighing between 300-500 g were implanted with chronic bipolar electrodes in the entorhinal cortex (4.8 mm anterior, 5.0 mm lateral, and 9.0 mm ventral to bregma), perforant path (7.2 mm anterior, 4.1 mm lateral and 3.3 mm ventral to bregma) and dentate gyrus (3.5 mm anterior, 2.2 mm lateral and 3.3 mm ventral to bregma)(Paxinos & Watson, 1982). The electrodes were held in place by dental acrylic and 4 stainless-steel screws inserted into the skull. Animals received 15 mg/kg atropine methyl nitrate and were anesthetized with 65 mg/kg pentobarbital. The bipolar electrodes consisted of two teflon coated Nichrome wires 127 μm in diameter. These were twisted together and had a vertical tip separation of 0.5 mm. Electrodes were lowered under physiological control to ensure optimal placement for the perforant path and the dentate gyrus. We chose a site in lateral, rather than medial,

entorhinal cortex because pilot experiments indicated that the evoked epileptiform discharge may propagate more strongly from these lateral placements.

2.2.2. Primary kindling

Following a 2 week post surgery recovery period, the animals were randomly assigned to entorhinal cortex (n=10), perforant path (n=37) or dentate gyrus (n=9) primary site kindling groups. Stimulation consisted of a 1 s train of 1 ms biphasic square wave pulses, at 60 Hz. These stimulation trains were administered once every 24 h at an intensity of 600 μA, which was reliably above threshold for all but a few animals. In those animals, the stimulation intensity was raised in 100 μA steps until AD was reliably triggered (maximum intensity was 1000 μA). EEG recordings were taken for all kindling sessions throughout primary and transfer kindling. The AD durations were measured daily for all sites. The motor responses were rated according to a 5 point scale (Racine, 1972b), culminating in a response that included head and facial automatisms, forelimb clonus, a clonic rearing and loss of postural control (stage 5). Stimulation was continued until two fully generalized seizures had occurred.

2.2.3. Transfer Kindling

After 2 generalized seizures had been triggered from the primary site, stimulation was begun at the transfer site and continued until 2 stage 5 seizures were triggered. The groups that received their initial stimulation in either the dentate gyrus or

entorhinal cortex served as primary site controls for animals that were transfer kindled in these sites following perforant path kindling. In addition, the dentate gyrus and entorhinal cortex kindled groups both received transfer kindling stimulation in the perforant path, which was begun 24 h after completion of primary site kindling. The animals that were initially kindled in the perforant path were divided into 4 groups. Two of these groups received transfer kindling in the entorhinal cortex which was started either 24 h (n=9) or 4 weeks (n=9) after completion of primary site kindling. The remaining 2 groups received transfer kindling in the dentate gyrus after a 24 h (n=10) or 4 week (n=9) delay. Stimulation intensity was again set at a level which reliably evoked an AD.

2.2.4. Statistical analysis

Nonparametric Mann-Whitney U-tests (Siegel, 1956) were used to compare transfer kindling against primary site kindling in the same site. For example, the rate of transfer kindling in the dentate gyrus following perforant path kindling was compared with the rate of primary site kindling in a separate group of animals kindled first in the dentate gyrus.

2.2.5. Histology

Upon completion of the transfer tests, all but 17 animals were anesthetized and perfused transcardially with saline and formalin and their brains removed, frozen and sectioned. The sections were stained with Cresyl Violet and Luxol Blue and examined

under the microscope to determine electrode placements. The remaining 17 animals served as subjects in an additional experiment after which their brains were sectioned, stained and examined as described above.

2.3. Results

In this paper, the term secondary site will refer to sites from which recordings were taken but which were not directly stimulated, and the term transfer site will refer to the second kindled site.

2.3.1. Electrode Placements

All electrodes were found to be accurately placed. The distribution of electrode tip locations is shown in Fig. 2.1.

2.3.2. Rates of Seizure Development

In accordance with previous reports (e.g. Goddard et al, 1969), the entorhinal cortex was found to kindle most rapidly (10.9 ADs) followed by the perforant path (15.1 ADs) and finally the dentate gyrus (53.4 ADs). The number of afterdischarges (ADs) required to kindle animals in the dentate gyrus (range 28-96 ADs) was greater than normally cited in the literature (Burnham, 1976) and greater than normally required in our

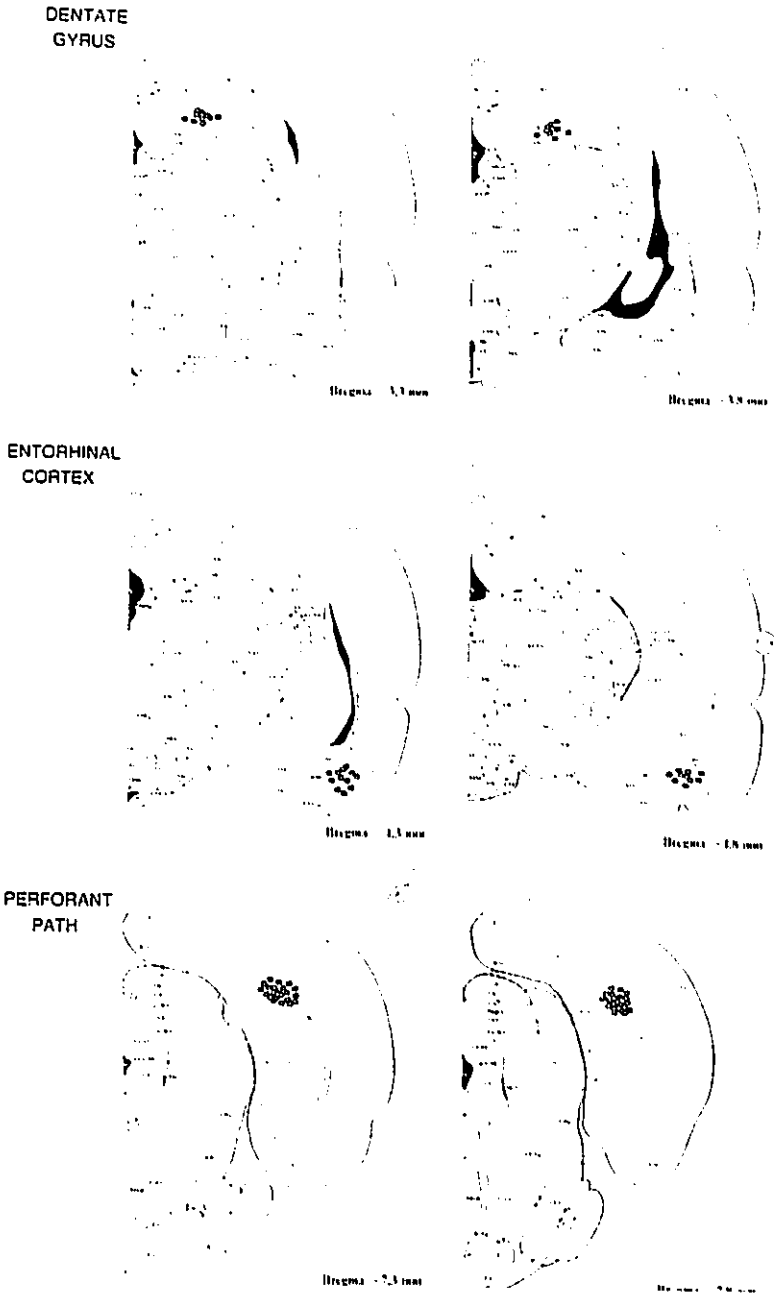


Fig. 2.1. The positions of the electrode tips, determined histologically, are shown for the entorhinal cortex, perforant path and the dentate gyrus. The anterior/posterior coordinate, with respect to bregma, is shown for each diagram. Atlas diagrams are from Paxinos and Watson (1982).

lab. The reason for this is unclear as the dentate electrodes were implanted under physiological control and histological examination of the electrode tracts, as shown in Fig. 2.1 revealed accurate electrode placements.

Table 2.1 presents the primary and transfer kindling rates for the sites studied in this experiment. Significance was calculated using the non-parametric Mann-Whitney U test (Siegel, 1956). Although there was a substantial positive transfer to the dentate gyrus following perforant path stimulation, the dentate gyrus still required a mean of 15.8 ADs and 13.9 ADs when transfer was started immediately and after a 4 week delay. That is, there was only a 70.4% and 74.0% savings in the number of afterdischarges required to reach criterion of up to but excluding the first stage 5 seizure when the dentate gyrus was transferred immediately and after a 4 week delay, respectively. The entorhinal cortex also showed a relatively weak transfer effect. The group that was started immediately after completion of primary site kindling did not even show a significant transfer, and the 4-week-delay group showed a savings of only 74.3%. When the perforant path was the transfer site, there was a 78.8% savings in number of afterdischarges needed to reach criterion immediately following dentate gyrus kindling but a non-significant increase in the number of discharges required immediately following entorhinal cortex kindling. Spiller and Racine (1994) calculated the savings using the criterion of 2 stage 5 seizures rather than the criterion of up to but excluding the first stage 5 seizure.

Table 2.1.

Primary and transfer kindling rates (ADs to up to and excluding the 1st stage 5 seizure)

Primary site	Kindling rate	Delay	Secondary site	Kindling rate	Prob	% Savings
DG	x = 53.4 (28-96) n = 9	24 h	DG after PP	x = 15.8 (4-37) n = 10	P < 0.01	+70.4
		4 weeks	DG after PP	x = 13.9 (3-48) n = 9	P < 0.01	+74.0
EC	x = 10.9 (6-16) n = 10	24 h	EC after PP	x = 8.6 (0-17) n = 9	P > 0.05	+21.1
		4 weeks	EC after PP	x = 2.8 (0-13) n = 9	P < 0.01	+74.3
PP	x = 15.1 (4-56) n = 37	24 h	PP after DG	x = 3.2 (0-8) n = 9	P < 0.01	+78.8
			PP after EC	x = 17.1 6-56) n = 10	P > 0.05	-13.2

2.3.3. Development of Afterdischarge

In accordance with reports of previous investigators (McIntyre and Goddard, 1973; Racine, 1972b), repeated stimulation of the entorhinal cortex (EC), perforant path (PP) or dentate gyrus (DG) led to the appearance of epileptiform afterdischarges which progressively became longer in duration, greater in amplitude and more complex in waveform. In the initial stage of the kindling process, the secondary sites often showed

weak or even absent epileptiform activity which was surprising given the synaptic relationships between these sites. As kindling continued, however, there was a rapid development of propagation of the AD to these sites. Concomitantly with AD growth, the behavioral response changed from no response to a convulsive response that progressed through some or all of the 5 stages of seizure development as described by Racine (1972b). About two-thirds of the animals in this series skipped over seizure stages, for example jumping from a stage 2 to a stage 5. The remaining one third progressed through all 5 seizure stages. This contrasts with the amygdala/piriform area in which all animals typically progress through all stages of seizure activity (Racine, 1972b).

AD duration, an easily measured index of growth, was used for a more detailed analysis of developing epileptogenesis. Mean AD durations during primary kindling for both stimulated and secondary sites are presented in Table 2.2A. The same measures for transfer kindling are shown in Table 2.2B. Transfer interval had no aparent effect on any of the AD measures and so the data were collapsed across transfer interval. Somewhat longer AD durations were achieved during transfer kindling than during primary kindling. The longest AD durations were found in the EC, regardless of stimulation site, and the shortest in the DG. In addition, the propagated AD to the DG was of longer duration in EC kindling than when the DG was directly stimulated.

Table 2.2A

Stimulated and reactive AD durations (seconds) during primary kindling

Kindling site	Site	First AD	Stage 5 AD	Longest AD
PP kindling	EC	18.7	61.1	71.9
		(±2.5)	(±6.2)	(±5.8)
	PP	27.6	50.7	61.7
		(±3.0)	(±5.2)	(±5.0)
	DG	14.2	35.7	48.7
		(±1.8)	(±3.5)	(±4.5)
EC kindling	EC	14.8	73.1	96.1
		(±2.1)	(±8.5)	(±8.0)
	PP	8.1	57.4	83.6
		(±2.3)	(±6.3)	(±8.8)
	DG	6.4	56.6	85.3
		(±1.8)	(±4.5)	(±8.7)
DG kindling	EC	4.8	53.7	88.4
		(±3.2)	(±11.8)	(±10.8)
	PP	8.4	38.8	66.7
		(±3.4)	(±9.2)	(±12.6)
	DG	13.1	30.3	46.2
		(±3.5)	(±10.1)	(±14.6)

Table 2.2B
Stimulated and reactive AD durations (seconds) during transfer kindling

Kindling site	Site	First AD	Stage 5 AD	Longest AD
PP kindling	EC	40.0	83.9	101.8
		(±9.4)	(±10.4)	(±9.0)
	PP	26.2	74.6	87.8
		(±7.0)	(±10.3)	(±9.6)
	DG	24.3	64.1	81.6
		(±2.9)	(±9.2)	(±9.7)
EC kindling	EC	35.4	82.1	88.0
		(±10.2)	(±11.2)	(±10.4)
	PP	29.4	57.5	71.6
		(±9.3)	(±10.2)	(±9.1)
	DG	28.1	54.2	63.6
		(±8.6)	(±9.1)	(±8.1)
DG kindling	EC	27.0	57.2	74.1
		(±6.4)	(±10.9)	(±9.6)
	PP	29.8	51.1	62.1
		(±5.6)	(±8.6)	(±8.0)
	DG	15.3	35.3	47.9
		(±2.7)	(±8.2)	(±8.2)

Fig. 2.2 shows an example of the curves plotted for the growth of AD duration throughout the kindling process from two animals kindled in the perforant path. The animals differed in the number of stimulations required to reach the criterion of 2 stage 5 seizures. The shapes of the curves become similar, however, as the animals approach the criterion. The AD durations remained relatively short in the initial stages of kindling but increased sharply a few stimulations before a stage 5 seizure was evoked. The longest AD durations were not always recorded during stage 5 seizure activity. Over half of the animals showed their longest ADs 1-3 days prior to the appearance of stage 5 seizures.

In an attempt to plot group data for AD growth, only the first AD, the last 5 ADs before and one AD after the first stage 5 seizure were plotted for animals kindled in the perforant path (Fig. 2.3). Only 3 ADs are shown for transfer kindling to allow the inclusion of the few animals that kindled rapidly in the secondary site. There was a collapse of the AD duration upon transfer and, as in the primary site, the response tended to stay relatively weak until stage 2 convulsive activity appeared. The AD duration then rapidly increased beyond that of primary site kindling (Fig. 2.3). Although the number of ADs required to kindle the transfer site was significantly less than for the primary site, the shape of the AD duration curve remained similar to that in the primary site, except that growth progressed more rapidly once it began. Similar curves were found for primary and transfer kindling of the DG and EC.

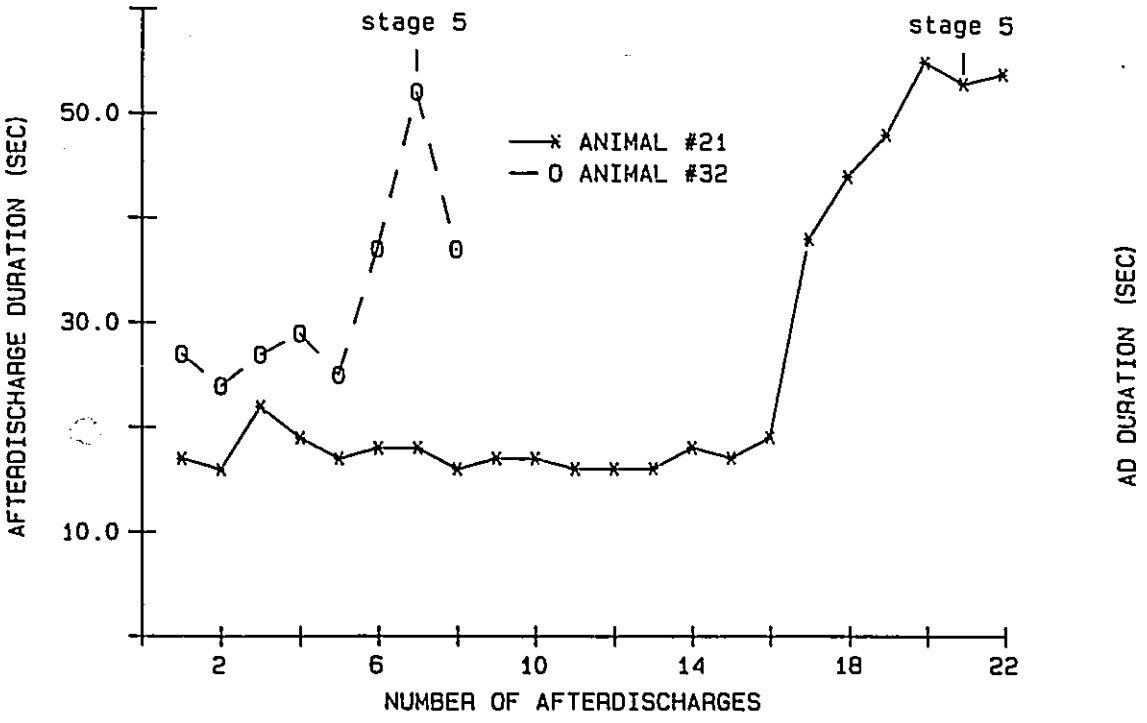


Fig. 2.2. This graph shows the number and duration of afterdischarges for two animals that were kindled in the perforant path. Although the number of after discharges required to evoke stage 5 seizures are different for these two animals, the general shape of the curves become similar as convulsive responses develop. Similar curves were found for animals kindled in the entorhinal cortex and the dentate gyrus.

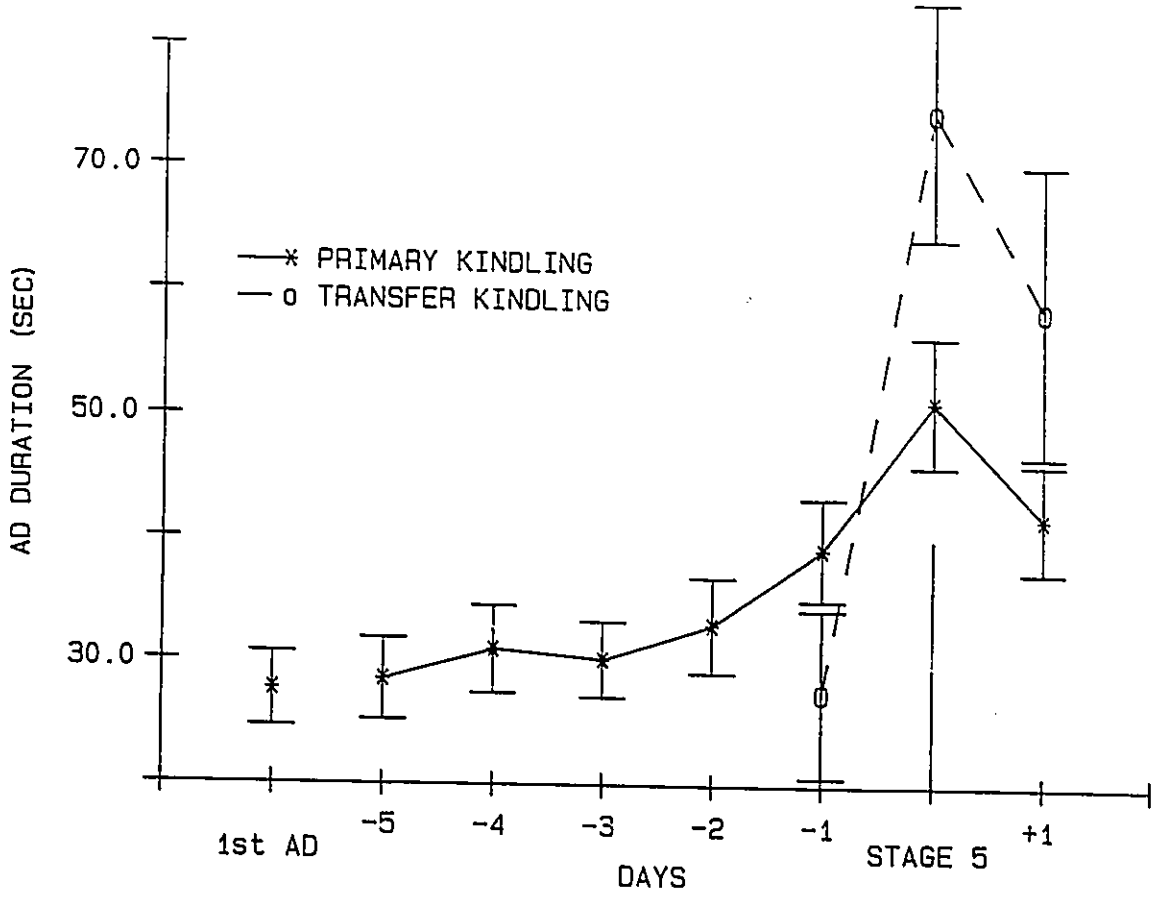


Fig. 2.3. Mean afterdischarge durations (± S.E.M.) for primary and transfer kindling in the perforant path are plotted against days relative to the first stage 5 seizure. Similar results were found for animals transfer kindled in the perforant path after primary site kindling in either the dentate gyrus or entorhinal cortex, so these groups were combined for this figure. For transfer kindling, only 3 ADs are shown because a few animals kindled very rapidly. The peak AD duration for the transfer kindling was significantly longer than it was for primary kindling. The transfer interval for this group was 24 h. Similar curves were also found for the entorhinal cortex and dentate gyrus kindled animals, although the AD growth was somewhat less abrupt in animals kindled in the entorhinal cortex.

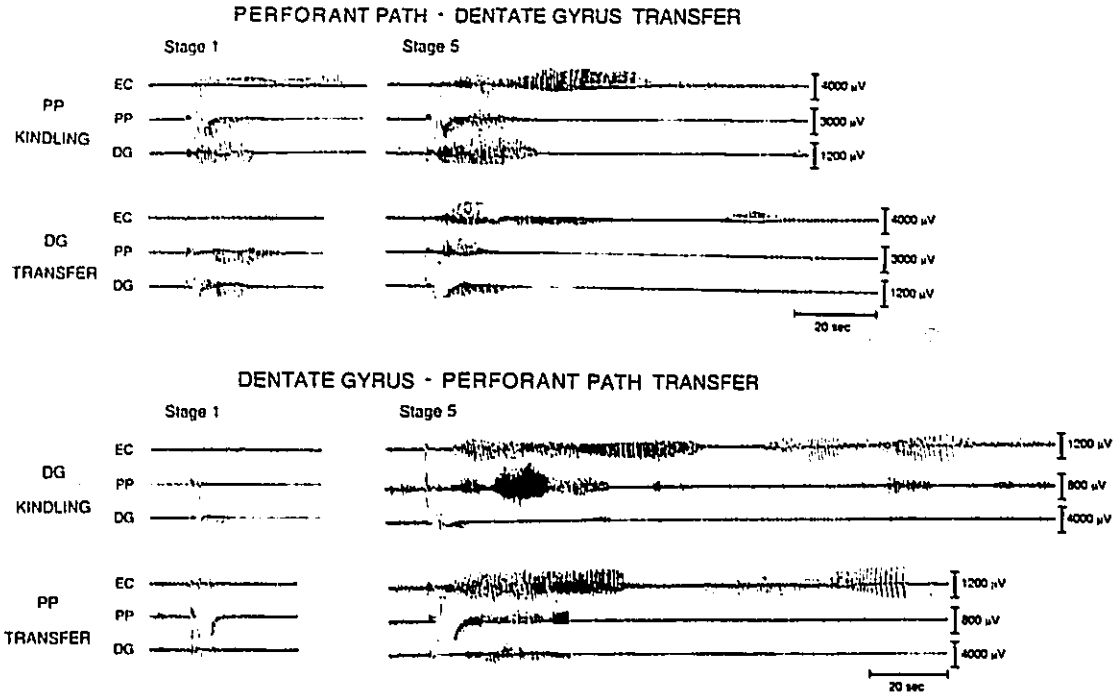


Fig. 2.4. Typical traces of the development of EEG activity for animals kindled in the perforant path and the dentate gyrus. The first section shows stage 1 and stage 5 EEG traces during primary site (perforant path) and secondary site (dentate gyrus) kindling. The second section shows an example in which the dentate gyrus served as primary site and the perforant path as secondary site. These examples are from animls in which transfer kindling began 24 h after completion of primary site kindling. The EEG effects were similar in the 4 week delay group.

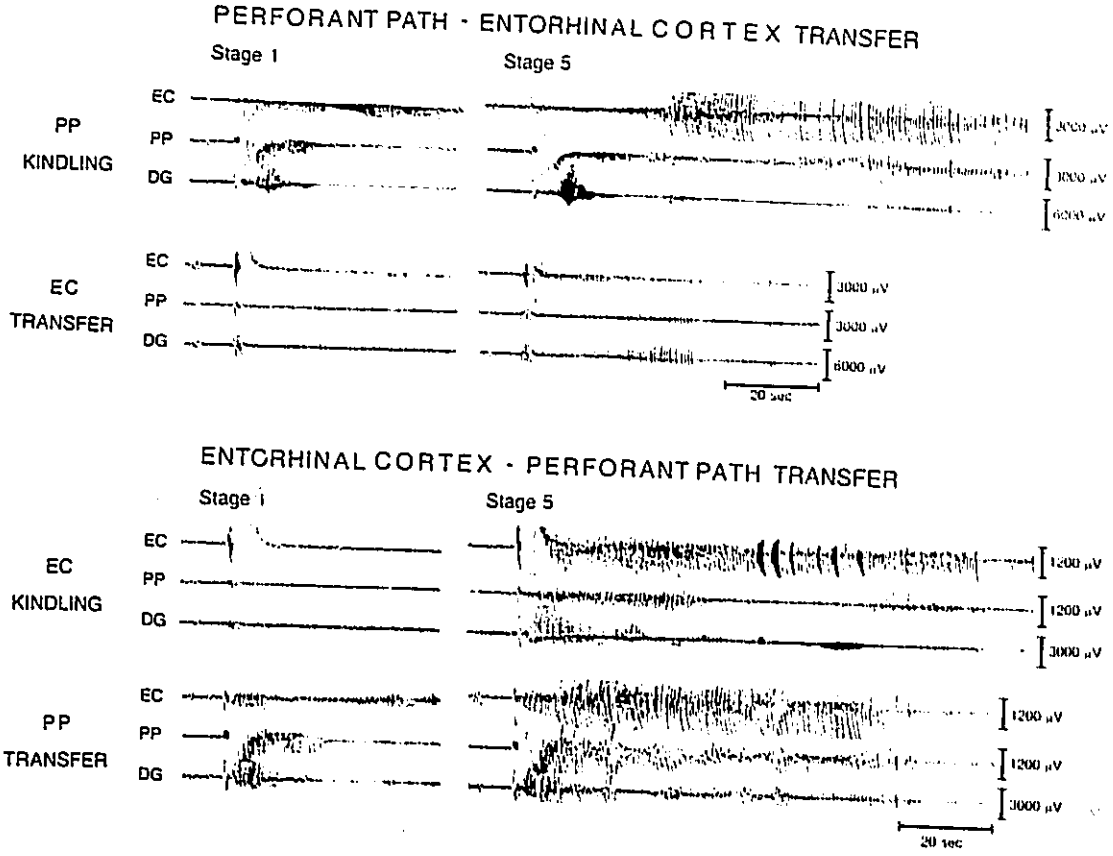


Fig. 2.5. Typical traces of the development of EEG activity for animals kindled in the perforant path and entorhinal cortex. The first section shows the EEG traces during the perforant path primary site kindling and the entorhinal cortex (secondary site) kindling. Similar effects were seen in both the 24 h and 4 week delay groups. These traces were taken from animls in which transfer kindling began 24 h after completion of primary site kindling.

Fig. 2.4 shows typical traces of the EEG activity for animals kindled in the perforant path with subsequent transfer to the dentate gyrus and in the dentate gyrus with subsequent transfer to the perforant path. Fig. 2.5 shows the same for animals kindled in the perforant path with entorhinal cortex transfer and in the entorhinal cortex with perforant path transfer. During both primary and transfer kindling, there was development in the duration and complexity of the afterdischarge in the stimulated and secondary sites as can be seen by comparing the EEG accompanying a stage 1 versus a stage 5 behavioral response. Stage 1 EEGs were used for initial comparisons in these figures, because many animals showed a stage 1 behavioral response during the first transfer stimulation. Both figures show the strength of the propagated discharge in the entorhinal cortex which tended to develop a much longer AD than the stimulated perforant path or dentate gyrus. Upon transfer, as mentioned previously, there was an initial collapse of the responses in all sites irrespective of stimulation site. There was no difference in the EEG recordings for animals that received immediate transfer as compared to animals that had a 4 week delay between primary and transfer kindling (not shown).

2.4. Discussion

The entorhinal cortex, perforant path and dentate gyrus make up a powerful monosynaptic system that shows a strong propagation of epileptiform discharge, regardless of which site is kindled. In fact, the dentate gyrus often showed a stronger

electrographic discharge as a secondary site (with either perforant path or entorhinal cortex kindling) than when kindled directly. The perforant path and entorhinal cortex discharges appeared to be at least as strong when these structures were secondary sites as when they were stimulated directly.

Even though these structures all appeared to participate strongly during the primary site kindling, the transfer kindling was surprisingly slow. The dentate gyrus, which appeared to have been more strongly activated during perforant path kindling than during dentate gyrus kindling itself, and which is the principal recipient of perforant path fibers, still required about 15 additional ADs to complete kindling following primary site kindling of the perforant path. In our pilot studies, we had found even less transfer from perforant path to dentate gyrus. The dentate gyrus control group in this experiment required more than the usual number of kindling stimulations, so we may actually be overestimating the amount of transfer shown between these sites. There was essentially no transfer to the entorhinal cortex, the source of perforant path fibers, when transfer kindling was begun 24 h after the completion of primary site perforant path kindling. There was also no transfer to the perforant path after entorhinal cortex kindling. A significant transfer did develop in the entorhinal cortex following perforant path kindling after a 4 week delay, but it was still not particularly strong (a 74% savings) considering the strong relationship between these sites.

The effect of the delay in transfer from the perforant path to the entorhinal cortex is similar to the effects reported by McIntyre and Goddard (1973) following kindling of

the amygdala and transfer to the contralateral amygdala. They suggested that a widespread and decaying after-effect occurred following the convulsions triggered from the primary site, leading to a reduction in the amount of transfer. This effect might be related to the 'kindling antagonism' model reported by Burchfiel and co-workers (Burchfiel and Applegate, 1989) in which alternating stimulation between 2 sites results in a blockade of the progression of kindling from one or both sites. This effect apparently does not occur in 16-day-old rats (Haas, Sperber and Moshé, 1990), so it might be interesting to test these transfer effects in young rats as well. There was, however, no effect of delay, on transfer from the perforant path to the dentate gyrus.

The entorhinal cortex is strongly connected to the dentate gyrus via the perforant path. Nevertheless, it is quite clear from these results that the spatio-temporal patterns of discharge, although they may appear superficially similar in the EEG recordings, must be expressed differently at the network level. If we ignore the strong converging/diverging connectivity, and the superficial similarities in the discharge patterns, it is not surprising that the spatio-temporal patterns of discharge should differ substantially. In the case of perforant path stimulation, the dentate gyrus is receiving a very diffuse input that presumably has immediate effects upon nearly the whole extent of that structure. This may account for the stronger dentate discharge when stimulation was applied to the perforant path or entorhinal cortex, rather than to the dentate gyrus directly. In the case of direct stimulation, the discharge presumably begins locally within the dentate gyrus and then propagates throughout the rest of the structure via quite different pathways.

Similarly, when stimulating the entorhinal cortex only one segment of input to the dentate gyrus is initially affected. The activation of the remaining portions of this input must depend upon the nature of the subsequent recruitment of the rest of the entorhinal cortex.

The prevailing view of seizure discharge propagation is that it follows "preferred" pathways. Providing that the mechanism of activation remain synaptic, this is not a particularly controversial point of view. This rather general notion, however, leaves a very broad range of possibilities. At one extreme we have complete activation of whole structures and propagation between structures over fixed pathways. At the other extreme, we could have a partial and more distributed, and modifiable, activation within structures, and propagation over pathways that are themselves modifiable. Our results suggest that the latter version may be closer to the truth. The pattern of neuronal remodelling that occurs during kindling appears to somewhat specific to the location of the kindling electrode. From pilot data, it appears that this may be the case even for different sites within the same structure (e.g. entorhinal cortex or hippocampus). This specificity suggests a sensitivity to precise spatio-temporal patterns of activation, which can differ with different kindling sites.

In any case, these results raise some interesting questions about the kindling phenomenon itself. Prior to this experiment, one of the more plausible explanations for transfer kindling was that it was dependent upon how much circuitry the respective discharge patterns had in common, i.e. the spatial overlap. The system tested in this study, however, was initially chosen because it was felt that there should be extensive

spatial overlap in the neuronal activation patterns. The electrographic data apppeared to confirm this assumption, but the transfer data indicate that the overlap might be more apparent than real. The circuitry may occupy the same location, but the specific networks are likely different. Also, it is possible that the temporal as well as spatial patterns of activation play an important role in the kindling process. For example, activation of 'A' followed by 'B' may not be equivalent to activation of 'B' followed by 'A'. It will not be easy to test for temporal dependencies in kindling, but one approach might be to maintain specific temporal patterns of electrical stimulation during the AD, which should bring the temporal patterning partly under experimental control.

Chapter 3

TRANSFER KINDLING BETWEEN SITES IN THE OLFACTORY BULB-LATERAL OLFACTORY TRACT-PIRIFORM CORTEX SYSTEM

3.1 Introduction

In Chapter 2, transfer effects were investigated in the monosynaptic entorhinal cortex-perforant path-dentate gyrus system. Even though all three sites appeared to participate strongly during the primary site kindling, the transfer kindling was surprisingly slow. Although there was significant transfer to the dentate gyrus following perforant path stimulation, the dentate gyrus still required a mean of 15.8 stimulations to reach criterion. In the entorhinal cortex, there was a significant positive transfer following primary kindling of the perforant path only in the group in which transfer kindling was delayed by 4 weeks. When the perforant path itself was the transfer site, there was a significant savings in the number of afterdischarges needed to reach criterion following dentate gyrus kindling but no savings following entorhinal cortex kindling. These results indicated that the spatio-temporal patterns of discharge, although they may appear superficially similar in the EEG recordings, must be expressed differently at the network level.

To further explore transfer effects in monosynaptic pathways, we have repeated the transfer kindling experiment. This time, we tested for transfer effects within the piriform cortex, the target structure, and the olfactory bulb, the source structure after

primary kindling of the lateral olfactory tract, the monosynaptic pathway. Based on our previous experiment, our hypothesis was that the spatio-temporal patterns of discharge during kindling of these sites would be sufficiently different to preclude immediate transfer kindling. In this case, however, we found transfer kindling to be immediate at both transfer sites.

3.2. Materials and methods

3.2.1 Animals and surgical procedures

A total of 49 male Long Evans hooded rats weighing between 280-450 g were implanted with chronic bipolar electrodes in the olfactory bulb (8.9 mm anterior, 1.2 mm, and 1.8 mm ventral to bregma), lateral olfactory tract (3.7 mm anterior, 3.4 mm lateral and 6.8 mm ventral to bregma) and piriform cortex (2.4 mm anterior, 3.2 mm lateral and 7.2 mm ventral to bregma). The electrodes were held in place by dental acrylic and 4 stainless-steel screws inserted into the skull. Animals received 15 mg/kg atropine methyl nitrate and were anesthetized with 65 mg/kg pentobarbital. The bipolar electrodes consisted of two teflon coated Nichrome wires 127µm in diameter. [illegible]e were twisted together and had a vertical tip separation of 0.5 mm. Electrodes were lowered under physiological control to ensure optimal placement for the lateral olfactory tract and piriform cortex.

3.2.2 Primary kindling

Following a 2 week post surgery recovery period, the animals were randomly assigned to olfactory bulb (n=13), lateral olfactory tract (n=23) or piriform cortex (n=13) primary site kindling groups. Stimulation consisted of a 1-4 s train of 1 ms biphasic square wave pulses, at 60 Hz. These stimulation trains were administered once every 24h at an intensity of 500 μA, which was reliably above threshold for all but a few animals. In those animals, the stimulation intensity was raised in logarithmic steps until an AD was reliably triggered (maximum intensity was 794 μA). EEG recordings were taken for all kindling sessions throughout primary and transfer kindling. The AD durations were measured daily for all sites. The motor responses were rated according to a 5 point scale (Racine, 1972b), culminating in a response that included head and facial movements, forelimb clonus, a clonic rearing and loss of postural control (stage 5). Stimulation was continued until two fully generalized seizures had occurred.

3.2.3 Transfer kindling

After 2 stage 5 seizures had been triggered from the primary site, stimulation was begun at the transfer site and continued until 2 additional stage 5 seizures were triggered. The groups that received their initial stimulation in either the piriform cortex or the olfactory bulb served as primary site controls for the animals that were transfer kindled in these sites following lateral olfactory tract kindling. In addition, the piriform cortex and olfactory bulb kindled groups both received transfer kindling stimulation in the lateral

olfactory tract, which was begun 24 hr after completion of primary site kindling. The animals that were *initially* kindled in the lateral olfactory tract were divided into 2 groups. One group received transfer kindling in the olfactory bulb which was started 24 h after completion of primary site kindling. The other group received transfer kindling in the piriform cortex after a 24 h delay. Stimulation intensity was again set a level which reliably evoked an AD.

3.2.4 Statistical analysis

Non parametric Mann-Whitney U-tests were used to compare transfer kindling against primary site kindling in the same site. For example, the rate of transfer kindling in the piriform cortex following lateral olfactory tract kindling was compared with the rate of primary site kindling in a separate groups of animals kindled first in the piriform cortex.

3.2.5 Histology

Upon completion of the transfer tests, all animals were anesthetized and perfused transcardially either with saline and formalin or with 50 cc of sodium sulfide. Their brains were removed, frozen, sectioned horizontally and stained either with Cresyl Violet/ Luxol Fast Blue or Timm stain. The sections were examined under the microscope to verify electrode placements.

3.3 Results

In this paper, 'secondary site' will refer to a site from which EEG recordings were taken but which was not directly stimulated, and 'transfer site' will refer to the second *kindled* site.

3.3.1 Electrode placements

Verification of all electrode placements was difficult because the brains were sliced horizontally in order to investigate changes in cell density and mossy fiber axonal sprouting (Chapter 5). However, in those animals where it was possible to identify the locations of the electrode tips, placements were found to be accurate.

3.3.2 Rates of seizure development

The olfactory bulb, lateral olfactory tract and piriform cortex all kindled at the same rate (about 11 ADs). Table 3.1 presents the kindling rates for the primary and transfer sites studied in this experiment. The non-parametric Mann Whitney U-test was used to calculate significance levels (Siegel, 1956). There was immediate positive transfer (stage 5 seizures within 1 or 2 stimulations) to the piriform cortex (98.2% savings) and olfactory bulb (96.5% savings) following lateral olfactory tract stimulation and immediate positive transfer to the lateral olfactory tract following either piriform cortex or olfactory bulb stimulation (99.1% and 98.4% savings respectively).

3.3.3 *Development of afterdischarge*

As previously reported, daily stimulation of the olfactory bulb, lateral olfactory tract or piriform cortex led to the appearance of epileptiform afterdischarges which became progressively longer in duration, greater in amplitude and more complex in waveform morphology (McIntyre and Goddard, 1973; Racine, 1972b). In the initial stage of kindling, the secondary sites showed similar epileptiform activity to that of the primary site in terms of AD duration, frequency and complexity. This contrasts with the entorhinal cortex- perforant path-dentate gyrus monosynaptic system in which the secondary sites initially showed very weak or even absent epileptiform activity (Chapter 2: Spiller and Racine, 1994a). Concomitantly with AD growth, the behavioural response changed from no response to a convulsive response that progressed through some or all of the 5 stages of seizure development as described by Racine (1972b). Only 7 out of the total of 49 animals progressed through all stages of seizure activity and 5 (out of 13) of these animals were kindled in the piriform cortex. This finding contrasts with those reported by Racine (1972b) who found that animals kindled in the amygdala/piriform cortex typically progressed through all 5 stages of seizure activity. Table 3.2 presents a breakdown of the number of stages of epileptiform activity that the animals progressed through during primary site kindling according to stimulation site.

AD duration was used as an index of growth of developing epileptogenesis. Mean AD durations during primary kindling for both stimulated and secondary sites are presented in Table 4.3A. The same measures for transfer kindling are shown in Table

4.3B. As previously reported, the longest AD tends to occur prior to reaching a stage 5 behavioural seizure (Racine, 1972b). Fig. 3.1 shows typical traces of EEG activity for animals kindled in the lateral olfactory tract with subsequent transfer to the piriform cortex and in the piriform cortex with subsequent transfer to the lateral olfactory tract. Fig. 3.2 shows the same for animals kindled in the lateral olfactory tract with olfactory bulb transfer and in the olfactory bulb with lateral olfactory tract transfer. The AD durations for both primary and transfer kindling were similar as were the AD durations between sites. This contrasts with the entorhinal cortex/perforant path/dentate gyrus system in which the longest AD durations were found in the entorhinal cortex, regardless of stimulation site, and the shortest in the dentate gyrus. Also, the propagated AD to the DG was found to be of longer duration in EC kindling than when the DG was directly stimulated.

Table 3.1
Primary and transfer kindling rates (ADs up to and excluding the first stage 5 seizure)

Primary site	Kindling rate	Secondary site	Kindling rate	Prob.	% Savings
PC	x = 9.9 (4-20) n=13	PC after LOT	x =.18 (0-1) n=11	P<0.001	98.2%
OB	x = 9.3 (4-18) n=13	OB after LOT	x =.22 (0-1) n=12	P<0.001	96.5%
LOT	x = 9.2 (4-20) n=23	LOT after PC	x =.08 (0-1) n=13	P<0.001	99.1%
		LOT after OB	x=.15 (2-3) n=13	P<0.001	98.4%

Table 3.2
Breakdown of the number of stages of epileptiform activity animals progressed through during primary kindling according to stimulation site

Primary kindling site	Number of stages	Number of animals
PC	5	5
	4	0
	3	7
	2	1
LOT	5	1
	4	15
	3	6
	2	1
OB	5	1
	4	4
	3	5
	2	3

Table 3.3A
Stimulated and reactive AD durations (seconds) during primary kindling

Kindling site	Site	First AD	Stage 5 AD	Longest AD
LOT kindling	OB	9.0	60.3	73.5
		(±1.1)	(±5.9)	(±5.3)
	LOT	9.0	62.0	74.5
		(±0.8)	(±5.8)	(±5.1)
	PC	9.4	62.2	75.0
		(±0.9)	(±5.7)	(±4.9)
OB kindling	OB	8.6	52.8	66.8
		(±0.9)	(±5.7)	(±6.5)
	LOT	8.6	52.8	68.0
		(±0.9)	(±5.7)	(±6.5)
	PC	8.6	53.6	67.3
		(±0.9)	(±5.9)	(±6.4)
PC kindling	OB	10.1	52.5	62.7
		(±1.1)	(±7.4)	(±7.9)
	LOT	10.2	56.3	69.5
		(±1.0)	(±7.5)	(±7.8)
	PC	10.2	58.0	68.2
		(±1.0)	(±7.6)	(±7.6)

Table 3.3B
Stimulated and reactive AD durations (seconds) during transfer kindling

Kindling site	Site	First AD	Stage 5 AD	Longest AD
LOT kindling	OB	57.4 (±4.7)	58.8 (±4.1)	64.0 (±4.6)
	LOT	55.3 (±4.7)	56.6 (±4.3)	62.8 (±4.4)
	PC	55.7 (±4.7)	57.0 (±4.3)	63.7 (±4.2)
OB kindling	OB	44.2 (±6.1)	50.4 (±5.6)	56.2 (±5.8)
	LOT	43.2 (±5.9)	49.2 (±5.5)	54.8 (±5.7)
	PC	43.7 (±5.9)	49.7 (±5.4)	56.0 (±5.5)
PC kindling	OB	46.1 (±6.2)	51.2 (±6.3)	55.8 (±6.3)
	LOT	44.7 (±4.8)	49.6 (±5.0)	52.6 (±4.9)
	PC	47.5 (±5.9)	52.4 (±5.9)	56.3 (±6.0)

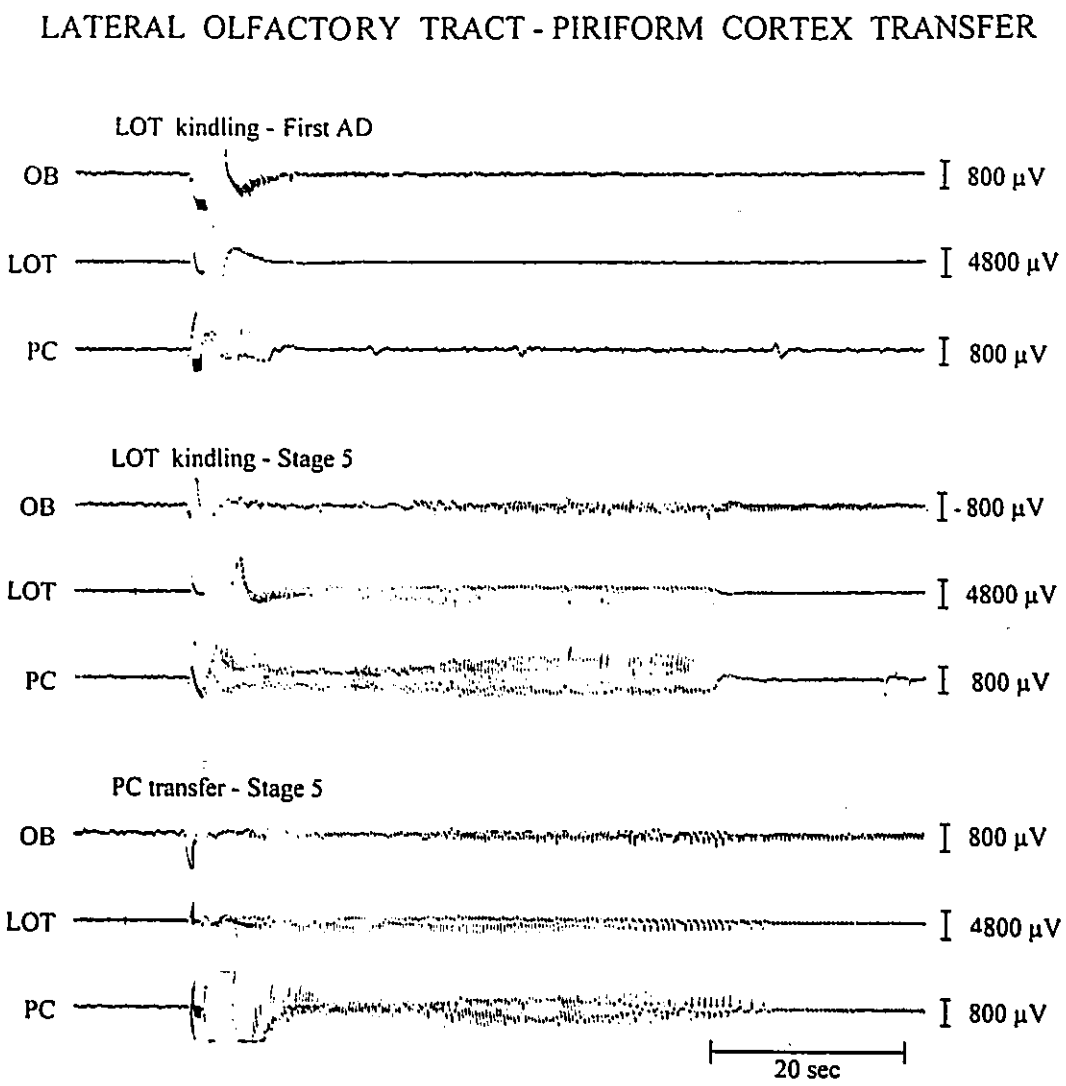


Fig. 3.1. Typical traces of the development of EEG activity for animals kindled in the lateral olfactory tract and the piriform cortex. The first section shows the first AD and the first stage 5 EEG traces during primary site (lateral olfactory tract) and secondary site (piriform cortex) kindling. The second section shows an example in which the piriform cortex served as primary site and the lateral olfactory tract as secondary site.

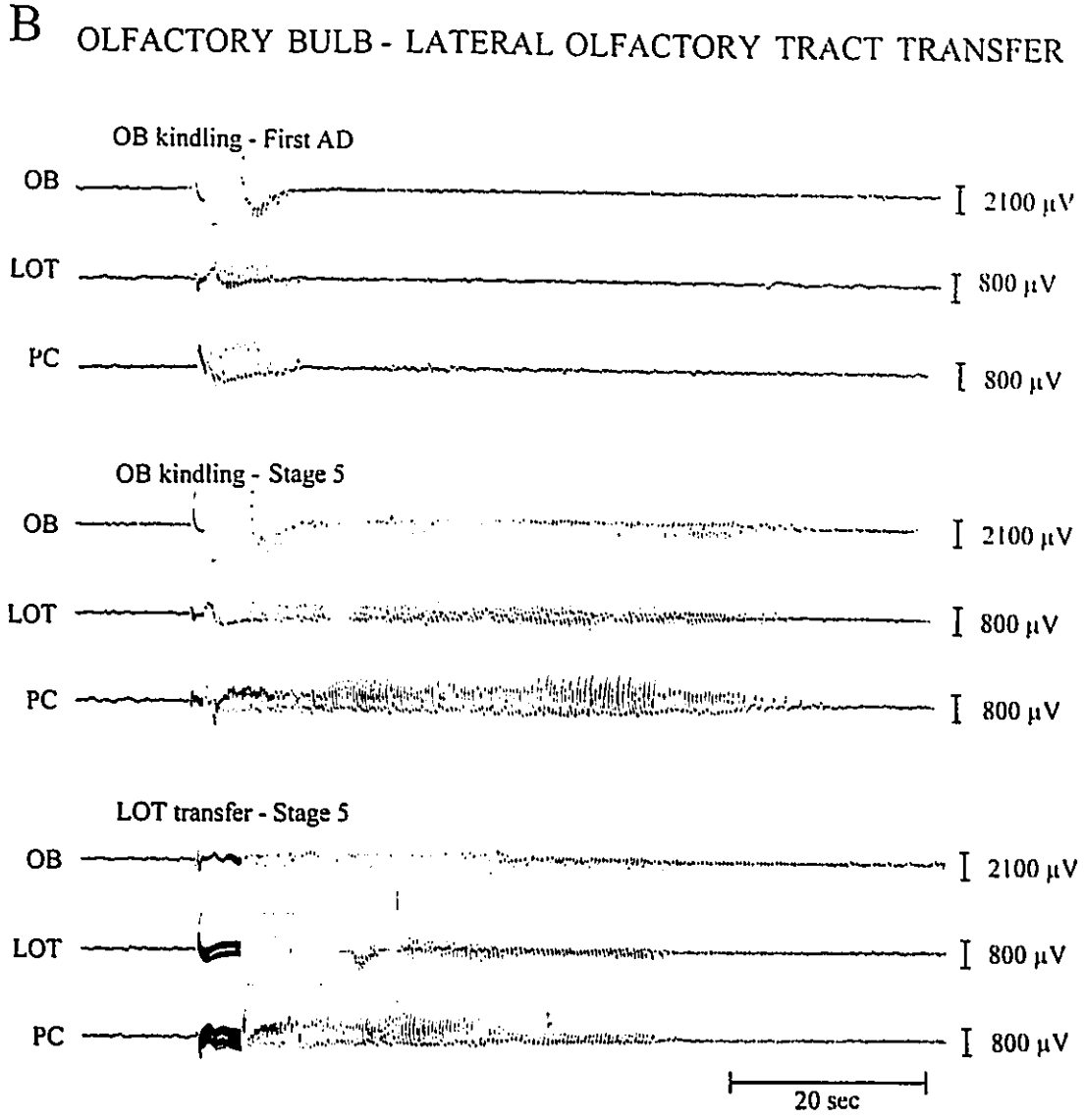


Fig. 3.2. Typical traces of the development of EEG activity for animals kindled in the lateral olfactory tract and olfactory bulb. The first section shows the first AD and the first stage 5 EEG traces during primary site (lateral olfactory tract) and secondary site (olfactory bulb) kindling. The second section shows an example in which the olfactory bulb served as primary site and the lateral olfactory tract as secondary site.

3.4 Discussion

The olfactory bulb-lateral olfactory tract-piriform cortex make up a powerful monosynaptic system that shows strong propagation of epileptiform discharge, regardless of which site is kindled. These structures all appeared to participate equally and strongly during primary site kindling, and most animals showed fully generalized seizures immediately following transfer kindling. In our previous study that looked at transfer effects in the entorhinal cortex-perforant path-dentate gyrus monosynaptic system, we found the transfer kindling to be surprisingly slow (Chapter 2). The dentate gyrus still required a mean of 15.8 stimulations prior to and excluding the first stage 5 seizure following primary site kindling of the perforant path. The entorhinal cortex required a mean of 8.6 stimulations in the transfer test (compared to 10.9 stimulations for primary kindling on that site).

When the perforant path itself was the transfer site, it only required 3.2 afterdischarges prior to and excluding the first stage 5 seizures following primary site kindling in the dentate gyrus. The perforant path required 17.1 afterdischarges, however, following entorhinal cortex kindling (compared to 15.1 stimulations for primary kindling of the perforant path). These results were interpreted to mean that the spatio-temporal patterns of discharge must be expressed differently with different kindling sites. For example, perforant path stimulation might be expected to result in diffuse input into the dentate gyrus with immediate effects upon nearly the whole extent of the structure, whereas direct stimulation of the dentate gyrus would result in localized discharge that

then propagates throughout the rest of the structure via quite different pathways.

The olfactory bulb is strongly connected to the piriform cortex via the lateral olfactory tract and the EEG recordings looked superficially similar in all three sites. Since fully generalized seizures occurred immediately following transfer kindling of the secondary site, this suggests the spatio-temporal patterns of discharge must be expressed similarly at the network level regardless of the site stimulated. The neural organization of the piriform cortex and its inputs may account for these findings. The horizontal distribution of both afferent and association fiber systems in the piriform cortex leads to sequential activation of this structure (Haberly, 1973). In other words, stimulation of the LOT results in the sequential activation of the piriform cortex and stimulation of a localized area of the piriform cortex results in a similar sequential activation of the piriform cortex. Thus, activation of the piriform cortex would proceed in a similar way regardless of the site of stimulation and may account for the immediate transfer effects found in this study. On the other hand, more caudally placed piriform cortex electrodes would be expected to show greater deviations from this common discharge pattern, and, perhaps, transfer more slowly from OB and LOT kindling.

Another possible explanation for these results may be the extent of the inhibition in this monosynaptic pathway. Experiments in which the paired pulse technique has been used to measure the amount of recurrent or feedforward inhibition in the piriform cortex have shown a net facilitation effect in this site (Racine, Moore and Evans, 1991, Chapter 5). That is, the amplitude of the second response was larger than the first response at all

inter-pulse intervals, suggesting that recurrent inhibition is not normally very strong. Following kindling, the piriform cortex showed a decrease in the paired pulse facilitation (Racine et al, 1991; Chapter 5), indicating an increase in inhibition. In most animals, however, there was still a net facilitation. The apparently weak inhibitory system might account for the fast kindling rates found in the piriform cortex as well as for the immediate transfer effect.

In any case, these results raise some interesting questions about the propagation of epileptiform discharge. The differences in transfer rates in these two monosynaptic systems indicates that the preferred route of propagation of epileptiform discharges and the development of secondary epileptogenesis are heavily dependent upon the site of the primary focus. That is, the architecture of the epileptogenic network will depend upon its precise location. From a mechanistic perspective, the pattern of neuronal remodelling that occurs during kindling appears to be somewhat more specific to the stimulating electrode location in the hippocampal system than in the olfactory system.

The pattern of cell loss that can develop may also be dependent upon the specific site of initial stimulation. For example, kindling the perforant path resulted in cell loss in the hilus of the dentate gyrus (Chapter 4; Spiller and Racine, 1994b) but kindling the lateral olfactory tract resulted in no observable cell loss in the hilus (Chapter 5). A small study by Babb, Lieb, Brown, Pretorius and Crandall (1984) looked at the distribution of pyramidal cell density and hyperexcitability in patients with temporal lobe epilepsy and found that focal signs of hyperexcitability in the hippocampus correlated with a selective

loss of pyramidal cell neurons in that region. Thus, focal excitability in the anterior hippocampus correlated with pyramidal cell loss in the anterior region whereas, more widespread hippocampal hyperexcitability was correlated with pyramidal cell loss throughout the hippocampus. We continue to explore the nature of the neuronal remodelling induced by kindling in the next chapter.

Chapter 4

THE EFFECT OF EXTENDED KINDLING ON PAIRED-PULSE DEPRESSION AND HILAR CELL COUNTS IN THE DENTATE GYRUS

4.1 Introduction

One of the mechanisms most frequently proposed by those who study epilepsy is failure of inhibition (Bruton, 1988; Burnham, 1989; Kapur and Lothman, 1989; Kapur, Michelson, Buterbaugh and Lothman, 1989; Kapur, Stringer and Lothman, 1989; Kamphuis, Wadman, Buijs, Lopes da Silva et al, 1986). It is likely that a transient failure of inhibition underlies the *triggering* of seizures by electrical stimulation (Morimoto, 1989; Tuff, Racine and Adamec, 1983), and by the application of epileptogenic agents which induce their effects by blocking inhibitory neurotransmitters (Goddard, 1967; Wood, 1975). These transient failures, however, cannot account for the permanent increases in susceptibility to epileptic responses seen in the kindled preparation. The evidence for more enduring alterations in the function of inhibitory systems is mixed.

Tests designed to provide such evidence have typically utilized the paired-pulse paradigm to investigate the characteristics of inhibition in forebrain sites, particularly the hippocampus. The first pulse delivered to the input pathway activates cell discharge which, via collaterals, activates interneurons that exert a recurrent inhibitory effect on the same population of cells. The second evoked response will then be depressed if the

interpulse interval (IPI) falls within the period of recurrent inhibition. One of the most frequently tested sites is the dentate gyrus (Adamec, McNaughton, Racine and Livingston, 1981; Matthews, McCafferty and Setler, 1981). In that system, there is also a second late phase of inhibition which is observed at IPIs ranging from 100 ms to several seconds (de Jonge and Racine, 1987; Tuff, Racine and Adamec, 1983). This component probably reflects various late afterhyperpolarizations (de Jonge and Racine, 1987). These various depression effects depend upon the evocation of cell discharge.

A number of studies, using the paired pulse technique, have monitored the effects of kindling on GABA-mediated inhibition in the dentate gyrus (de Jonge and Racine, 1987; King, Dingledine, Giacchino and McNamara, 1985; Maru and Goddard, 1987; Oliver and Miller, 1985; Racine, Milgram and Hafner, 1983; Tuff, Racine and Adamec, 1983), including several that followed the time course of these effects throughout the kindling process (de Jonge and Racine, 1987; Racine, Milgram and Hafner, 1983). In all cases, kindling was found to produce an increase in the amount of paired pulse depression during the period of recurrent inhibition. Several of the studies also reported an increase in the late depression (100 ms to several seconds), suggesting a possible increase in one or more of the late afterhyperpolarization components (de Jonge and Racine, 1987; Tuff, Racine and Adamec, 1983). Similar kindling-induced increases in paired-pulse depression have recently been reported in the piriform cortex, although the increased depression is primarily seen as a reduction in paired-pulse facilitation which is the predominant effect at that site (Stripling, Patneau and Gramlich, 1988; Racine, Moore and

Evans, 1991).

One site which has consistently shown a kindling-induced *decrease* in paired pulse depression is area CA1 of the hippocampus (Kapur and Lothman, 1989; Kapur, Stringer and Lothman, 1989; Kamphuis, Huisman, Veerman and Lopes da Silva, 1991; Kamphuis and Lopes da Silva, 1990). It has been suggested that this might serve as a mechanism underlying the kindling phenomenon, but it has been shown that the hippocampus can be removed without having much effect on kindling from other sites (Racine, Paxinos, Mosher and Kairiss, 1988).

Although kindling appears to produce increases in inhibition in the dentate gyrus, the picture appears to be more complex for models of status epilepticus. Several studies have used the paired pulse technique to monitor inhibition in the dentate gyrus during and after episodes of status epilepticus (Cornish and Wheal, 1989; Kapur and Lothman, 1989; Milgram, Yearwood, Khurgel, Ivy and Racine, 1991; Sloviter, 1987; Sloviter, 1991; Tuff, Racine and Adamec, 1983). In these models, an epileptogenic agent is applied at sufficient levels or for a sufficiently prolonged period to induce a state of continually recurring seizures. These can be relatively mild or severe. In either case, if prolonged they can lead to brain damage and death. Sloviter (1987) reported a decrease in paired-pulse inhibition in the dentate gyrus following induction of status by prolonged periods of electrical stimulation. Milgram et al (1991), on the other hand, found an increase in recurrent or feedforward inhibition following kainic acid induced status epilepticus. Upon histological examination in both of these studies, there was similar damage found in

the hilar region of the dentate gyrus and to pyramidal cells of CA3.

Cavazos and Sutula (1990) looked at neuronal loss in the hilar polymorphic region of the dentate gyrus following kindling. They found a 12.7% loss of neurons after 3 stage 5 seizures and a 40.1% loss after 30 stage 5 seizures. The hilar damage is at least superficially similar to that reported in the status models. It is possible that induction of seizure activity produces degenerative effects, and that kindling and status are simply at two ends of a continuum of damaging activation levels. The excessive kindling used by Cavazos and Sutula may begin to produce effects that are similar to the relatively mild status induced by Sloviter. If the same cells are lost in both cases, and if Sloviter is correct in his proposal that these cells are driving inhibitory interneurons and that their loss accounts for the reduced inhibition (Sloviter, 1987), then the induction of 30 or more stage 5 seizures should reverse the kindling-induced increase in inhibition.

The present study was designed to monitor the changes in inhibition in the dentate gyrus that occur throughout a kindling process which culminates in the induction of 44 stage 5 seizures. These measures were also monitored during a post-kindling recovery period spanning 5 weeks. In addition, cell counts were taken in the hilar region of the dentate gyrus in an attempt to confirm the results reported by Cavazos and Sutula (1990). The additional objective of these experiments was to establish a baseline of inhibition and cell loss effects that could be used for comparison against the effects on these measures of kindling other sites. If these structures are part of common seizure propagation systems, then similar effects might be expected regardless of kindling site. It is clear from the

literature, for example, that both enhanced dentate gyrus inhibition (Tuff, Racine and Adamec, 1983) and decreased hilar cell density (Cavazos and Sutula, 1990) can be induced by either amygdala or perforant path kindling.

4.2 Materials and methods

A total of 24 male Long Evans hooded rats, 17 of which had been utilized previously in a transfer experiment, were used in this study. In the transfer study, primary kindling occurred either in the dentate gyrus (n=3), perforant path (n=11) or entorhinal cortex (n=3) and then the animals that were kindled in the dentate gyrus or entorhinal cortex were transfer-kindled in the perforant path while the animals that were kindled in the perforant path were transferred to either the dentate gyrus or the entorhinal cortex. The criterion for kindling was 2 stage 5 seizures (Racine, 1972b) for both the primary and transfer sites, so each animal came into the present study having had 4 stage 5 seizures, 2 of which were triggered from the perforant path.

These rats, weighing between 300-500g, were implanted with chronic bipolar electrodes, in the entorhinal cortex, perforant path and the dentate gyrus, which were held in place by dental acrylic and 3 stainless steel screws inserted into the skull. Animals received i.p. injections of 15 mg/kg atropine methyl nitrate and were anesthetized with 65 mg/kg pentobarbital. The electrodes were made from two teflon coated Nichrome wires 127μm in diameter. These were twisted together with a vertical tip separation of 0.5 mm. Electrodes were lowered under physiological control to ensure optimal placement for the

perforant path and the dentate gyrus. The additional 7 animals were prepared with electrodes in the same 3 sites and served as implanted, non-kindled controls.

Following the transfer experiment, animals were divided into 2 experimental groups. One group ('4-stage 5' group), received no additional kindling stimulations, while the animals in the other group ('+40-stage 5' group) were kindled in the perforant path until they experienced 40 additional stage 5 seizures. The kindling parameters consisted of a 1 s train of 1 ms 60 Hz biphasic square wave pulses given once every 24 h at an intensity of 600 μA. This was sufficient to reliably trigger afterdischarges (ADs) in all but 2 animals. Stimulation intensities were increased in 100 μA steps in these animals until ADs were reliably evoked (max 800 μA).

4.2.1 Paired-pulse tests

Every animal used in this experiment showed stable characteristic evoked field potentials in the dentate gyrus when stimulation pulses were applied to the perforant path. Awake and freely moving animals were given paired-pulse tests with the stimulation intensity set at 1000 μA, and 10 sweeps were averaged at each of 12 interpulse intervals: 20, 30, 40, 50, 70, 100, 150, 200, 250, 300, 500, 750 ms. The intervals were tested sequentially. We have found in unpublished experiments that the order of testing has no effect on these measures. Pulse pairs were separated by 10 s (0.1 Hz). Two of these paired-pulse tests were given prior to primary site kindling in the kindled groups. They were given again after completion of primary site kindling and again after transfer

kindling. Paired-pulse measures were then taken once a week. In the +40-stage 5 group, these measures continued until an additional 40 stage 5 seizures had been evoked and 5 weeks of subsequent recovery time had elapsed. The 4-stage 5 animals had similar paired-pulse measures taken except that no additional stage 5 seizures were induced. The number of weeks of paired-pulse measures following transfer kindling in this group was determined by the median time required for the experimental group to show the additional 40 stage 5 seizures and the rate at which the paired-pulse response returned to baseline levels. The implanted control group was tested over a period which also included the median time required for the primary and transfer kindling of the kindled groups and subsequent recovery. Consequently, the paired-pulse measures in this group were distributed comparably to those in the kindled groups. Both EPSP slope and population spike measures were taken from the paired pulse data. The population spike amplitudes were measured from the tangent line, falling on the onset and offset of the spike, to the peak of the spike. The depression/facilitation ratios were determined by dividing the 2nd (test) response by the 1st (conditioning) response. The results were then multiplied by 100 for plotting.

4.2.2 Hilar cell counts

Upon completion of the kindling and paired pulse tests, the rats were deeply anesthetized and perfused transcardially with saline and formalin. The brains were soaked in a glucose solution overnight and then frozen and sliced horizontally into 20 μm

sections using a cryostat. Every 5th slice was mounted on a slide and stained with Cresyl Violet and Luxol Blue.

From the stained horizontal sections of hippocampus. 6 sections were selected at approximately 400 μm intervals along the dorsal-ventral axis. Photographs of the hilar polymorphic region of the dentate gyrus were taken of each section. Both ipsilateral and contralateral hilar regions were photographed making a total of 12 samples for each animal. Special effort was made to choose sections from equivalent locations along the dorsal-ventral axis in each animal and in each group. Neuron counts were obtained from neurons found within a 74,925 μm^2 grid and were counted by a technician who was blind to the experimental hypothesis and the group membership. Cells that were on the boundary of the grid were counted only if at least half of the cell body fell within the grid. Neurons were distinguished primarily by size, shape and presence of a nucleus.

4.3 Results

4.3.1 *Kindling and paired-pulse tests*

The smallest and largest number of stimulations required to complete the initial primary site plus transfer site kindling was 17 and 78, respectively. The comparable delay period utilized in the implanted control group was 29 days. Animals that were kindled to an extra 40 stage 5 seizures, also differed in the number of additional stimulations they required to reach this criterion. The animal that reached the criterion the fastest required an additional 42 AD-invoking stimulations to accumulate 40 additional generalized motor

seizures while the slowest animal required 70 (AD-invoking) stimulations. Both the implanted control group and the 4-stage 5 group received weekly paired-pulse tests over about 70 days to approximate the time necessary to complete +40 stage 5 seizures and to recover. This number of paired-pulse tests was still somewhat less than the number taken for many of the animals in the +40-stage 5 group, but it was sufficient to cover the period during which critical changes were seen in this group.

The conditioning responses showed quite good intra-session stability in all tests and in all animals (Fig. 4.1). The test responses showed the characteristic depression of the population EPSP from 20 ms to about 200-500 ms. The population spike amplitudes were depressed at short IPIs (20-30 ms), then facilitated at intermediate intervals (30-100 ms) and finally depressed again at long intervals (150-750 ms). These three phases were seen in all animals tested. It is the early component of depression that has been attributed to the activation of basket cells and possibly other interneurons resulting in recurrent and feedforward inhibition. The spike onset latency, or latency to peak, showed only a depression (i.e. an increased latency) which generally had not fully recovered by 750 ms. Fig. 4.1 shows the sweeps for a typical IPI run.

Typical paired-pulse IPI curves before, during and after kindling for the implanted control, 4-stage 5 and +40-stage 5 groups are shown in Fig. 4.2. These curves are based upon population spike amplitude measures. The baseline curves for the two kindled groups show less facilitation and late onset inhibition than the implanted control group,

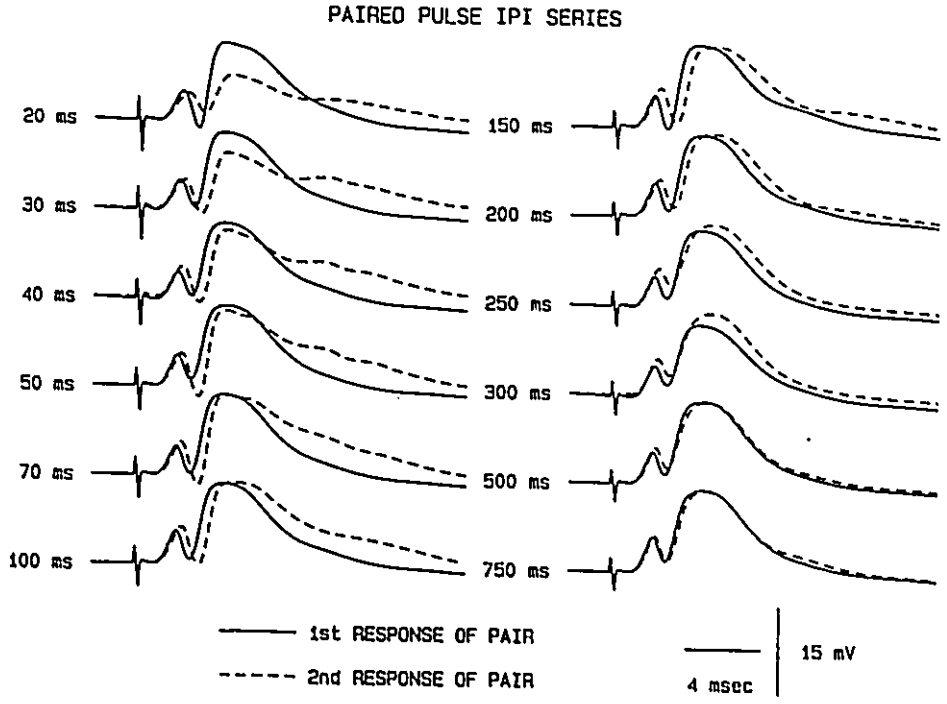


Fig. 4.1. An example of field potentials evoked in the dentate gyrus by paired-pulse stimulation of the perforant path using 12 interpulse intervals ranging from 20 to 750 ms. Solid line: an average of 10 conditioning evoked potentials. Dashed-line: an average of 10 test evoked potentials at the indicated intervals.

Price, 1978). Many structures of the olfactory cortex are reciprocally connected with each other. For example, the piriform cortex, olfactory peduncle and amygdaloid cortex also send fibers to the olfactory bulb (Luskin and Price, 1983). Efferent pathways from comparable in the three groups. Significant changes were evident in this component of inhibition following kindling. For example, following initial transfer kindling (row 1 in Fig. 4.2), the early inhibition was increased and prolonged (20-70 ms) in both kindled groups. Equivalent increases in inhibition were seen in the 4 stage 5 group whether the perforant path stimulation was delivered during the primary kindling or during the transfer kindling. All subsequent stimulations, of course, were delivered to the perforant path. The paired-pulse measures remained unchanged in the implanted control group over a comparable time period. These two IPI curves are retained as reference curves in rows 2, 3 and 4 of Fig. 4.2. The levels of inhibition in the 4-stage 5 group returned to baseline levels over a period of 4-5 weeks (33 days, row 3) and was completely back to baseline following 61 days of recovery (row 4). The inhibition remained potentiated in the +40-stage 5 group throughout the subsequent kindling to 40 additional stage 5 seizures (row 2, column 3) and then returned to near baseline levels after 4 weeks (row 4). The measures for the implanted control group remained stable over the full course of the experiment (rows 2, 3 and 4). There was no change in inhibition at the longer IPIs (150-750) during kindling in contrast to the results previously reported by Tuff et al (1983) and deJonge and Racine (1987).

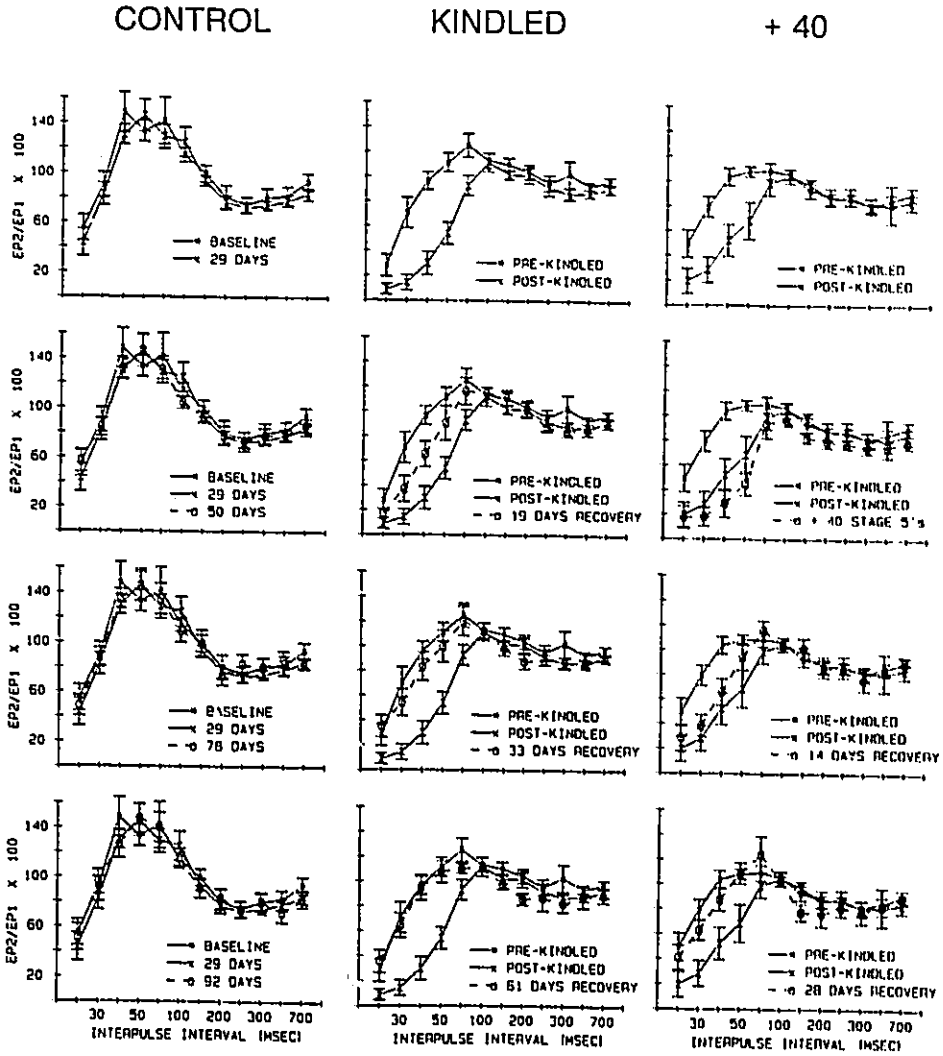


Fig. 4.2. The IPI curves are shown for each group at selected points over the course of the experiment. The measures are based upon population spike amplitudes. The ratios were calculated by dividing the amplitude of the population spike of the first evoked potential (EP1) by the population spike amplitude of the second evoked potential (EP2). The first row shows the baseline (PRE-KINDLED) curves and the curves following 4 stage 5 seizures (POST-KINDLED) for the 4-stage 5 (KINDLED) group and the 40-stage 5 (+40) group. The corresponding curves for the implanted control group (CONTROL) were separated by 29 days. These curves were then replotted as a reference in each of the subsequent panels. The 2nd row shows the stability in the implanted controls at a delay of 50 days, the partial recovery in the 4-stage 5 group after a delay of 19 days, and the maintained enhancement of inhibition in the +40 group after 40 additional stage 5 convulsions. The remaining panels show the continued stability in the implanted control group (up to 92 days), the continued recovery to baseline in the KINDLED group and the recovery to near baseline levels in the +40 group.

Fig. 4.3 (column 3) shows an example of response pairs at a 30 ms interpulse interval at baseline, following 4 stage 5 seizures, following an additional 40 stage 5 seizures, and after a recovery period for one animal in the +40-stage 5 group. Sample responses are shown for comparable time periods in the implanted control (column 1) and the 4-stage 5 (column 2) groups. The animal representing the +40-stage 5 group was well matched to the control for both response morphology and amount of baseline inhibition. We selected an animal showing a stronger baseline inhibition for the 4-stage 5 group to show that the kindling and recovery effects are not dependent upon initial levels of paired pulse depression.

Fig. 4.4 shows similar progressions based on group averages of measures at the 30 ms IPI. In this figure, we see that primary site kindling produces near maximal increases in paired-pulse depression. There is some additional increase after transfer kindling. The continuation of kindling to a total of 44 stage 5 seizures did not have much additional effect on the paired-pulse response except to maintain the increased depression. Both kindling groups began to recover towards baseline as soon as the kindling stopped. The rate of recovery for the 2 kindling groups was also comparable (the last 2 intervals represented on the curves are approximately double the duration of the earlier intervals).

A repeated measures ANOVA was run for both the EPSP slope and population spike heights using the data from the 30 ms interval. The analysis of slope data showed significant main effects for GROUP ($p<0.002$) and for SESSIONS ($p<0.001$), but there was no significant interaction ($p>0.1$) because of the variability in these measures. The

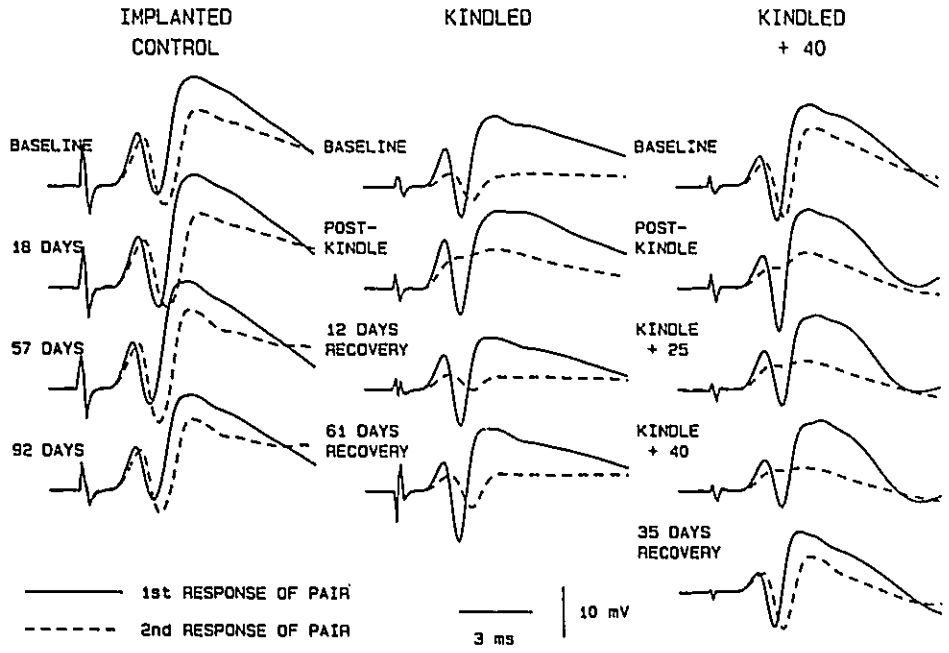


Fig 4.3. Sample sweeps from selected points over the course of the experiment are shown. The solid lines represent the conditioning response and the dashed lines represent the test responses. The interpulse interval is 30 ms. The left hand colum shows the stability of the evoked responses and the paired-pulse effect over a 92 day test period in the implanted control group. The middle column shows the increase in paired-pulse depression that results from kindling to 2 stage 5 seizures and the recovery seen at 12 and 61 days. The data for 2 stage 5 seizures are shown because that is one of the standard criteria for completion of kindling in the literature. The right hand column shows the increased depression post-kindling (2 stage 5 seizures) and, in this animal, a further small increase in depression following 25 and 40 additional stage 5 seizures. Most of this additional increase actually developed over 2-4 seizures. Also shown is the recovery at 35 days.

analysis of spike data, however, showed significant main effects for GROUP ($p<0.001$), SESSIONS ($p<0.001$) and a significant interaction of GROUP by SESSION ($p<0.001$). Fig. 4.4 shows that this interaction is due to the effect of kindling on the paired-pulse measure and to the maintenance of the enhanced depression in the +40-stage 5 group (which delayed the onset of recovery in that group).

4.3.2 Hilar cell counts

Photographs of dorsal and ventral sections of the hilar region of the dentate gyrus are displayed in Fig. 4.5 for an implanted control (CONTROL) animal and an animal from the +40-stage 5 (40 STAGE 5s) group. Fig. 4.6 represents graphically the findings of the cell counts in the 3 groups. A repeated measures ANOVA showed that there was no significant hemisphere effect, so these data were combined for Fig. 4.6. There was, however, a significant effect of section depth. There were 26.8% fewer cells counted in the most dorsal sections then in the most ventral sections for all groups of animals (main effect of section depth: $p<0.001$). There was also a significant effect of kindling. The 4-stage 5 group showed an 11.5% decrease in the number of cells counted in the hilus, while the +40-stage 5 group showed a 15.2% decrease (main effect of kindling: $p<0.009$). There was no significant interaction between section depth and kindling.

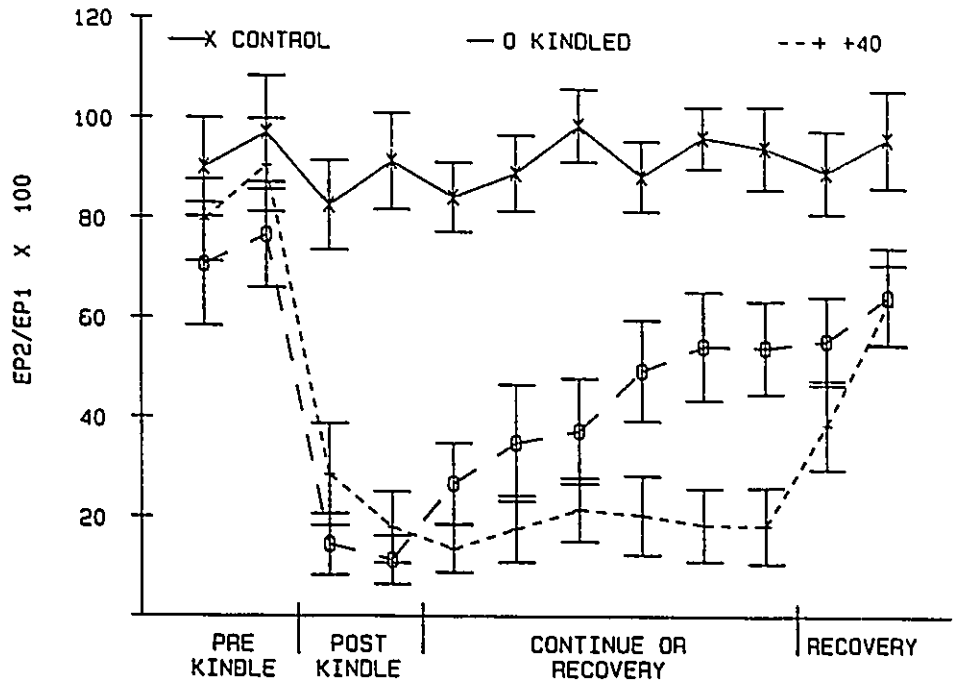


Fig. 4.4. This figure shows the group paired-pulse depression measures (± S.E.M.) for the 30 ms IPI. Each point follows the previous point by 1-2 weeks, except for the last 2 points which follow at 2 and 3 weeks. There was no significant change in response over time for the implanted control (CONTROL) group. Both kindled groups show a kindling-induced increase in paired-pulse depression followed by a post-kindling recovery to near baseline levels. The recovery for the 4-stage 5 (KINDLED) group began after the completion of primary and transfer kindling (during the period labelled CONTINUE or RECOVERY). The 40-stage 5 animals continued to receive kindling stimulations during this period and retained their increase in paired-pulse depression. When kindling was stopped, however, the responses in this group also recovered to near baseline levels (RECOVERY).

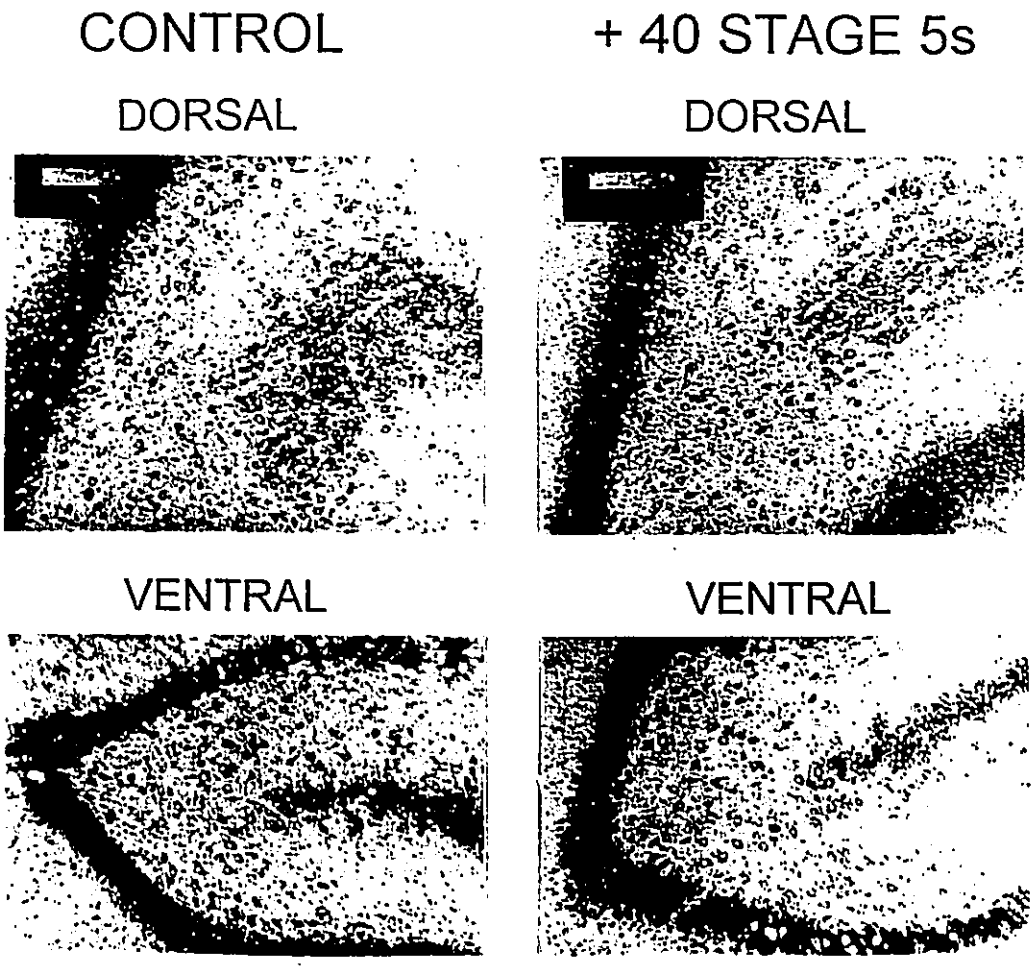


Fig 4.5. Examples of dorsal and ventral sections of the hilus of the dentate gyrus for both an implanted control and a 40-stage 5 animal are shown in the micrographs above. Cells within a 74,925 μm^2 grid, which was placed in similar locations in the hilus for each section, were counted. Bar = 200 μm.

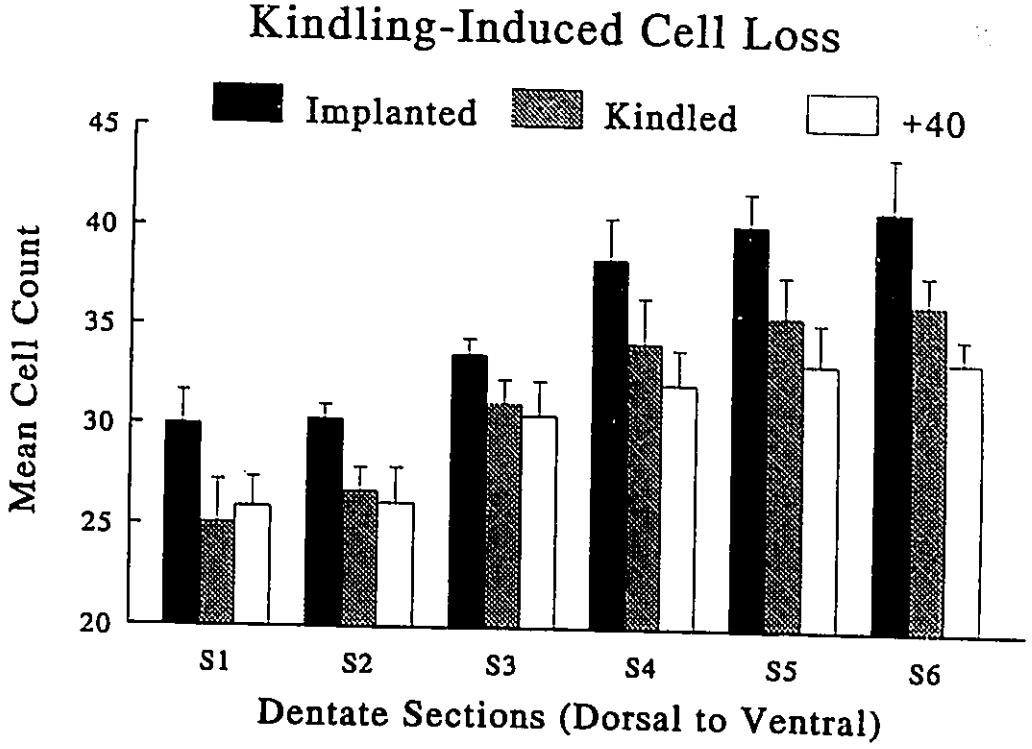


Fig. 4.6. The above graph shows the extent of kindling-induced cell loss in the hilus of the dentate gyrus. The most dorsal sections had 26.8% fewer cells overall across all groups than the most ventral sections. Between groups, there was an 11.5% decrease in the number of cells counted in sections from the 4-stage 5 (KINDLED) group and a 15.2% decrease in the nuber of cells counted in the sections from the 40-stage 5 (+40) group as compared to the implanted control group ($P < 0.009$).

4.4 Discussion

Milgram, Yearwood, Khurgel, Ivy and Racine (1991) found an increase in paired-pulse inhibition, following recovery from kainic acid induced status epilepticus, which lasted for at least one month. Similarly, in this study, we found that kindling animals to 44 stage 5 seizures produced a progressive increase in recurrent inhibition in the dentate gyrus in freely moving animals. This effect continued throughout the kindling process but decayed to near baseline levels after 4-5 weeks without AD invoking stimulations. Both of these epileptogenic models induce excessive seizure activity, but in a controlled fashion. For example, Milgram et al. (1991) terminated the ongoing status by administering an anticonvulsant 1 h after the onset of motor seizures. With more prolonged episodes of status epilepticus (Sloviter, 1987; Sloviter, 1991; Sloviter and Damiano, 1981) a decrease in paired-pulse inhibition has been reported.

We also demonstrated a decrease in the number of cells in the hilus of the dentate gyrus following kindling. There was an 11.5% decrease in the number of cells following the induction of 4 stage 5 seizures and a 15.2% decrease following an additional 40 stage 5 seizures. This reduction was not as large as that reported by Cavazos and Sutula (1990) who found a 12.7% decrease after 3 stage 5 seizures and a 40.1% decrease following 30 stage 5 seizures.

Sloviter (1991) used immunocytochemical staining to address the question of which cell types are lost following seizure activity. He found a nearly complete loss of mossy cells and somatostatin immunoreactive interneurons. He also reported

electrophysiological evidence for a decrease in GABA mediated recurrent inhibition following recovery from status. Sloviter suggested that the mossy cell normally excite GABA-containing basket cells of the dentate gyrus, and their loss could explain the seizure-associated loss of inhibition he found. Although they did not distinguish between the types of cells lost, Milgram et al. (1991) found damage to the hilar region of the dentate gyrus which was similar to that reported by Sloviter. Cavazos and Sutula (1990) found cell losses in this same region as a result of kindling and we have confirmed this effect in the present paper. It seems likely that the same type of cells may be susceptible to all of these epileptogenic treatments. They do not, however, produce a consistent effect on inhibition. We found only an increase in inhibition with kindling and Milgram et al. (1991), using a status model, also found an increase in inhibition. Sloviter (1991) has claimed that mossy cells are highly susceptible to damage making it likely that their damage is common to all these seizure models. The very different effects on measures of inhibition, however, renders it unlikely that mossy cell loss can account for increased epileptogenesis via a denervation of GABA interneurons. Obviously, more research is needed to resolve the question of which types of cells are lost in each of these models and how that loss is related to seizure activity and to subsequent increases in seizure susceptibility. In addition, Sloviter (1992) has recently reported that a kainic acid induced status resulted in a transient decrease in inhibition which was replaced by normal or enhanced inhibition. These results are consistent with those outlined in the Milgram, et al. (1991) paper. Although Sloviter (1992) attempted to relate the restoration and

enhancement of inhibition in the kainic acid model to sprouting in the mossy fibers, Milgram et al. (1991) found that failed inhibition could recover and progress to an enhanced state within hours of exposure to kainic acid precluding any contribution of functional axonal sprouting.

In conclusion, these results show some effects that appear to be common to each of the kindling sites tested in Chapter 2. Measures of inhibition showed equivalent enhancements of inhibition regardless of which site received primary site kindling. The literature shows similar effects (ie enhanced inhibition in the dentate gyrus) even when the kindling stimulations are applied to the amygdala. Similarly, decreases in hilar cell density can be found with kindling of either the perforant path or amygdala. These results indicate that at least some seizure-induced effects are common across kindling sites or even seizure models. This question will be addressed again in Chapter 5. Furthermore, the results indicate that hilar damage is not necessarily accompanied by a decreased inhibition, and decreased inhibition, at least in the dentate gyrus, is not likely to provide an explanation for the increased epileptogenesis seen in kindling.

Chapter 5

THE EFFECT OF EXTENDED KINDLING ON PAIRED-PULSE DEPRESSION IN THE PIRIFORM CORTEX AND ON HILAR CELL DENSITY AND MOSSY FIBER SPROUTING IN THE HIPPOCAMPUS

5.1 Introduction

As described in Chapter 4, a number of studies have reported results that are consistent with a failure of inhibition as a mechanism of epileptogenesis. For example, kindling studies looking at the effect of kindling on recurrent or feedforward inhibition in the CA1 of the hippocampus found decreases in inhibition (Kamphuis, Gorter, Wadman and Lopes da Silva, 1992; Zhao and Leung, 1991; Kapur, Stringer and Lothman, 1989; Kapur, Michelson, Beterbaugh and Lothman, 1989; Kamphuis, Lopes da Silva and Wadman, 1988; King, Dingledine, Giacchino and McNamara, 1985; Krnjevic, 1983; Ben-Ari, Krnjevic and Reinhardt, 1979) which persisted for at least 3 weeks (Zhao and Leung, 1991). The magnitude of the depressed inhibitory response was positively correlated to the number of seizures induced (Kapur, Stringer and Lothman, 1989; Lancaster and Wheal, 1984). Similar losses of paired-pulse inhibition in CA1 have been found using kainic acid (Cornish and Wheal, 1989; Lancaster and Wheal, 1984). A persistent loss of paired pulse inhibition was also found in the *dentate gyrus* following an episode of electrically-induced status epilepticus (Sloviter, 1987; Kapur and Lothman, 1989).

Milgram, Yearwood, Khurgel, Ivy and Racine (1991), using a more controlled kainic acid induced status epilepticus model, found a transient loss of inhibition in the dentate

gyrus which disappeared within 24 hours. With subsequent testing, they found an increase in inhibition above baseline levels and this increase lasted for at least 1 month. Using the kindling paradigm, an increase in inhibition has been demonstrated in the dentate gyrus (Oliver and Miller, 1985; Tuff, Racine and Adamec, 1983; Stringer and Lothman, 1989; de Jonge and Racine, 1987, Lothman, Stringer and Bertram, 1992; Milgram, Michail, Cammisuli, Head, Ferbinteanu, Reid, Murphy and Racine, 1995) which recovered toward baseline levels about 5 weeks after the last kindling stimulation. The increased inhibition in the DG would be expected to act in opposition to the developing epileptogenesis (Kamphuis and Lopes da Silva, 1990). Few paired pulse measures have been taken outside of the hippocampus in kindling experiments. Racine, Moore and Evans (1991) reported a net facilitation effect in baseline paired pulse measures taken in piriform cortex when stimulation was applied to the lateral olfactory tract. There was a kindling-induced *decrease* in facilitation, possibly reflecting an increase in inhibition. These effects were long-lasting but recovered towards baseline levels over about 3 months.

Recently, there have been reports of actual structural changes in the brains of kindled animals. Cell loss, for example, has been found in the hilus of the dentate gyrus (Cavazos and Sutula, 1990; Cavazos, Das and Sutula, 1994; Chapter 4: Spiller and Racine, 1994b) and in area CA3 (Cavazos and Sutula, 1990; Cavazos, Das and Sutula, 1994) following kindling. Using immunocytochemical staining techniques, Sloviter (1987) determined that the GABA containing interneurons thought to mediate inhibition

in the dentate gyrus were not damaged in this status epilepticus model but that somatostatin-containing interneurons and mossy cells were almost completely lost. He suggested that the mossy cells normally excite GABA-containing basket cells of the dentate gyrus, and their loss could explain the seizure-associated loss of inhibition he found. On the other hand, Bertram and Lothman (1993) found cell loss following an episode of status epilepticus but no cell loss associated with kindling. They did, however, find an increase in the volume of the dentate gyrus following kindling which they felt could explain the apparent cell loss reported by Cavazos and Sutula. Similarly Khurgel, Switzer, Teskey, Spiller, Racine and Ivy (1995) found proliferation and hypertrophy of astrocytes following kindling but no kindling-specific degenerative changes in neural cells.

Recently, there has been much interest in the phenomenon of sprouting in the mossy fibers of the dentate gyrus granule cells as assessed with Timm stains. It is not yet clear what constitutes a necessary and sufficient stimulus to induce mossy fiber sprouting. Neural damage appears to be an effective stimulus for mossy fiber axonal sprouting (Ben-Ari, 1985; Nadler, Perry and Cotman, 1978), but the hypersynchronous activity associated with seizures may also serve as a trigger for neural growth. In kainic acid-induced status models, it is thought that the destruction of pyramidal cells in the CA3/CA4 of the hippocampus (Ben-Ari, 1985; Nadler, Perry and Cotman, 1978) and of hilar cells in the dentate gyrus (Sloviter, 1992) triggers the sprouting of the mossy fibers of the dentate granule cells. These fibers then cross the granule cell layer and form a plexus in the inner

molecular layer of the granule cell dendrites (Sundstrom, Mitchell and Wheal, 1993; Cronin and Dudek, 1988). In kindling models, researchers have found similar sprouting effects, including the formation of novel synaptic contacts between the sprouting mossy fibers and the basilar dendrites of the CA3 pyramidal neurons (Represa and Ben-Ari, 1992). The familiar plexus in the inner molecular layer (IML) of the granule cell dendrites was also seen following kindling (Cavazos, Golarai and Sutula, 1991; Sutula, Xiao-Xian, Cavazos and Scott, 1988). Initially, these effects were seen in the absence of any overt hippocampal damage (Sutula et al, 1988, Represa and Ben-Ari, 1992). In human temporal lobe epilepsy, there have been reports of both moderate to severe loss of neurons in the hilus, CA1 and CA3 (Meldrum and Corsellis, 1984; Bruton, 1988) and of mossy fiber sprouting into the supragranular layer of the dentate gyrus (Sutula, Cascino, Cavazos, Parada and Ramirez, 1989; Masukawa, Uruno, Sperling, O'Connor and Burdette, 1992). However, Qiao and Noebels (1993), using a mutant mouse with inherited spike-wave seizures, found that mossy fiber axonal sprouting into the IML followed the onset of hypersynchronous discharge, not cell loss.

The functional significance of mossy fiber sprouting and its role in epileptogenesis has yet to be determined. Sutula and his colleagues (Sutula et al, 1988; Cavazos, Golarai and Sutula, 1991) found alterations in the circuitry early in the course of kindling, before the development of generalized seizures. They suggested that the abnormal synchronous activity induces a structural reorganization that promotes epileptogenesis. This hypothesis assumes that the sprouting fibers form the majority of their synapses with

other excitatory granule cells, thus enhancing recurrent excitation and producing a permanent hyperexcitability. However, the increased recurrent inhibition found in the dentate gyrus of kindled rats has suggested that the sprouted mossy fibers may form synapses mainly with the dendrites of inhibitory, GABAergic basket cells (Seress and Ribak, 1983; Ribak and Seress, 1983). However, evidence against this account of the functional significance of sprouting is the fact that the increased inhibition found in the dentate gyrus returns to baseline levels following discontinuation of kindling stimulations (Chapter 4: Spiller and Racine, 1994) even though sprouting has been observed as long as 8 months after the last evoked seizure (Cavazos, Golarai and Sutula, 1991).

The present study was designed to assess the changes in inhibition in the piriform cortex that occur throughout the kindling process. These measures were also monitored during a post-kindling recovery period of about 12 weeks following a period of extended kindling (34 stage 5 seizures). In addition, cell counts and area measures were taken in the hilar region in the dentate gyrus, and the density of Timm granules, thought to represent mossy fiber axonal sprouting, were measured in the supragranular layer of the dentate gyrus and in the stratum oriens of the CA3/4.

5.2 Materials and methods

A total of 95 male Long Evans hooded rats, 49 of which had been utilized previously in a transfer experiment, were used in this study. In the transfer study, primary kindling occurred either in the piriform cortex (n=13), lateral olfactory tract (n=23) or olfactory

bulb (n=13). Then the animals that were kindled in the piriform cortex or olfactory bulb were transfer-kindled in the lateral olfactory tract while the animals that were kindled in the lateral olfactory tract were transferred to either the piriform cortex or olfactory bulb. The criterion for kindling was 2 stage 5 seizures (Racine, 1972b) for both the primary and transfer sites, so each animal came into the present study having had 4 stage 5 seizures, 2 of which had been triggered from the lateral olfactory tract.

Sixty-four of these rats, weighing between 280-450 g were implanted with chronic bipolar electrodes in the olfactory bulb, lateral olfactory tract and piriform cortex and the remaining 31animals were implanted with chronic bipolar electrodes in the lateral olfactory tract only. These electrodes were held in place by dental acrylic and 4 stainless-steel screws inserted into the skull. Animals received i.p. injections of 15 mg/kg atropine methyl nitrate and were anesthetized with 65 mg/kg pentobarbital. The electrodes were made from two teflon coated Nichrome wires 127μm in diameter. These were twisted together and had a vertical tip separation of 0.5 mm. Electrodes were lowered under physiological control to ensure optimal placement for the lateral olfactory tract and piriform cortex. Twenty-four of these animals, 16 with 3 electrodes and 8 with 1 electrode,served as implanted, non-kindled controls.

Following the transfer experiment, animals were divided into 3 experimental groups. One group ('4-stage 5' group), received no additional kindling stimulations, the second group ('+10-stage 5' group) were kindled in the lateral olfactory tract until they experienced 10 additional stage 5 seizures while animals in the third group ('+30-stage 5'

group) were kindled in the lateral olfactory tract until they experienced 30 additional stage 5 seizures. The kindling parameters consisted of a 1-4 s train of 1 ms 60 Hz biphasic square wave pulses given once every 24 h at an intensity of 500 μA. This was sufficient to reliably trigger afterdischarges (ADs) in all but a few animals. Stimulation intensities were increased in logarithmic steps in these animals, to a maximum intensity of 794 μA, until ADs were reliably evoked .

5.2.1 Paired-pulse tests

The thirty-two animals used in this experiment, showed stable characteristic evoked field potentials in the piriform cortex following lateral olfactory tract stimulation. The paired-pulse tests involved the application of double pulse stimulation to the lateral olfactory tract at an intensity of 1000 μA. Five piriform cortex responses were averaged at each of 12 interpulse intervals: 20, 30, 40, 50, 70, 100, 150, 200, 250, 300, 500, 750 ms. The intervals were tested sequentially. Pulse pairs were separated by 10 s (0.1 Hz). Two baseline paired-pulse measures were taken prior to primary site kindling in the kindled groups and one paired-pulse measure was taken after primary site kindling, transfer site kindling, and once a week thereafter. In the +10-stage 5 group and +30-stage 5 group, these measures continued until an additional 10 and 30 stage 5 seizures, respectively, had been evoked and 6 weeks of subsequent recovery time had elapsed. The 4-stage-5 animals had similar paired-pulse measures taken except that no additional stage 5 seizures were induced. A final paired pulse measure was taken from all animals 24

hours prior to sacrificing them. The implanted control group was tested over a period which included the median time required for the initial primary and transfer kindling of the kindled groups and for recovery. Consequently, the paired-pulse measures in this group were distributed comparably to those in the kindled groups. EPSP slope measures were taken from the paired-pulse data. The depression/facilitation ratios were determined by dividing the 2nd (test) response by the 1st (conditioning) response. The results were then multiplied by 100 for plotting.

5.2.2 Cell counts

Following kindling and paired-pulse tests, 45 of the animals were deeply anesthetized and perfused transcardially with formalin and saline. The brains were soaked in a glucose solution overnight and then frozen and sliced horizontally into 40 μm sections using a cryostat. Every 4th slice was mounted on a slide and stained with Cresyl Violet and Luxol Blue.

From the stained horizontal sections of hippocampus, 6 sections were selected at approximately 500 μm intervals along the dorsal-ventral axis. Both ipsilateral and contralateral hilar and granule cell regions of the dentate gyrus were counted making a total of 12 samples for each location and for each animal. Special effort was made to choose sections from equivalent locations along the dorsal-ventral axis in each animal and in each group. Neurons were counted under a microscope using the X20 objective. Hilar neurons were counted within a 10.3 cm X 7.8 cm grid (equivalent to 265 by 200 μm)

which was superimposed on to the counting area using a camera lucida. Cell counts were done by A.S who was blind to the group membership. Cells that were on the boundary of the grid were counted only if at least half of the cell body fell within the grid. Neurons were distinguished primarily by size, shape and presence of a nucleus.

5.2.3 Mossy Fiber Axonal Sprouting

Following the completion of kindling and paired-pulse measures, 40 rats were prepared for Timm staining in order to examine the mossy fiber trajectories (Haug, 1973). The rats were deeply anesthetized and perfused transcardially with 50 cc of a medium containing sodium sulfide (Na_2S), anhydrous NaH_2PO_4 and sucrose. The brains were removed, quickly frozen using CO_2 and maintained in a -70°C freezer until ready for slicing. Horizontal sections, 40 μm thick, were cut in a cryostat and every fourth slice was mounted on acid-cleaned slides coated with gelatin and chromium potassium sulfate. Dried (> 1 day) sections were fixed in 100% ethanol at room temperature for 20 minutes and then were hydrated in graded ethanol prior to physical development. The development was carried out in a solution of gum arabic, citrate buffer, hydroquinone, and silver nitrate in a dark box at room temperature for 60 minutes. The slides were then rinsed in running tap water for 30 minutes, dehydrated in graded ethanol, cleared in xylene, and coverslipped with Permount.

Nine sections were selected at aproximately 500μm intervals along the dorsal-ventral axis for analysis. Using a Microcomputer Imaging Device (Imaging Research Inc., St.

Catharines, Ontario), digitized pictures of the CA3 and inner molecular layer of the dentate gyrus (IML) were taken both ipsilaterally and contralaterally to the side of the electrodes. Therefore, a total of 18 samples were analyzed for each animal in both the CA3 and IML.

The amount of Timm stain taken up in the CA3 and IML was analyzed by measuring the proportion of Timm stain relative to background. In the CA3, the proportion of Timm stain was measured by placing 16 circular cursors, each 12354μm^2 in area, in the stratum oriens starting at CA4 and moving anteriorly through the CA3. Sixteen cursors, placed exactly side by side, measured a 2mm strip of stratum oriens. In the IML, the proportional area of Timm to background was measured using 9 circular cursors placed around the genu of the dentate. The proportional area of Timm stain was analyzed for statistical significance using a repeated measures ANOVA (groupXsectionXcursor position).

5.3 Results

5.3.1 Kindling and paired-pulse tests

The smallest and largest number of stimulations required to complete the initial primary site plus transfer site kindling was 9 and 25, respectively. The comparable delay period utilized in the implanted control group ranged from 11 to 30 days. Animals that were kindled to an extra 10 and an extra 30 stage 5 seizures only required between 10-11 and 30 - 32 AD-evoking stimulations, respectively, to reach those criteria.

Haberly and Bower (1984) described the response of piriform cortex pyramidal cells following LOT stimulation as consisting of an initial EPSP followed by a long-lasting IPSP. The initial EPSP can be divided into two components: an initial monosynaptic component triggered by the LOT inputs, followed by a disynaptic component, attributed to activation of association fibers within the piriform cortex. All field potentials in this study were of the standard negative/positive form reported previously (Haberly and Bower, 1984). However, some animals did not show an inflection point in the field potential that could distinguish the two components of the initial EPSP, while others showed a morphology suggestive of multiple different surface-negative components.

Data from all kindled animals were combined in Fig. 5.1 to show group paired-pulse curves before and after kindling as compared to the control animals. As previously reported (Stripling, Patneau and Gramlich, 1988; Racine, Milgram and Hafner, 1983; Racine, Moore and Evans, 1991) the paired pulse technique produced a net facilitation in the piriform cortex, as evidenced by EP2/EP1 X100 values greater than 100 at almost all interpulse intervals. Following kindling, the animals showed a small decrease in paired pulse facilitation, that is thought to reflect an increase in inhibition, at the first 4-5 C-T intervals. A repeated measures ANOVA was run for EPSP slope using the data from the 30 ms interval. The analysis showed a marginally significant group by session interaction ($F_{24,224}$=1.58, P<0.05). When the kindled groups were combined and only prekindling and post kindling values were compared, the significance level increased ($F_{1,30}$=5.54,P<0.03).

Consistent with previous findings (Racine, Moore and Evans, 1991), a kindling-induced potentiation effect was evident following the completion of transfer kindling (see the top figure of Fig. 5.2). This effect was seen as an increase in the amplitude of the evoked conditioning response. Standardization of the conditioning responses before and after kindling , allowed us to confirm the reduction in facilitation of the test response following kindling (see arrow).

Fig. 5.3 shows group averages of measures at the 30 ms IPI. In this figure, we see again that kindling produces a small depression in paired-pulse facilitation. The continuation of kindling to a total of 14 or 34 stage 5 seizures did not have any additional effect on the paired-pulse response except to maintain the decreased facilitation. All 3 kindling groups began to recover towards baseline once the kindling stopped but at different rates. The 30 stage 5 seizure group did not show much recovery until 6 weeks after kindling had stopped whereas the 10 stage 5 seizure group and 4 stage 5 seizure group started recovering in 2 weeks and 1 week respectively. Recovery did not appear to be complete in the 30 stage 5 group at the time testing was completed.

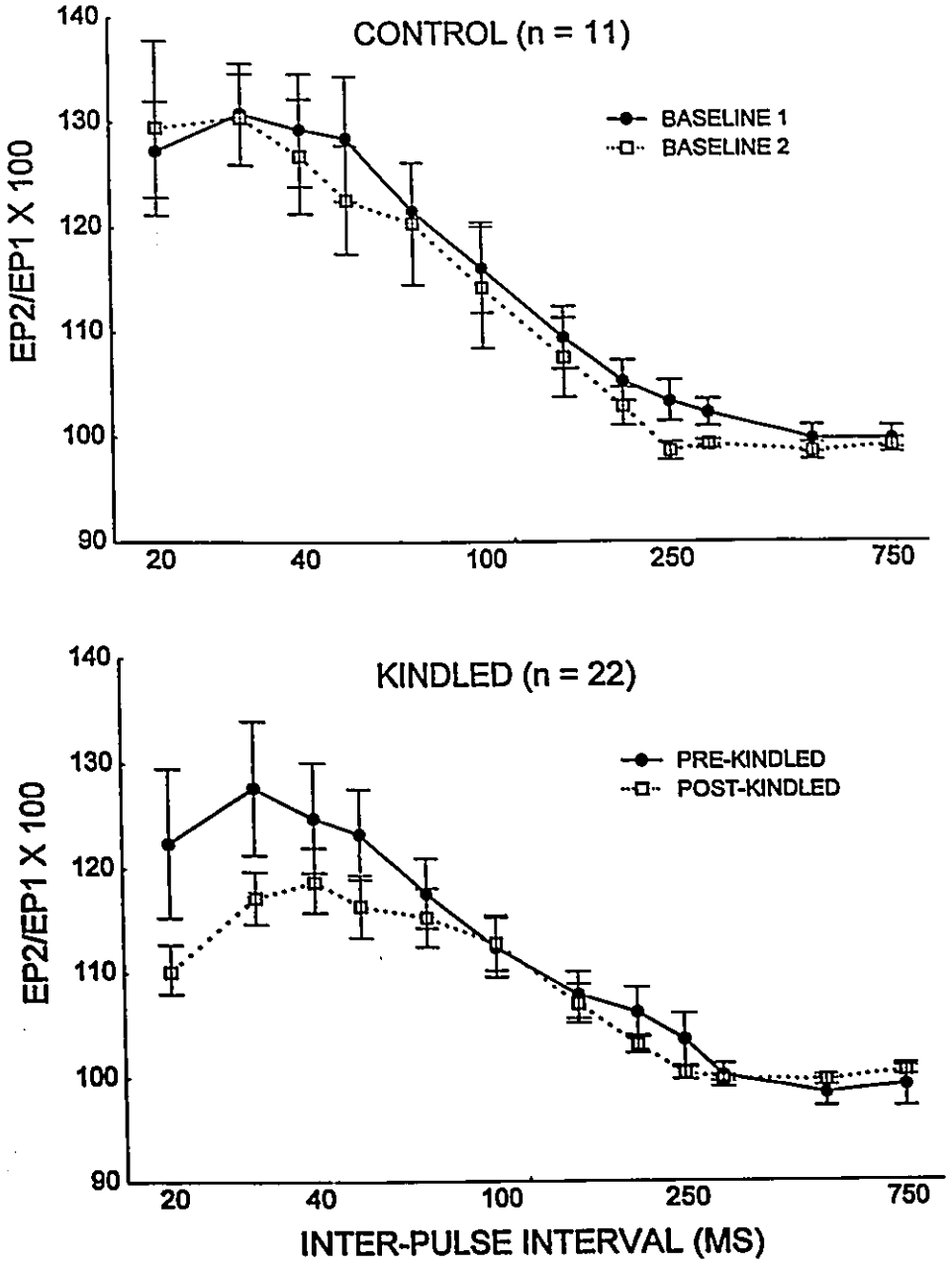


Fig. 5.1. The group averages of the paired-pulse measures are shown. The amplitude of the first response evoked by the conditioning pulse was divided into the amplitude of the second response and the result multiplied by 100 ('EP2/EP1X100'). These measures are plotted against the interpulse interval (ms). Values above 100 indicate facilitation of the second response and values below 100 indication depression. The averages for the control ('CONTROL') group are shown in the upper panel and the averages for the kindled ('KINDLED') group are shown in the bottom panel.

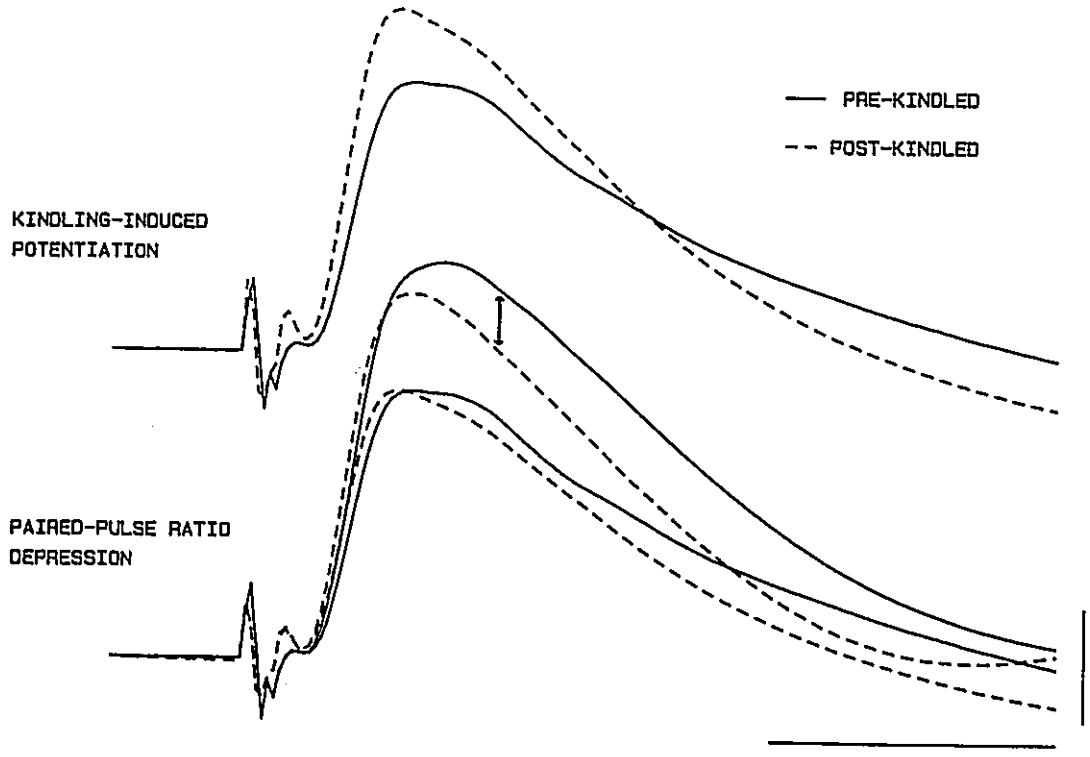


Fig. 5.2. The top panel of this figure shows a kindling-induced potentiation effect when the conditioning responses before and after kindling were compared. The bottom panel of this figure shows that with standardization of the conditioning responses before and after kindling, there is a depression in the test response (response to 2nd pulse) following kindling as compared to baseline measures (see arrow). The calibration bars represent 2mV and 5 ms.

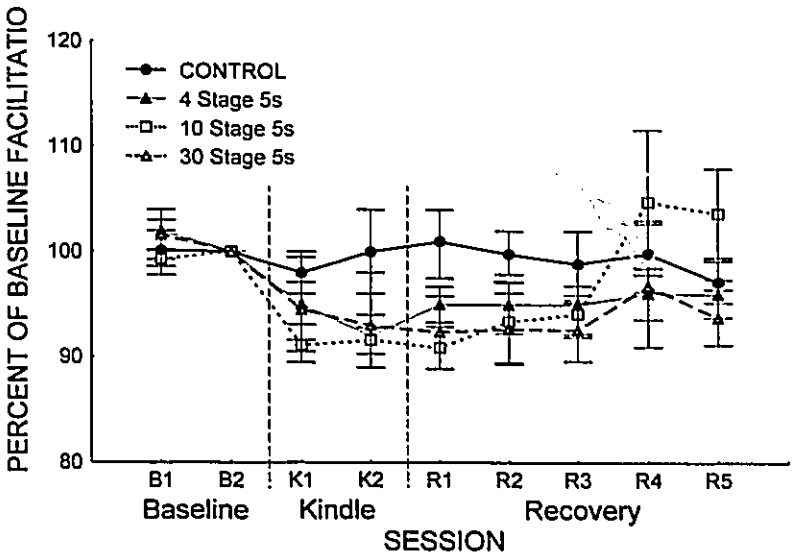


Fig. 5.3. This figure shows the group paired-pulse depression measures (±S.E.M.) for the 30 ms IPI. The baseline measures were taken 24 hours apart. K1 and K2 represent the response measures taken after primary kindling and after last kindling stimulation. The recovery measures were taken 1 week apart except for R4 and R5 which follow at 3 weeks and 3-8 weeks, respectively. There was no significant change in response over time for the implanted control (CONTROL) group. The 3 kindling groups all show a decrease in the level of facilitation that tends to recover towards baseline over the 4-month test period. Recovery was not complete by the end of the test period.

5.3.2 Hilar cell counts

A repeated measures ANOVA showed there was a significant effect of section depth, as previously reported in Chapter 4, but no significant effect of kindling and no significant interaction between section depth and kindling. There were 50.3% fewer cells counted in the most dorsal sections then in the most ventral sections for all groups of animals ($p<0.001$). A repeated measures ANOVA of hilar area showed that there was a significant main effect of side and section but no significant group or interaction effect. The right side of the brain tended to have a greater hilar area than the left side and the more ventral sections had a smaller hilar area than the more dorsal sections, in these horizontal planes.

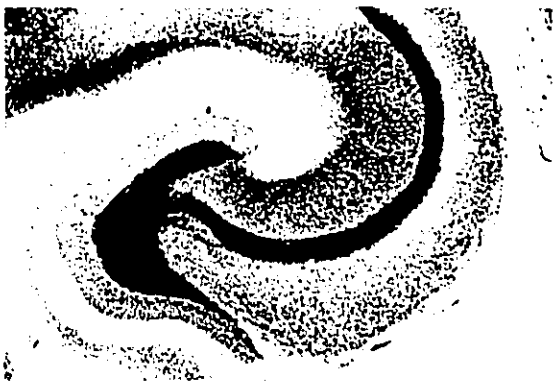
5.3.3 Mossy fiber sprouting

Photographs of dorsal and ventral sections of the dentate gyrus/CA3 region are displayed in Fig. 5.4 for a +30 stage 5 seizure animal. In the CA3, a repeated measures ANOVA showed no significant group effect but did show a significant section ($F_{8,288}=7.46$, $p<0.0001$) and cursor position (distance from the hilus) ($F_{15,540}=186.81$, $p<0.0001$) main effect as well as a significant section by cursor position interaction ($F_{120,4320}=35.07$, $p<0.0001$). Fig. 5.5 shows photographs of dorsal and ventral section of the CA3 for an implanted control and an animal from the +30 stage 5 seizure group. The density of Timm granules in the experimental group did not differ significantly from that of the implanted control. Fig. 5.6 shows the interaction between

section and distance from the hilus. The more dorsal sections (sections 1-4) showed *more* Timm stain than the more ventral sections (sections 6-9) for the first 750 μm from the hilus (first 6 cursor positions). After that, although not as great, the more dorsal sections tended to show *less* Timm stain than the more ventral sections. Also, the amount of Timm stain in the stratum oriens decreases as you measure further away from the hilus.

Photographs of dorsal and ventral sections of the IML are shown in Fig 5.7 for an implanted control animal and a '+30 stage 5 seizure' animal. A significant group by section interaction ($F_{15,155} = 2.49$, $p<0.003$) (see Fig. 5.8) and a significant section by distance from the "genu" interaction ($F_{40,1240} = 1.49$, $p<0.03$) were found with sprouting of mossy fibers into the IML of the granule cell dendrites (see Fig. 5.9). Sprouting was greatest in the '+30 stage 5 seizure' group especially in the more ventral sections. Also, the amount of Timm staining was greatest at the "genu" and decreased as you moved away from it on either side.

DORSAL



VENTRAL

Fig. 5.4. Examples of dorsal and ventral sections of the dentate gyrus/CA3 region for a +30 stage 5 seizure animal. Timm granules densities were measured in the IML of the dentate gyrus and in the stratum oriens of the CA3.

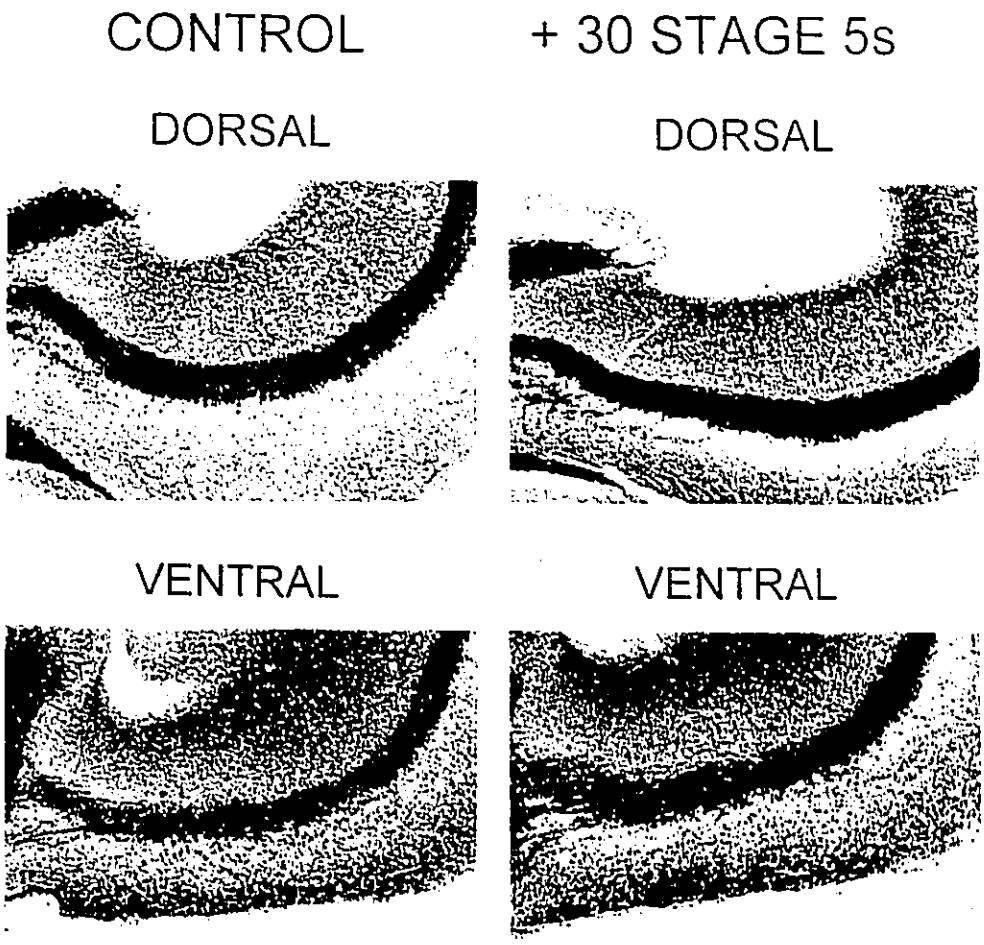


Fig 5.5. This figure shows photographs of dorsal and ventral sections of the CA3 for an implanted control animal and an animal from the +30 stage 5 seizure group. The density of Timm granules in the experimental group did not differ significantly from that of the implanted control.

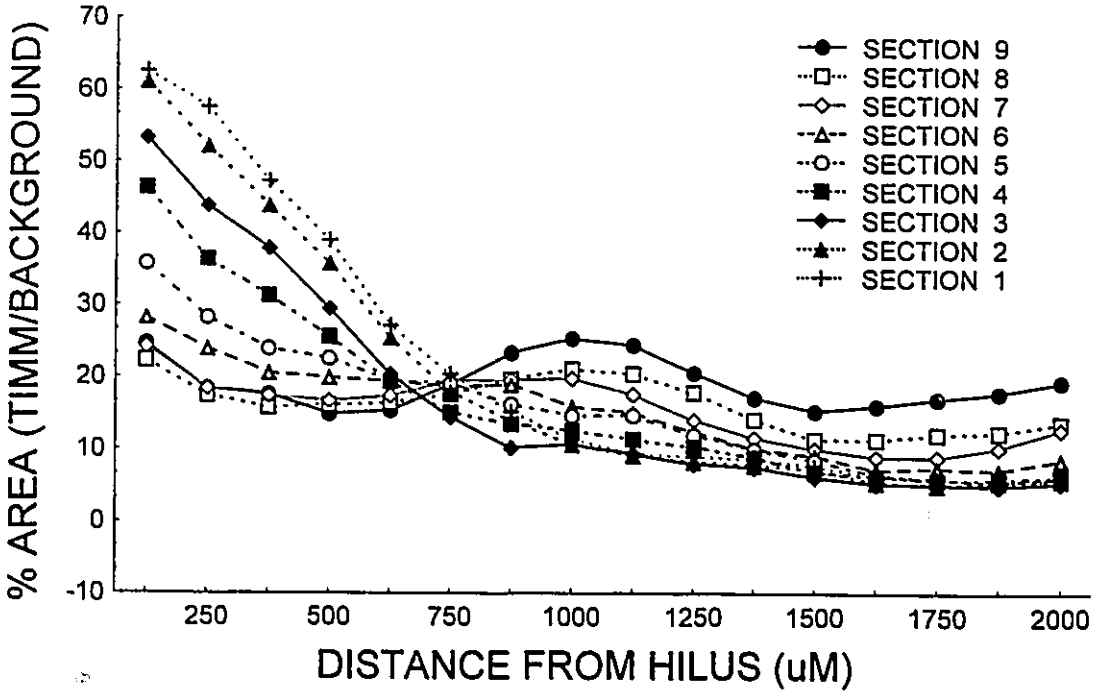


Fig. 5.6. This figure shows the interaction between section and distance from the hilus when measuring the proportion of Timm granules to background staining in the CA3 of the hippocampus. The more dorsal sections (sections 1-4) showed more Timm stain than the more ventral sections (sections 6-9) for the first 750 μm from the hilus. After that, although not as great, the more dorsal sections tended to show less Timm stain than the more ventral sections. Also, the amount of Timm stain in the stratum oriens decreased as you measure further away from the hilus. The diameter of each circular cursor was 125 μm.

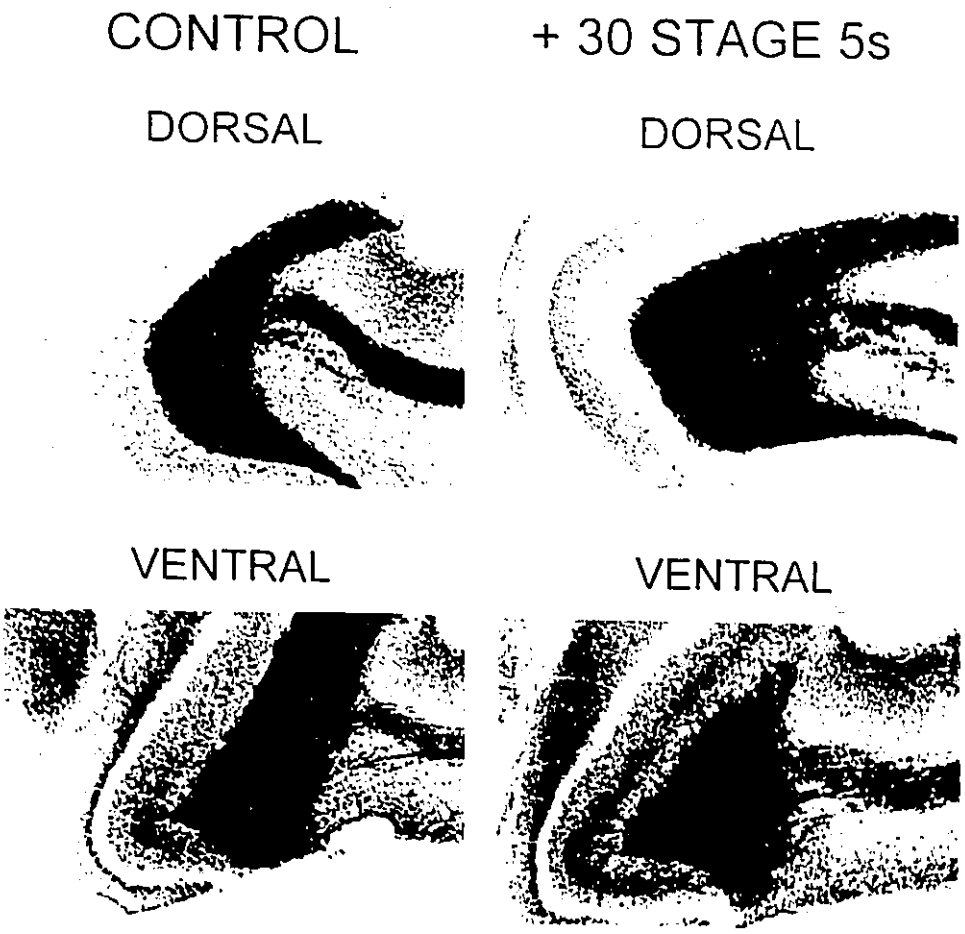


Fig. 5.7. Examples of dorsal and ventral sections of the IML are shown in the micrographs above for an implanted control animal and a '+30 stage 5 seizure' animal. Timm granule densities were measured for 500 μm on either side of the 'genu'.

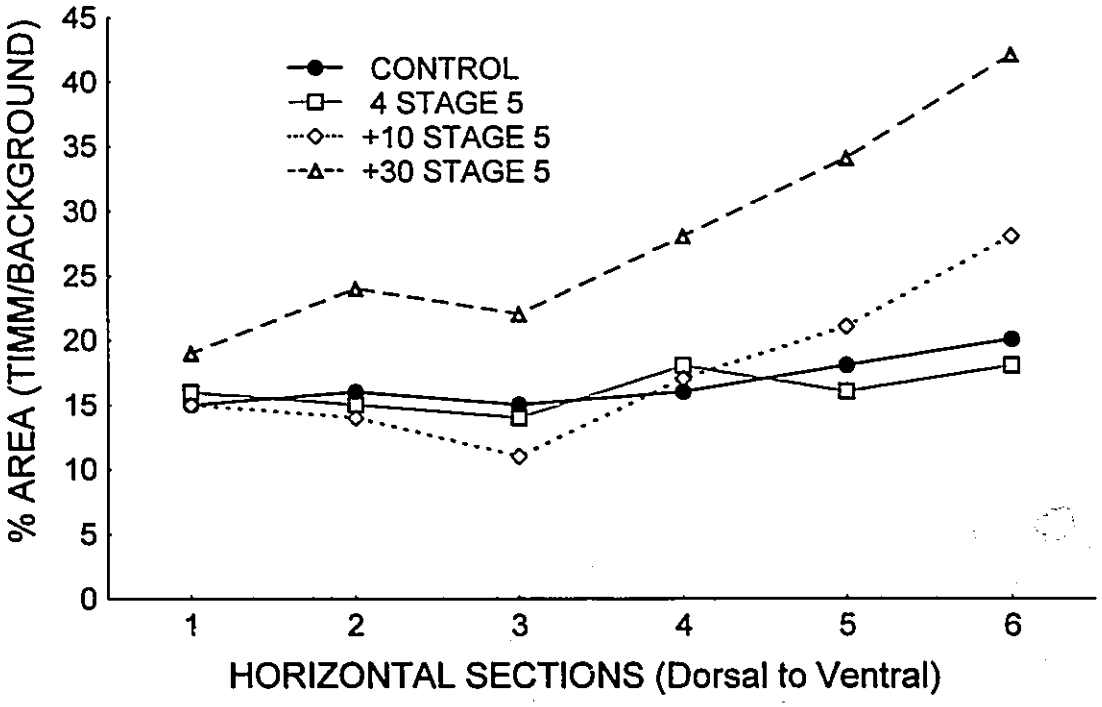


Fig. 5.8 shows the interaction between group and section when measuring the proportion of Timm granules to background staining in the IML of the dentate gyrus. The "+30 stage 5 seizure" group showed significantly more mossy fiber sprouting in all sections. Overall the dorsal sections showed less Timm staining than the ventral sections.

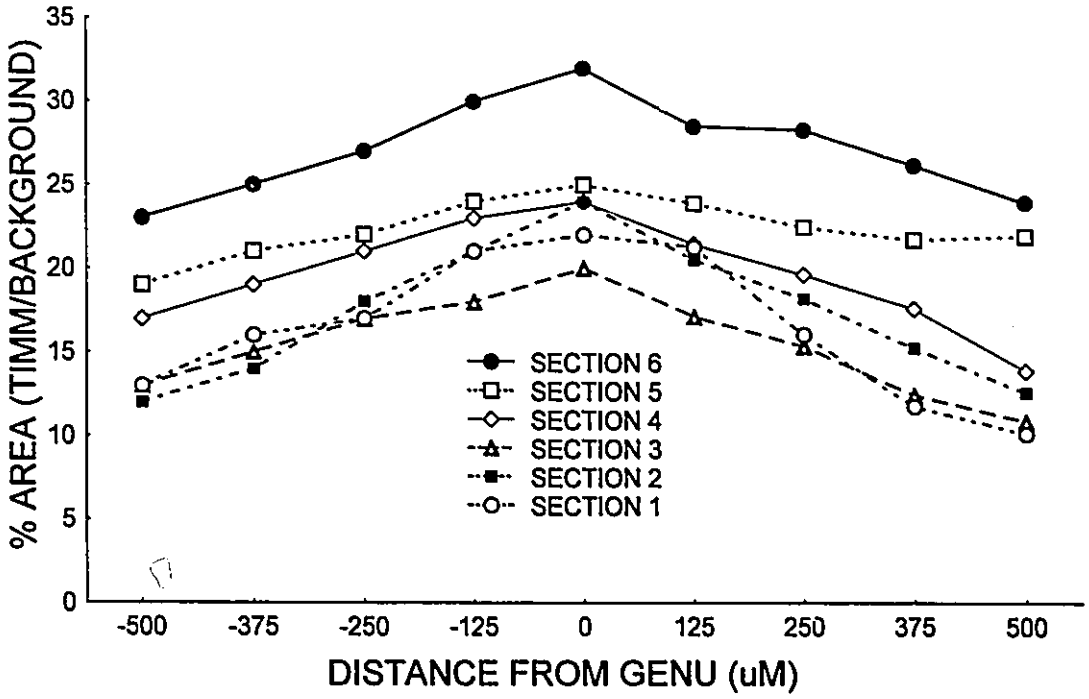


Fig. 5.9. shows the interaction between section and distance from the genu when measuring the porportion of Timm granules to background staining in the IML of the dentate gyrus. Mossy fiber sprouting is highest at the "genu" and decreases on either side as you move away from the genu. Overall, the ventral sections showed more Timm granules than the dorsal sections. Section 1 represents the most dorsal slice.

5.4 Discussion

Racine et al (1991) kindled animals in the lateral olfactory tract and measured paired-pulse inhibition in the piriform cortex before and after 12 kindling stimulations. They found a decrease in paired-pulse facilitation following kindling which was thought to reflect an increase in inhibition. In this study, paired-pulse inhibition was measured in LOT kindled animals that had experienced up to 34 stage 5 seizures and these measures were followed for 3-4 months after the completion of kindling stimulations. We found a similar, though smaller decrease in facilitation in the piriform cortex which remained until the kindling stimulations were stopped. The maximum decrease in facilitation was in place after the first 4 stage 5 seizures and subsequent stimulations produced no further decrement. Following discontinuation of the kindling stimulations, the measures tended to return to baseline levels over the next four months of testing.

A similar increase in paired-pulse inhibition was found in the dentate gyrus when the perforant path was kindled to 44 stage 5 seizures (Chapter 4: Spiller and Racine, 1994b). That is, there was a decrease in the P2/P1 ratio following kindling, extended kindling produced no further changes, and the ratio returned to baseline levels over 5-6 weeks following the discontinuation of kindling stimulations. It is likely that in both systems, olfactory cortex and dentate gyrus, the increase in inhibition could serve as a compensatory response to the induced hypersynchronous activation and slow down epileptogenesis (Kamphuis and Lopes da Silva, 1990).

There was also a kindling-induced potentiation effect in both sites (eg. Fig. 2). The

enhanced recurrent inhibition may have been an indirect consequence of this potentiation effect. That is, the potentiated conditioning response (1st response) could activate more pyramidal cells which would recruit more inhibitory interneurons resulting in a more depressed test response (2nd response). In previous experiments in the dentate gyrus (Tuff et al, 1983), this mechanism was ruled out by adjusting pulse intensities so that the levels of activation matched those of the pre-kindled responses.

We also looked for a kindling-induced decrease in the number of cells in the hilus of the dentate gyrus. We found no effect of kindling the lateral olfactory tract on hilar cell counts, although such effects had been reported following perforant path kindling (Chapter 4: Spiller & Racine, 1994b; Cavazos & Sutula, 1990) or amygdala kindling (Cavazos & Sutula, 1990). The olfactory bulb projects via the lateral olfactory tract directly to the entorhinal cortex (Shepherd, 1991) which projects directly to the hippocampus (Shepherd, 1991). Nevertheless, the discharges triggered by lateral olfactory tract kindling may be weakened by the time they reach the hippocampus. Alternatively, the discharge patterns (eg. spiking frequency) of LOT-induced seizures may be less damaging.

The spacing of kindling stimulations may also be an important variable in these measures. B. Adams (personal communication) found decreased cell density in the hilus following amygdala kindling to a criterion of 22 kindling stimulations. They used a twice daily kindling protocol which perhaps drives the system harder than once daily kindling stimulations. Khurgel et al (1995), however, using a degeneration-sensitive cupric silver

stain, found no degenerative changes specific to the kindling process following massed kindling of the amygdala in which a maximum of 1 stage 5 seizure or 21stimulations were applied over 5 days.

We also found no significant sprouting of mossy fibers into the stratum oriens of the CA3 following kindling of the LOT. This contrasts with the findings of Represa and Ben-Ari (1992) who found that rats kindled in the *amygdala* to a criterion of 3 stage 5 seizures showed sprouting of mossy fibers which made synaptic contact with the basilar dendrites of the CA3 pyramidal cells. Again a difference in methodology could account for these opposing findings. Represa & Ben-Ari kindled their rats twice a day while in this study rats were kindled only once a day. Ebert & Löscher (1995) found differences in mossy fiber sprouting during conventional versus rapid amygdala kindling. Rapid kindling involved stimulating rats 10 s every 30 min, 10 times a day for 2 days while conventional kindling involved stimulating rats for 1s once a day. Conventional amygdala kindling resulted in no significant mossy fiber sprouting but rapid kindling resulted in "a massive induction of mossy fiber sprouting". They offered this finding as support for the hypothesis that the two kindling protocols followed different routes of epileptogenesis (Ebert & Löscher, 1995; Lothman & Williamson, 1994).

LOT kindling did produce a marginally significant group by section interaction effect ($F_{15,135}$= 1.74, p=0.05) of mossy fiber sprouting into the IML of granule cell dendrites. Sprouting was significantly greater in the '+30 stage 5 seizure' and '+10 stage 5 seizure' groups only for the 2 most ventral sections measured. Cavazos, Golarai and Sutula

(1991) also found an increase in Timm granules in the IML of the dentate gyrus following perforant path kindling but they found significant differences after induction of only 5 afterdischarges. Again the difference found in these two studies could be due to the fact that Cavazos et al. kindled the rats twice a day while we kindled the rats once a day. Ebert and Löscher (1995) found no sprouting of mossy fibers into the supragranular layer after only 7 kindling stimulations given once a day to the amygdala but massive sprouting after 10, 10s kindling stimulations a day for 2 days. Our own data indicate that somewhere between 4 and 14 stage 5 seizures must be evoked in order to induce detectable sprouting in the IML of the dentate gyrus when stimulations are applied once a day to the LOT. Using a twice-a-day kindling protocol may drive the system harder and require only about 5 kindling stimulations to induce detectable sprouting. Of course, the differences in mossy fiber sprouting could also be explained by the different kindling sites used or by differences in rat strain.

In conclusion, it appears that axonal sprouting of mossy fibers, as measured by an increase in Timm granules, occurs more readily into the IML of the dentate gyrus than into CA3. With once-a-day stimulation of the lateral olfactory tract, IML sprouting required at least 14 stage 5 seizures. This sprouting into the IML occurred in the absence of any observable structural change or cell loss in the hilus, which provides a dissociation between sprouting and hilar cell loss (or volume change). A similar dissociation was found by Burnham et al (submitted) in their analysis of hippocampal tissue from animals exposed to multiple electro-convulsive stimulations. Increased mossy fiber sprouting was

found in the absence of hilar cell loss. These results indicate that hilar cell loss is not likely to be a mechanism for kindling and perhaps not for any form of developing epileptogenesis.

Chapter 6

GENERAL DISCUSSION

In this thesis, a comparison was made between the effects of kindling on two monosynaptic systems, the EC-PP-DG system and the OB-LOT-PC system in terms of transfer kindling effects, recurrent or feedforward inhibition and cell density and sprouting. The purpose of studying the transfer phenomenon in monosynaptic systems was to help elucidate whether different stimulation sites produced sufficiently different temporo-spatial patterns of activation to affect the rates of transfer kindling. If so, it is reasonable to conclude that changes in stimulation site, even within monosynaptic systems, can have a considerable effect on the further elaboration and propagation of the seizures discharge. The second comparison monitored the effects of extended kindling on recurrent and feedforward inhibition in each of these systems. A difference in either the pattern of activation of inhibitory systems or plastic alterations in those systems could contribute to shaping the temporo-spatial pattern of discharge.

Direct comparisons could not be made for changes in cell density and sprouting. In both studies, cell loss was measured in the hilus of the dentate gyrus because no comparable area has been found in the piriform cortex. Sprouting was not measured when I studied the EC-PP-DG system, because our lab was not equipped at that time with

the essential imaging system. Also, the observation that *perforant path* stimulation resulted in mossy fiber sprouting was not controversial. When I studied the OB-LOT-PC system, I measured sprouting of the mossy fibers into the IML of the dentate gyrus and into the CA3 of the hippocampus. Amygdala kindling , and perforant path kindling, had already been reported to induce such sprouting, and no comparable sprouting effects have yet been demonstrated within the piriform cortex. In any case, by taking our measures from the same sites in each case, we provided additional data for comparing the points of similarity and dissimilarity between kindling processes triggered from 2 different stimulation sites.

6.1 Transfer

Axonal pathways can not directly support cell discharge. The assumption, therefore, is that either the source or the target serves as the primary kindling site when stimulations are applied to a pathway. This assumption leads to the prediction that following primary kindling of an axonal pathway, transfer kindling to either the source or the target should lead to immediate transfer. In the experiments described in this thesis, we tested this hypothesis in two monosynaptic systems. Differences were observed in the number of kindling stimulations required to elicit generalized seizures from the transfer sites for these two systems. The olfactory bulb-lateral olfactory tract-piriform cortex system showed strong propagation of epileptiform discharge from the onset of kindling, regardless of which site was kindled. These structures all appeared to participate equally

and strongly during primary site kindling, and most animals showed fully generalized seizures in response to the first transfer kindling stimulation. The entorhinal cortex-perforant path-dentate gyrus system, on the other hand, initially showed relatively weak propagation of the afterdischarge to the secondary sites during primary kindling (Chapter 2: Spiller & Racine, 1994a). With transfer kindling, the dentate gyrus still required a mean of 15.8 stimulations (compared to 53.4 stimulations during primary kindling) to reach up to and excluding the first stage 5 seizure and the entorhinal cortex still required a mean of 8.6 stimulations after immediate transfer (compared to 10.9 stimulations for primary kindling) but only 2.8 stimulations after a 4 week delay. When the perforant path itself was the transfer site, it only required 3.2 afterdischarges to reach up to and excluding the first stage 5 seizures following dentate gyrus kindling but it required 17.1 afterdischarges following entorhinal cortex kindling (compared to 15.1 stimulations for primary kindling). This suggests that the pattern of discharge (and neuronal reorganization) may differ most when the EC is kindled or that kindling-induced inhibitory influences resulting from EC kindling are stronger than from DG kindling.

The positive effect of delaying transfer kindling by 4 weeks in the entorhinal cortex is likely due to a decay of such an inhibitory process. McIntyre & Goddard (1973) first observed this decaying inhibitory "aftereffect" following amygdala kindling and transfer to the contralateral amygdala. They found that the amount of transfer was greater in the group which received a 2 week rest period before transfer kindling was initiated. Similarly, on rekindling the primary site, an inhibitory process resulting from the

discharges triggered from the secondary site prevented immediate induction of fully generalized seizures. This suppression effect also decayed after two weeks. In Burchfiel and Applegate's 'kindling antagonism' model (1990), alternating stimulation between 2 sites results in a blockade of the progression of kindling from one of the two sites. If subsequent kindling of the suppressed site alone starts immediately after discontinuation of the antagonism paradigm, it takes longer to kindle the site than if a delay of 2-3 weeks is imposed. This suggests that there is a transient inhibitory phenomenon occurring that is expressed most strongly with immediate transfer.

We hypothesized that the differences in transfer rates between these two different monosynaptic pathways were due to differences that exist in the spatio-temporal patterns of discharge at the network level. Since fully generalized seizures occurred immediately following transfer kindling of all secondary sites in the OB-LOT-PC monosynaptic system, this suggests the spatio-temporal patterns of discharge must be expressed similarly throughout this system regardless of the site stimulated. We suggested that the neural organization of the piriform cortex and its inputs may account for these findings. The horizontal distribution of both afferent and association fiber systems in the piriform cortex leads to sequential activation of this structure (Haberly, 1973) such that stimulation of the OB, LOT or the anterior piriform cortex results in a similar sequential activation of the piriform cortex.

Since fully generalized seizures did not occur immediately following transfer kindling of the secondary site in the EC-PP-DG monosynaptic pathway, this suggests that

the spatio-temporal patterns of discharge must be expressed differently depending upon the stimulation site. For example, perforant path stimulation would be expected to produce a diffuse input into the dentate gyrus which presumably has immediate effects upon nearly the whole extent of the structure, whereas direct stimulation of the dentate gyrus presumably results in a localized discharge that then propagates throughout the rest of the structure via different, presumably associational, pathways. Similarly, antidromic activation of the entorhinal cortex from perforant path stimulation is also likely to be diffuse and thus affect the whole structure whereas local activation of the lateral entorhinal cortex likely recruits a somewhat different network, or at least activates its components in a different sequence.

These results lead to an interesting prediction for Applegate and Burchfiel's kindling antagonism model. Since the kindling substrate within the OB-LOT-PC monosynaptic system appears to be the same, regardless of the stimulation site, then kindling antagonism should not occur if two of these sites were alternately kindled (e.g. olfactory bulb and piriform cortex). That is, kindling one site is similar to kindling the other, and therefore, both sites should progress to fully generalized seizures. Conversely, since the kindling substrate within the EC-PP-DG monosynaptic system is different, kindling antagonism should occur (e.g. when kindling entorhinal cortex and dentate gyrus). That is, kindling one site should progress to fully generalized seizures whereas the other site will be suppressed at a lower stage of seizure activity.

6.2 Inhibition

Another possible explanation for the differences in transfer rates may be that there are differences in the levels of inhibition expressed within each of these monosynaptic pathways. A comparison of the amount of recurrent or feedforward inhibition in the two systems was made using the paired pulse technique. Previous experiments have demonstrated a net facilitation in the piriform cortex at all inter-pulse intervals, suggesting that recurrent inhibition in this structure is normally relatively weak (Racine et al, 1983; Stripling et al, 1988; Racine et al, 1991). Following kindling, we showed that the paired-pulse facilitation in the piriform cortex decreased slightly and remained decreased until kindling stimulations were stopped (after 4, 14, or 34 stage 5 seizures). Overall however, most animals still showed a net facilitation following kindling. Racine (unpublished observations) found a similar depression of facilitation following the administration of diazepam, an inhibitory agonist. Consequently, the decrease in facilitation found with kindling may reflect a small potentiation in recurrent inhibitory systems. In summary, the apparently weak inhibitory system in the piriform cortex, and the lack of a strong kindling-induced enhancement of that system, might account for the fast kindling rates found in the piriform cortex as well as for the immediate transfer effect.

Experiments using the paired pulse technique to study inhibition in the dentate gyrus typically have shown a depression of the population spike at short IPIs (20-30 ms), then facilitation at intermediate intervals (30-100ms) and finally depression again at long

intervals (150-750ms). It is the early component of depression that has been attributed to the activation of basket cells and possibly other interneurons resulting in recurrent and feedforward inhibition. It appears that inhibition in the dentate gyrus is normally rather strong. There also appears to be a relatively large enhancement of this inhibition following kindling. The dentate gyrus showed an increase and a prolongation (20-70 ms) of the early inhibitory component which was maintained during extended kindling but returned toward baseline levels over a period of about 5 weeks following the termination of kindling. Therefore, the apparently strong inhibitory system in the dentate gyrus might account for the slower kindling rate and the lack of immediate transfer. The recovery from inhibition might account for the facilitated transfer effect seen in the entorhinal cortex after a 4 week delay.

Whatever the mechanism underlying the differences in the rate of transfer in the two different monosynaptic pathways, these results raise some interesting questions about propagation of epileptiform discharge. It appears that the preferred route of propagation of epileptiform discharges and the development of secondary epileptogenesis are heavily dependent upon the site of the primary focus. The pattern of neuronal remodelling that occurs during kindling appears to be somewhat more specific to the stimulating electrode location in the hippocampal system than in the olfactory system.

6.3 Hilar Cell Density

One of the more dramatic forms of neuronal "reorganization" is the alteration in

actual cell numbers. This type of network change also appears to be dependent upon the site of initial stimulation. Specifically, cell density changes that can develop in the hilus of the dentate gyrus differ between perforant path and olfactory tract kindling. Perforant path kindling resulted in significant reductions of cell density in the hilus of the dentate gyrus after either 4-stage 5 or 44-stage 5 seizures, (Chapter 4: Spiller & Racine, 1994b) whereas lateral olfactory tract kindling resulted in no observable density change even after the induction of 34 stage 5 seizures (Chapter 5). Similarly, Cavazos and Sutula (1990) reported a significant decrease in hilar cells in the dentate gyrus after kindling the perforant path to 30 stage 5 seizures. The extent of hilar cell loss or decreased cell density, has varied considerably between reports. Cavazos and Sutula (1990) found a 12.7% and 40.1% decrease in hilar cells after 3 and 30 generalized seizures, respectively whereas we found (Chapter 4:Spiller and Racine,1994b) found an 11.5% and 15.2% decrease in hilar cells after 4 and 44 stage 5 seizures, respectively. In contrast to our findings, however, Cavazos et al (1994) found a 37% decrease in hilar cells (after 30 stage 5 seizures) following *olfactory bulb* kindling.

The discrepancy between these two studies could be explained by the differences in the kindling protocols used. Cavazos, Das and Sutula (1994) used a twice daily kindling protocol, while we used a once daily kindling protocol. Although, the olfactory bulb projects via the lateral olfactory tract to the entorhinal cortex which projects directly to the hippocampus, the effects of lateral olfactory tract kindling are perhaps sufficiently removed so that decreased cell density in the dentate gyrus would only occur when the

system was driven harder by using a twice daily kindling protocol.

6.4 Sprouting

Both hippocampal and amygdala kindling have been reported to induce sprouting in the mossy fibers of the hippocampus. This hippocampal system, then, provides another point of comparison in the neuronal reorganization produced by perforant path as compared to olfactory tract kindling. A more complete design would include sprouting measures within *both* systems, but there has been no comparable sprouting effect observed, yet, in the olfactory cortex. Consequently, our sprouting measurements were taken from the hippocampus. In addition, we took these measures only from animals kindled in the OB-LOT-PC system, because the imaging methodology had not been established in our lab during the experiments on the EC-PP-DG system. Sutula and his colleagues, however, have published data on mossy fiber sprouting into the supragranular layer of the dentate gyrus following perforant path kindling (Sutula et al, 1988; Cavazos et al, 1991). As far as we know, these results have not been contradicted.

Following kindling of the lateral olfactory tract, we found a significant increase in Timm granules in the IML of the dentate gyrus but not in the CA3 of the hippocampus. This suggests that kindling induces mossy fiber sprouting into the dendritic layer in the IML more readily than into CA3. In the IML, sprouting was significantly greater in the '+30 stage 5 seizure' and '+10 stage 5 seizure' groups only for the 2 most ventral sections measured. Cavazos et al (1991) also found an increase in Timm granules in the IML of

the dentate gyrus following kindling but they found significant differences after evoking only 5 afterdischarges. Our lack of kindling-induced mossy fiber sprouting into the CA3 contrasts with the significant sprouting found by Represa and Ben-Ari (1992). They found that rats kindled in the *amygdala* to a criterion of 3 stage 5 seizures showed sprouting of mossy fibers which made synaptic contact with the basilar dendrites of the CA3 pyramidal cells.

Differences in stimulation protocol could account for these opposing findings. Ebert & Löscher (1995) found differences in mossy fiber sprouting when they compared conventional versus rapid amygdala kindling. Rapid kindling involved stimulating rats 10 s every 30 min, 10 times a day for 2 days while conventional kindling involved stimulating rats for 1s once a day. Conventional amygdala kindling resulted in no significant mossy fiber sprouting but rapid kindling resulted in "a massive induction of mossy fiber sprouting". They suggested that each kindling protocol induces a different kindling mechanism (Ebert & Löscher, 1995; Lothman & Williamson, 1994). Using the conventional kindling protocol, Ebert and Löscher (1995) only measured sprouting in the supragranular layer after 7 kindling stimulations and found no sprouting. We, on the other hand, measured sprouting into the IML after 4, 14 and 34 stage 5 seizures and found a small but significant amount after the induction of 14 stage 5 seizures. This suggests that using a once-a-day kindling protocol, somewhere between 4 and 14 stage 5 seizures must be evoked from the LOT in order to induce detectable sprouting in the IML of the dentate gyrus. Using a twice-a-day kindling protocol, which drives the system a little

harder, fewer stimulations would be required to induced detectable sprouting. A similar argument can be used to explain the differences between our findings and those of Represa & Ben-Ari, as they also kindled their rats twice a day. A twice-a-day kindling protocol, although it doesn't drive the system as strongly as rapid kindling, may still drive the system hard enough to account for the differences.

To summarize, axonal sprouting of mossy fibers, as measured by an increase in Timm granules, appeared to occur more readily into the IML of the dentate gyrus than into CA3 but it still required evoking at least 14 stage 5 seizures. This sprouting into the IML occurred in the absence of any observable changes in either hilar cell number or hilar volume. Again, the differences between our results and those of others may be due to differences in the kindling procedure whereby twice daily kindling drives the system harder resulting in more hilar cell loss, and increased sprouting into both the supragranular layer and area CA3.

As was outlined in the general introduction, there are 2 main theories regarding the functional significance of mossy fiber sprouting and its role in epileptogenesis. The first hypothesis assumes that the sprouted mossy fibers form the majority of synapses with other excitatory granule cells, thus enhancing recurrent excitation and producing permanent hyperexcitability (Sutula et al,1988; Cavazos et al, 1991). However, we found no mossy fiber sprouting in area CA3 and only a small amount of sprouting in the IML following the induction of 14 stage 5 seizures. These results indicate that the sprouting is not essential for epileptogenesis. The second hypothesis, uses the sprouting to explain the

increased recurrent inhibition found in the hippocampus. The sprouted mossy fibers, it is proposed, form synapses mainly with inhibitory cells such as the GABAergic basket cells (Seress & Ribak, 1983; Ribak & Seress, 1983). This hypothesis assumes that sprouting is a compensatory response to the increased levels of activation. The increased inhibition found in the dentate gyrus, however, was found to return to baseline levels following the discontinuation of kindling stimulations (Chapter 4: Spiller and Racine, 1994b) even though sprouting has been observed as long as 8 months after the last evoked seizure (Cavazos et al, 1994).

In conclusion, this thesis examined a number of variables contributing to the determination of seizure circuitry. Transfer was immediate in the OB-LOT-PC system regardless of the sites stimulated while it was delayed in the EC-PP-DG system indicating that the patterns of neuronal reorganization depend upon the kindling site. That is, within the EC-PP-DG system, kindling one site leads to the activation of a specific network that is different from the pattern activated by kindling another site within the same system whereas in the OB-LOT-PC system, kindling any of the sites results in extensive spatial overlap in the neuronal activation patterns. Recurrent inhibition was increased throughout the kindling process in both monosynaptic systems and decayed towards baseline levels when stimulation ceased. These effects may reflect a long-term potentiation of inhibitory synapses. Changes in inhibition in these systems, therefore, are not likely to contribute to the seizure circuitry. It also seems unlikely that structural changes, within the hippocampus, such as decreased hilar density or mossy fiber

sprouting, were necessary for the development of the seizure circuitry. Decreased cell density was evident after perforant path kindling but not after LOT kindling even after the induction of 34 stage 5 seizures. Mossy fiber sprouting into the IML of the granule cells was evident after LOT kindling but only after the induction of 14 or more stage 5 seizures. Mossy fiber sprouting into the CA3 of the hippocampus was not evident after LOT kindling, even after the induction of 34 stage 5 seizures. Although cell loss and sprouting can occur with kindling, they are not necessary for kindling to occur.

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