HIPPOCAMPAL SLICE STUDIES
OF
KINDLING-INDUCED EPILEPSY

by

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HIPPOCAMPAL SLICE STUDIES OF KINDLING-INDUCED EPILEPSY

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DESCRIPTIVE SUMMARY

Epilepsy is a disorder of the nervous system which is characterized by recurring episodes of paroxysmal, synchronized nerve cell activity, often accompanied by convulsions. The basic mechanisms responsible for the seizures are unclear, and the experiments described in this thesis have utilized an animal model of epilepsy to study the cellular abnormalities associated with this state.

The experimental epilepsy was created by daily repetition of mild electrical stimulation to certain brain regions which, through an effect known as kindling, produced an increased disposition to seizures. Our experiments involved the hippocampus, a brain structure which has a low threshold for seizures and which is suspected to be a critical site for the generation of epileptic seizures.

Slices of hippocampus were dissected from unkindled and kindled rats, and maintained in an artificial apparatus mimicking the fluid environment of the brain. This technique permitted ready access to the tissue for purposes of stimulation and recording, and enabled the manipulation of the ionic environment of the nerve cells.

Our major findings may be summarized as follows: a) Hippocampal slices from kindled rats did not display an increased tendency to generate spontaneous epileptiform discharges, either in normal perfusing fluid, or in that containing elevated levels of potassium. In some experiments, however, electrical stimulation evoked responses which reflected an increase in the excitability of the nerve cells generating the response. b) Electrical recordings from single nerve cells showed

that the basic properties of hippocampal cells from kindled rats are unchanged. However, several more subtle alterations in the responses of these cells to electrical stimulation were observed. These included an increased inhibitory response and an enhanced response to direct stimulation of the nerve cell membrane.

These findings suggest that the hippocampus may not develop strong epileptogenic properties in a chronic form of epilepsy. More subtle alterations in nerve cell properties, however, may be components of the epileptic process.

ABSTRACT

Repeated electrical stimulation of certain brain sites in animals leads to the progressive development of clinical seizures, a phenomenon known as kindling. The basic neuronal mechanisms underlying this effect may be similar to those involved in human epilepsy, but are not well understood. We have examined the properties of single neurones and populations of neurones in hippocampal slices derived from rats kindled in the hippocampus, fimbria/fornix, perforant path, or amygdala, and compared them with those of unstimulated animals.

At the neuronal population level, tissue from kindled rats did not display an increased tendency to generate spontaneous epileptiform activity. This was also the finding in ionic substitution experiments, where the levels of extracellular potassium were increased as high as 9 mEq/l. Electrical stimulation of afferents to the CA1 region evoked synaptically-mediated population responses which were similar to those of controls, except for tissue derived from rats kindled in the fimbria or in the amygdala. In these experiments, there was a tendency for multiple population spikes to appear in the responses to stimulation and this may reflect an increased excitability of the neuronal population.

Intracellular studies of hippocampal CA1 neurones from amygdala-kindled rats revealed that the basic active and passive membrane properties did not differ from those of controls. However, the amplitude of the afterhyperpolarization following synaptic activation was increased in these neurones. In addition, the response to intracellular injection of depolarizing current was altered in some

neurones, observed as an increased action potential discharge during the initial phase of depolarization.

These findings are discussed in terms of an apparent immunity of the hippocampus towards the development of chronic epileptogenic properties, and the ionic and membrane mechanisms which may be responsible for the observed differences between kindled and unkindled tissue.

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TABLE OF CONTENTS

CHAPTER 3 INTRACELLULAR STUDIES

CHAPTER 4 GENERAL DISCUSSION

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

1.0 The Objectives of the Present Study

The human epilepsies are disorders of the nervous system, characterized by recurring episodes of neuronal seizure activity which may generalize to produce convulsions. The basic cellular mechanisms responsible for the abnormal neuronal discharge, and the means by which seizures propagate to involve many areas of the brain, are not well understood. Clinical data describe precipitating factors, neuropathological characteristics of biopsy and autopsy tissue. The experimental study of epilepsy in the human, however, faces severe limitations. Ethical and technical considerations limit the range of experimental manipulations which may be attempted with human subjects. As a result, numerous animal models of epilepsy have been developed, which offer varying degrees of control over (a) the nature and site of the epileptogenic lesion and (b) the duration and severity of the seizures.

A particularly interesting method for the experimental production of epilepsy involves the repeated induction of seizure activity in certain brain regions. The stimulation may be electrical current or a chemical convulsant, and the parameters are chosen such that the initial application of a low intensity stimulus produces only a local epileptiform afterdischarge. With daily repetitions, however, the seizures intensify, propagate to other areas and eventually generalize.

This progressive alteration in brain excitability, termed "kindling" by Goddard, McIntyre and Leech (1969), reflects apparently permanent modifications at one or more levels of nervous system organization. The use of kindling as an experimental model for human epilepsy rests on the assumption that these changes may be analogous to those involved in certain types of clinical seizures, particularly to complex partial seizures of temporal lobe origin (Adamec, Stark-Adamec, Perrin and Livingstone, 1981; Albright and Burnham, 1980; Perrin and Hoffman, 1979; Wada, 1978).

Many of the important questions in this form of epilepsy concern the concept of focus, its origin, localization, and neurophysiology. Is there only one primary focus, or are there several foci? What is the relationship between the pathology (in clinical epilepsy) and the focus of epileptiform discharge? What are the properties of neurones in a focus? What are the mechanisms underlying the development of focal hyperexcitability, and how do these contribute to the generalization of seizures?

The hippocampus is often suspected of playing a key role in temporal lobe epilepsy. Medically-intractable seizures of temporal lobe origin are often treated neurosurgically by resection of temporal lobe structures (Purpura, Penry, and Walter, 1975). The most commonly employed technique involves removal of varying amounts of hippocampal tissue as well as other limbic and cortical tissue (Falconer and Taylor, 1968; Olivier, 1983). Glaser (1980) maintains that removal of the hippocampus is critical for successful surgical therapy of complex partial seizures. Ammon's horn sclerosis, or hippocampal neuronal loss

is the most common neuropathological finding in epileptic patients (Mathiesen, 1975; Mouritzen Dam, 1982).

Experimental studies of normal hippocampus have demonstrated that (a) it is one of the most seizure-prone areas of the brain (Green, 1964); (b) it is highly sensitive to many convulsant agents, such as kainic acid (Lothman, Collins and Ferrendeli, 1981; Nadler, Perry and Cotman, 1978a, 1978b; Nadler, Perry, Gentry and Cotman, 1980) and penicillin (De Feo, Cherubini, Mecardli, Dicorato and Ricci,1982; Gloor, Hall and Coceani, 1966); (c) it displays properties characteristic of hyperexcitable neurones, such as paroxysmal cell bursts (Kandel and Spencer, 1961; Fujita, 1975) and spontaneous electroencephalographic (EEG) spikes (Vanderwolf, Kramis, Gillespie and Bland, 1975; Wadman, Lopes da Silva and Leung, 1983); and (d) it displays a high degree of synaptic plasticity (Bliss and Lomo, 1973; Lynch, Gall and Dunwiddie, 1978).

Direct evidence that the hippocampus develops epileptogenic properties and plays a pacemaker role in seizures has been difficult to obtain. One reason for this difficulty is the extensive interconnectivity of the hippocampus with limbic and other structures, such that the origin of epileptiform activity is difficult to ascertain with precision. In addition, measurement of neuronal properties in the in situ hippocampus is hampered by many methodological obstacles. For example, high quality intracellular measurements are difficult to obtain, and the ionic microenvironment of neurones is difficult to control. The development of in vitro brain slice preparations suitable for electrophysiology (Yamamoto and McIlwain, 1966) offered an

opportunity to examine the mechanisms of seizure generation with a degree of resolution not available *in vivo*. The hippocampal slice (Skrede and Westgaard, 1971; Yamamoto, 1972), while maintaining much of the intrinsic circuitry of the hippocampus, is free from the influences of other brain structures. In addition, its ionic and pharmacological environment can be manipulated with relative ease.

The cellular and synaptic substrates required for seizure generation are present in the hippocampal slice preparation. Application of convulsants such as penicillin or bicuculline can trigger robust spontaneous seizure activity (Schwartzkroin and Prince, 1977). The mechanisms of epileptiform discharge generated by acute exposure to convulsants are now understood in considerable detail (e.g. reviews by Andersen, 1983; Schwartzkroin, 1980), but the relevance of these findings to the chronic epileptic state remains to be tested.

The experiments in this thesis address the role of the hippocampus in the generation of epileptiform activity in kindled rats. Slices of hippocampus were taken from animals subjected to kindling stimulation in a variety of brain areas, and their *in vitro* electrophysiological properties were compared with those of normal animals. Our hypothesis was that the hippocampus, being predisposed to seizures, should display an increased incidence of epileptiform activity in the kindled preparation. The mechanisms underlying this activity could be pursued utilizing the advantages afforded by the *in vitro* technique. Specifically, the responses of neuronal populations to specific afferent inputs were examined, and the membrane and synaptic properties of individual neurones were measured.

1.1 An Overview Of Human Epilepsy

There are two major reasons why it is important to search for the neural mechanisms of epilepsy. It has been estimated that 0.2%-0.5% of the world's population suffers from epilepsy. While some forms of epilepsy are relatively innocuous, its most severe manifestations can produce total invalidism, or even status epilepticus and death. Epileptic individuals may also experience social and psychological problems, as well as side-effects of anticonvulsant medication. A better understanding of the neuronal basis of epilepsy should be of value in designing more effective strategies for diagnosis and therapy.

The second reason, perhaps of more importance to a neuroscientist, is a heuristic one. It has been discussed quite eloquently by Arthur Ward:

"The application of [the Aristotelian approach] to biological problems has not been easy. As a consequence, there is a segment of modern research in the neurosciences which consists of the documentation of elegant data obtained with sophisticated instrumentation under precisely controlled conditions which may ultimately make little contribution to our understanding of the functioning brain. However, there is a research strategy that almost ensures that the data generated will be relevant. This is to utilize the experiments of nature to provide clues, which can now be taken to the laboratory and studied with our modern, powerful tools. Epilepsy is such an experiment of nature...

Experiments generated utilizing the clues provided by a study of the condition of epilepsy in the human brain may not necessarily yield data and hypotheses that increase our understanding of epilepsy, but it

is probable that they will add relevant knowledge to the neurosciences. It has been said that physics owes more to the steam engine than the steam engine owes to physics. It was, after all, an attempt to understand the physics of the steam engine that led to the development of thermodynamics. Thus neuroscience may ultimately owe more to epilepsy than epilepsy owes to neuroscience." (Ward, 1980, page 1)

This 'prophecy' had already seen some degree of fulfillment. Perhaps the best example of this was the knowledge gained about the localization of function in the cortex as a result of stimulation mapping during neurosurgery for medically intractable epilepsy (e.g. Penfield, 1969).

Epilepsy has proven to be a most difficult phenomenon not only to diagnose and treat, but simply to define. Although Hippocrates was convinced of a natural origin, it has been attributed to gods, demons and unnatural acts (Temkin, 1971). It was first perceived as a distinctly neurological phenomenon by nineteenth century physicians such as William Gowers and Hughlings Jackson. Jackson's contributions were many, but two stand out in importance. First, he clearly distinguished between seizures which were of unilateral origin and spread throughout a limb in a stereotypical fashion, from those which showed a nearly simultaneous onset on both sides of the body. A second, and perhaps more fundamental contribution, was the postulate that all forms of epilepsy were due to a common pathology which resulted in a "sudden excessive temporary discharge of neural tissue" (in Taylor, 1931). This "epileptic neurone" hypothesis continues to generate controversy in experimental epileptology, and is central to the experiments in this

thesis. Gowers, on the other hand, differentiated "idiopathic" from organic forms of epilepsy, and argued that an "imbalance of nervous energy" was responsible for epileptic seizures (Gowers, 1901). In spite of considerable progress in clinical and experimental methodology, our present-day understanding of the cellular mechanisms underlying all forms of epilepsy has advanced remarkably little over that of these pioneering neurologists.

1.1.1 Basic Features of Human Epilepsy

The principal characteristic of epilepsy is the epileptic seizure, or ictus. Our present-day concept of a seizure owes much to the ideas forwarded by Hughlings Jackson, who proposed that: "Convulsions and other paroxysms are owing to sudden, excessive, and temporary nervous discharges..." (in Taylor, 1931). The electroencephalogram (EEG) of an epileptic individual can display a wide variety of abnormal patterns representative of seizure activity. The simplest is a transient epileptiform "spike" or "spike-wave complex", which may be observed during the period between seizures. These are called "interictal" spikes (IIS), and their EEG localization in the brain is an important technique for identifying the site of an epileptic focus. The neurones responsible for IIS generation experience sudden and excessive depolarizations of membrane potential, usually accompanied by a high frequency discharge of action potentials. This "paroxysmal depolarization shift" (PDS; Matsumoto and Ajmone-Marsan, 1964) is now considered to be the hallmark of an acute epileptiform event, although the mechanisms of its generation are the subject of some controversy

(see section 1.2). Ictal EEG phenomena are characterized by a phase of periodic, high voltage, synchronous, oscillations (tonic phase) followed by bursts of complex waveforms interrupted by silent periods (clonic phase). The ictus may last from several seconds to many minutes, and is followed by a period of depressed neuronal and EEG activity. If electrical stimulation is applied to a tissue which is predisposed to epileptic activity, then a seizure which outlasts the stimulation may result. This event is referred to as an afterdischarge (AD), and is seen in the EEG as a prolonged series of sharp deflections on the order of 100 uV and at a frequency of about 1/second.

The behavioural events associated with seizures vary, depending on the anatomical location of the discharge, its duration, and its pattern of propagation throughout the nervous system. Interictal events may not be perceived by the individual experiencing them. Alternatively, if they involve an area of the brain which is associated with control of movement, a brief muscular contraction or twitch may occur. A seizure which does not generalize, but remains restricted to a particular region of the brain (focal seizure), will produce a behaviour representative of the functional output of that region. A generalized seizure which involves brain areas extensively may produce a convulsion and loss of consciousness.

1.1.2 Seizures vs Epilepsy

Two important distinctions must be made in the study of epilepsy. One is the distinction between non-epileptic and epileptic seizures; the other is to differentiate between a seizure and epilepsy. The first issue is of considerable importance in the clinic, for

epileptics are sometimes misdiagnosed as having other conditions. Equally often, disturbed forms of behaviour are mistaken for epilepsy. Examples are anxiety attacks, hysterical (or "psychogenic") attacks, and fits due to a variety of biochemical and vascular disorders (transient ischemia, hypoglycemia, etc.). These often mimic behaviourally, the major components of petit mal, absence, and other forms of partial seizure. Only the specific high voltage electroencephalographic discharge characteristic of epilepsy should be accepted as evidence of an epileptic basis to any particular symptom. Pedley (1984) points out, however, that the EEG recorded at the cortical surface is not an unequivocal indicator of the presence or absence of epilepsy, since patients with confirmed epilepsy have shown normal EEGs. As an operational definition, however, it is useful to associate a "genuine epileptic seizure" with the "EEG spike", the signature of epileptic hypersynchrony.

The other distinction to be made, that between epileptic seizures and epilepsy, is of primary importance in the development of experimental models of epilepsy. A single, isolated occurrence of a seizure cannot be taken to represent genuine epilepsy. For this purpose, the definition of epilepsy most commonly adopted is one which emphasizes the recurrence of epileptic seizures. "By definition, human epilepsy, or the epilepsies, are recurrent self-sustained paroxysmal disorders of brain function characterized by excessive discharge of cerebral neurons." (Jasper, 1972, p. 585). Similarly, there are many agents which can induce epileptiform seizures in an experimental animal, but unless the seizures recur over an extended period of time, the

preparation is of value only as a model of epileptic seizures, not of epilepsy.

1.1.3 The Diversity of Epilepsies

The recurrent seizures of epilepsy are seen in a wide variety of clinical and electrographic manifestations. As pointed out by Jasper (1972), this may be attributed to the inherent diversity of the neuronal circuitry of the nervous system. It may also reflect a heterogeneity of mechanisms underlying the generation and propagation of seizures. Jasper supports the view that the human epilepsies may be symptoms of a variety of diseases of the brain, rather than a disease in itself.

The perplexing diversity of epileptic phenomena is evident from 2 points of view: neuropathology or precipitating causes, and the different clinical manifestations of seizures.

Meldrum (1981) has surveyed the numerous genetic, biochemical and metabolic factors which are known to trigger seizures, or create a predisposition to seizures. Precipitating factors such as trauma or disease could act via a number of possible pathways to initiate seizures. Neuronal loss and/or deformation, and astrocytic proliferation are the most common neuronal pathologies associated with seizure activity, and could be a result of the seizures as well as a cause.

Epileptic seizures can usually be classified according to their clinical and electroencephalographical characteristics (Gastaut, 1970), and include: (a) partial seizures or seizures beginning locally; (b) generalized seizures or seizures without local onset; and (c)

predominantly unilateral seizures. Partial seizures may possess elementary symptamology such as localized epileptic myoclonus or somato-sensory signs. Depending on the anatomical substrate, they may also be associated with ideational and affective disturbances, and a loss of consciousness. Generalized seizures do not display any sign referable to a specific anatomical system, and are usually bilaterally synchronous and symmetrical. They may result in "absences" and loss of consciousness, or tonic clonic ('grand mal') convulsions.

Since an epileptic patient may have several different kinds of seizures, a classification of the epilepsies is more difficult to establish. A modification of the seizure classification has been proposed by Gastaut (1969) and basically divides the epilepsies into primary generalized, secondary generalized, and partial epilepsy. A study of over 6000 patients according to this classification indicated that 23.5% of all cases were unclassifiable. The remaining classifiable cases consisted of 37.7% generalized epilepsy and 62.3% partial epilepsy (Gastaut, Gastaut, Goncalves de Silva and Sanchez, 1975). The single largest subgroup (39.7% of all classifiable cases) was that identified as partial epilepsy with complex symptomology ('temporal lobe epilepsy').

1.1.4 What are the important questions?

As with any other phenomenon involving the nervous system and behaviour, a satisfactory understanding of epilepsy must involve explanation at, and extrapolation between, levels of organization. In

the case of human epilepsies, many questions appear at each level of nervous system organization.

a) Molecular: What is the genetic locus for the inherited predisposition to certain forms of seizure? What are the molecular mechanisms whereby many drugs and metabolites trigger seizures?

b) Cellular: Some neurones in an epileptic focus display paroxysmal bursts of high-frequency discharge. Is this due to the abnormal development of membrane hyperexcitability? Is it due to an imbalance of synaptically-mediated inhibition and excitation?

c) Neuronal Aggregate: What are the mechanisms by which large ensembles of neurones display hypersynchronous discharge (interictal activity) between periods of seizure activity (ictal activity)? How does the transition from interictal to ictal activity take place? How does seizure activity terminate?

d) Neural Subsystem: Why are some regions of the brain more seizure-prone than others? Are there certain regions of the brain which tend to develop into primary foci and act as pacemakers for other areas? What is the nature of an epileptic focus in terms of number of participating neurones and their intrinsic abnormalities?

e) Nervous System and Clinical Expression: Why are many cases of epilepsy not associated with a detectable pathology? When a certain pathology is present, why does it result in epilepsy in one individual, but not in others? Why do many patients with abnormal EEGs never experience clinical seizures, while many seizure-sufferers have apparently normal EEGs? Why do some epilepsies appear suddenly, with no obvious precipitating factor, while others show spontaneous remission?

Why do some seizures generalize? Why are some generalized from their onset?

1.2 Experimental Approaches to Epilepsy

The role of experimental models is a central issue in the investigation of the mechanisms of epilepsy. Our ultimate goal is to understand epileptic phenomena in the human, and to use this knowledge to develop effective diagnostic and therapeutic techniques. However, experimentation with human epileptic patients poses many ethical and methodological problems. The development of good animal models of epilepsy has become a major challenge for experimental neuroscience, and has resulted in a broad array of preparations, each with its advantages and disadvantages. Wada (1977) has proposed the following properties as indicators of a good experimental model: (1) It should be possible to exercise precise control over the volume of neural tissue which becomes epileptic. (2) The process of creating an epileptic focus should not involve destruction of tissue. (3) The time course of seizure development should be subject to manipulation by the experimenter. (4) It should be possible to trigger seizure activity in a controlled manner and with a clearly identifiable stimulus. (5) The preparation should eventually develop spontaneous seizure activity. (6) The chronology of development of the pathophysiology should mimic that of many human epilepsies, i.e. possess characteristics of persistence, progression, and even spontaneous remission.

Jasper (1972) offers similar considerations, and stresses the importance of verifying that the electrical and behavioural properties

of the model can be tightly linked to the analogous events in the human condition. That this is not always a straightforward task is illustrated by the observation, in an animal preparation, that electrical seizures and the corresponding behavioural events can be uncoupled during certain behavioural states. Therefore, both the electrical activity and its relationship to convulsive movements must resemble those in the human.

The many different experimental models currently being studied are extensively reviewed in the volumes by Chalazonitis and Boisson (1978), Jasper, Ward and Pope (1969), and Purpura, Penry, Tower, Woodbury and Walter (1972). The following sections summarize the results obtained using two models in particular, the penicillin focus and the alumina gel primate model. Section 1.3 will examine the kindling model in greater detail.

1.2.1 Acute *vs* Chronic Models

Two very different models of seizure activity have contributed a substantial portion of our understanding of the mechanisms underlying epilepsy. The first is a model of focal motor epilepsy and is created by the subpial injection of aluminum hydroxide in regions of sensorimotor cortex in monkeys (reviewed by Ward 1969; 1972; Lockard and Ward, 1980). This technique produces, after some delay, a chronic, recurrent, spontaneous epileptic state which closely resembles the human condition in terms of neuropathology (Westrum, White and Ward, 1964; Harris, 1980), seizure development (Ward, 1972), and electrophysiology (Wyler, Burchiel and Ward, 1978; Wyler and Ward, 1980).

Several important findings have emerged from studies using this model.

1. Neurones in the focus display a spectrum of hyperexcitability, ranging from normal to highly epileptic (Wyler et al., 1975). The highly epileptic neurones generate all-or-none bursts and may recruit surrounding neurones into a collective seizure discharge.

2. The proportion of pacemaker neurones within a focus is logarithmically proportional to the frequency of ictal events (Wyler et al., 1978).

3. Gamma-aminobutyric acid (GABA) is probably the major inhibitory neurotransmitter in the mammalian central nervous system (Krnjevic, 1974). The alumina focus is characterized by reduced GABA levels and a loss of GABAergic receptors (Bakay and Harris, 1981). These findings correspond to a loss of GABAergic nerve terminals in the focus (Ribak, Harris, Anderson, Vaughn and Roberts, 1979; Ribak, Bradburne and Harris, 1982).

4. A variety of subtle neuronal changes are seen in the focus, but the most prominent are loss of dendritic spines and bulbous distortions of the dendrites (Westrum et al., 1964).

In spite of these findings, the model suffers from several drawbacks: Its effectiveness is limited to cortical sites in primates; it is expensive; and the focus develops only after a delay of 35 to 60 days (Ward, 1972). An aluminum granuloma or gliotic scar develops (Ward; 1972), which presents technical difficulties to intracellular recording. Finally, the experimenter possesses little control over the

intensity of, and the pattern of propagation of, the electrical seizures.

Most of our knowledge concerning cellular events in focal epilepsy is derived from another popular experimental model, the penicillin focus (see reviews by Ajmone-Marsan, 1969; Ayala, Dichter, Gumnit, Matsumoto and Spencer, 1973; Prince, 1969, 1972, 1983). Topical application of penicillin to the cat neocortex or hippocampus results in synchronous epileptiform discharges generated by the underlying tissue. This is an acute focus in the sense that the interictal and/or ictal episodes persist only during the presence of the drug, and thus lacks the persistence and recurrence characteristic of human foci. Another disadvantage of this model is that most experiments using topical convulsants also employ general anesthetics, thereby introducing a potential source of interference. The major advantages of the preparation are rapidity of onset, and control over the intensity and the anatomical extent of the epileptiform response. Unlike the alumina focus, intracellular recording is unhampered by scar tissue, and numerous studies have described the behaviour of neurones in the penicillin focus under a variety of conditions.

Understanding of the cellular mechanisms which are responsible for epileptiform activity has been the major contribution of studies with the penicillin model. The following is a summary of the important findings, derived from <u>in situ</u> studies of cat neocortex and hippocampus, and from more recent work on <u>in vitro</u> brain slices exposed to penicillin (see also section 1.5.4, below).

1. The precise mode of action of penicillin in generating IISs remains uncertain. It has been shown to reduce GABA-mediated inhibition (Curtis, Game, Johnston, McCulloch and McLacchlan, 1972; Davidoff, 1972; Dingledine and Gjerstad, 1979, 1980; Hill, Simmonds and Straughan, 1976; MacDonald and Barker, 1977; Meyer and Prince, 1973; Pellmar and Wilson, 1977; Van Duijn, Schwartzkroin and Prince, 1973; Wong and Prince, 1973), induce repetitive firing in presynaptic terminals (Noebels and Prince, 1977), decrease resting chloride conductance (Futamachi and Prince, 1975; Hochner, Spira and Werman, 1976), and increase the excitability of neuronal membranes (Ayala, Lin and Vascorretto, 1970).

2. Exposure of neocortex to penicillin produces IIS's of 20-30 mV amplitude and 50-100 ms duration (Matsumoto and Ajmone-Marsan, 1964). Simultaneously, the intracellularly-recorded membrane potential reveals a PDS of up to 30 mV depolarization, and 90-150 ms in duration (Matsumoto and Ajmone-Marsan, 1964). These potentials occur spontaneously, and can also be evoked by afferent stimulation. Qualitatively similar events have been seen in other brain areas exposed to penicillin or other convulsants (e.g. Ajmone-Marsan, 1969; Schwartzkroin, Brimley and Shimada, 1977; Schwartzkroin and Prince, 1977).

3. The membrane properties of neurones exposed to penicillin appear to be normal. Input resistance, membrane potential and action potential characteristics are within the normal range (Andersen, 1983; Ayala et al., 1973; Matsumoto, Ayala and Gumnit, 1969; Schwartzkroin and Prince, 1978). There appears to be no change in the threshold,

conduction velocity, or refractory period of afferent fibres (Gjerstad, Langmoen and Andersen, 1977), although Futamachi and Prince (1975) have observed an increase in the presynaptic fibre volley.

4. The PDS in neocortical and hippocampal neurones cannot be triggered by antidromic stimulation (Matsumoto et al., 1969; Mesher and Wyler, 1976) or depolarization of the membrane by direct current injection (Matsumoto et al., 1969; Prince, 1968a; Courtney and Prince, 1977). However, Kao and Crill (1972) have triggered PDSs by current injection in motoneurons exposed to penicillin.

5. The effects of penicillin on excitatory post-synaptic potentials (EPSP) are controversial. No effect has been reported in pencillin treated spinal cord (Dunn and Somjen, 1977; Davenport, Schwindt and Crill, 1977), Mauthner fibre/giant synapse (Spira and Bennett, 1972), Aplysia neurones (Wilson and Escueta, 1974), hippocampal neurones (Schwartzkroin and Prince, 1980), and neocortex (Matsumoto et al., 1969). However, Walsh (1971) and Gjerstad, Andersen, Langmoen, Lundervold and Hablitz (1981), have observed an increase in the amplitude and duration of EPSPs in motor cortex and hippocampus, respectively.

6. The effects of penicillin on inhibitory post-synaptic potentials (IPSP) are also ambiguous. Matsumoto and Ajmone-Marsan (1964), Matsumoto et al., (1969), Prince (1968b), and Davenport et al., (1977) have reported no significant effects of penicillin on IPSP's in contrast to most studies which report a suppression of GABA-mediated IPSPs. Andersen (1983) suggests that the effects of penicillin on IPSPs (in hippocampus) are likely to be complex in view of the presence of several types of GABA receptors on both the cell bodies and dendrites of

pyramidal neurones (Andersen, Dingledine, Gjerstad, Langmoen and Mosfeldt-Laursen, 1980).

7. The ionic mechanisms underlying penicillin-induced PDS generation are controversial. The 'giant EPSP' hypothesis (Ayala et al., 1973) is based on evidence that the PDS has many properties of an EPSP (e.g. Dichter and Spencer, 1969b; Johnston and Brown, 1981; Matsumoto et al., 1969; Prince, 1968a, 1969). The "intrinsic mechanisms" hypothesis (Prince, 1983; Schwartzkroin, 1982; Schwartzkroin and Wyler, 1980) is derived largely from in vitro studies on hippocampal slices perfused with penicillin and proposes that certain neurones possess intrinsic burst generating mechanisms which are "disinhibited" by penicillin. These mechanisms may involve the depolarizing afterpotentials (DAPs) of spikes, fast prepotentials, and calcium-mediated dendritic depolarizations (Schwartzkroin and Prince, 1978; 1980). Reduction of GABA-mediated inhibition by penicillin (or other means) gives rise to more intense EPSP-induced depolarizations of these cells which generate PDSs through these intrinsic mechanisms.

1.2.2 Hypotheses

The above discussion summarized the evidence that experimental modification of synaptic and/or nonsynaptic properties can result in epileptiform activity. Two very general hypotheses have emerged from this work. Schwartzkroin and Wyler (1980) have combined the data from the alumina and penicillin foci studies into a unitary hypothesis of epileptiform neuronal discharge. They propose that (1) neurones possess intrinsic burst-firing properties which are "released" or "triggered" by extrinsic influences; (2) the PDS is an exaggeration of the normal

burst-producing mechanisms; and (3) an excess calcium ion (Ca^{++}) influx underlies PDS generation.

The imbalance between hyperpolarizing and depolarizing influences which leads to excess Ca^{++} influx can be brought about in a number of ways. In the alumina focus, changes in cell morphology could shorten the electrotonic length of the neurone. A more effective depolarization of the action potential initiation zone (at the initial segment) by synaptic and voltage-dependent Ca^{++} currents would result. The loss of GABA-ergic influences seen in the alumina focus would also favour depolarization of the neuron. Similarly, inhibitory blockade, and possible enhancement of excitatory synaptic drive, could trigger excess Ca^{++} influx and lead to cell bursts in the penicillin-treated preparation. Other factors, such as changes in the extracellular milieu, specific membrane properties, and dendritic ionic conductances are also proposed as contributing towards PDS generation.

The second hypothesis postulates that the PDS is a giant EPSP (Ayala et al., 1973; Johnston and Brown, 1981). The strongest direct support for this hypothesis was the confirmation of the synaptic nature of the PDS (Johnston and Brown, 1981) by showing that it fulfilled all of the characteristics one would expect of a synaptic potential. For example, the probability of occurrence of the spontaneous PDS was found to be uninfluenced by changes in membrane potential. However, PDS amplitude was dependent on membrane potential in a manner consistent with the existence of a synaptic driving potential. Furthermore, it could be reversed by depolarization of the neurone beyond the synaptic equilibrium potential. Finally, voltage clamping of the neurone

revealed the presence of synaptic currents larger than those associated with spontaneously-occurring EPSPs. This scheme proposes that a decrease of inhibition leads to abnormally large EPSPs, but is not specific with respect to these mechanisms. While these observations do not contradict the presence of intrinsic mechanisms in PDS generation, they do demonstrate that synaptic currents make an important contribution.

To what extent are these hypotheses applicable to human epilepsy? Both propose that disinhibition is a major factor in the generation of epileptiform activity, although they disagree with respect to the underlying mechanisms of both the disinhibition and the nature of the PDS. Roberts (1980), drawing on a broad range of observations complementary to those discussed above, also proposes that a loss of GABA-ergic function is consistent with many clinically and experimentally observed correlates of seizures, and that the transition from an excitable to an epileptic focus hinges on a loss of GABA-ergic efficacy.

1.3 The Kindling Phenomenon

The term "kindling" refers to both a procedure and a process. The kindling procedure consists of the repeated induction of epileptiform afterdischarges by discrete electrical or chemical stimulation of the brain, spaced over an extended period of time. The kindling process is the sequence of widespread morphological, chemical, and physiological changes which result from these afterdischarges, and which are specifically responsible for the development of an

increasingly epileptic (kindled) state. The intriguing characteristic of this state is that a stimulus which produced only a relatively brief, local epileptiform event in the normal state now triggers widespread seizure activity, even convulsions, and this effect appears to be permanent. Something in the nervous system has changed, and the goal of research on the kindling phenomenon is to determine the nature and locus of the change (or changes) and secondarily, to demonstrate how this change generates the kindled state.

1.3.1 Basic Features of Kindling

The basic kindling procedure consists of the intermittent application of low-level electrical stimulation to a brain region by means of chronically-implanted electrodes. Typical stimulation parameters are a one-second train of 60 Hz biphasic constant current pulses. The intensity of the pulses is usually adjusted to be slightly above the threshold for the production of a local afterdischarge. If the electrographic events during and after the stimulation are monitored, it is found that the initial stimulations produce only a brief, non-propagating AD of low-frequency (1-2 Hz) spikes. With daily repetition of the same stimulus, however, a series of electrographic and behavioural events develops. The locally-recorded AD becomes longer and increases in amplitude and complexity. Other brain areas, which had previously showed no response to the stimulation, begin to develop epileptiform activity. Behavioural manifestations of the seizures begin to appear, initially as facial and forelimb clonus, and increase in severity until fully generalized convulsions develop. These are

accompanied by widespread seizure activity throughout the brain. Long-term repetition of the kindling stimulus can result in spontaneously-recurring motor seizures.

Kindling possesses a number of interesting features. The effect is long-lasting, since an animal that has been kindled and left unstimulated for a period of up to several months, will still respond to stimulation with a convulsion. Kindling is not associated with major structural damage at the site of stimulation (Goddard and Douglas, 1975; Goddard et al., 1969; Racine and Zaide, 1978; Racine, Tuff and Zaide, 1975), although more subtle ultrastructural alterations may be present. Not all brain areas kindle equally rapidly. The amygdala and pyriform regions of the brain require the fewest stimulations to develop generalized convulsions, while the hippocampus requires the most (Goddard et al., 1969).

All of the conditions necessary for kindling have not yet been identified, but two factors appear to be critical. The stimulation must trigger AD activity for kindling to develop (Racine, 1972a), although repeated subthreshold stimulation can reduce AD threshold and subsequently lead to kindling. Secondly, protein synthesis is necessary, since its inhibition by anisomycin blocks the development of kindling (Cain, Corcoran and Staines, 1980).

In examining the kindling phenomenon, it is important to distinguish among the immediate, the short-term, and the long-term effects of kindling stimulation. Peterson and Albertson (1982) propose a division of the phenomenon into (1) the kindling process (or acquisition phase); (2) the kindled seizure; and (3) the kindled state.

According to this scheme, repeated stimulation triggers various biochemical and physiological changes, and these represent the dynamic phase of kindling, or the kindling process. The end-product of this phase is the establishment of the kindled state, which is characterized by generalized convulsions, spontaneous seizures, etc. Kindled seizures represent a distinctive state of the animal, as the physiological alterations which take place during the seizure and the post-ictal period may be quite different from those which distinguish the kindled state from the normal state. Peterson and Albertson (1982) emphasize the importance of this fact in evaluating experimental evidence; data derived from an animal several hours post-ictally may not be comparable with that taken several weeks after the last kindled seizure.

Several reviews have summarized most of the available kindling data (Goddard, 1983; McNamara, Byrne, Dashieff and Fitz, 1980; Peterson and Albertson, 1982; Racine, 1978; Wada, 1978). Much of the work to date has been directed towards exploring the phenomenology of kindling in greater detail. Two specific topics will be discussed in detail below: the electrophysiology of kindling, and the hypotheses concerning kindling mechanisms.

1.3.2 Electrophysiological Aspects of Kindling

The first thorough studies of the electrical correlates of kindling were performed by Racine (1972a,b) and Racine, Okujava, and Chipashvili (1972). These studies described the properties of development and spread of the afterdischarge, and the subsequent patterns of motor involvement. The basic electrographic phenomena

associated with kindling have since been verified by other laboratories, and in other species (cf. review by Racine, 1978).

Racine, Gartner, and Burnham (1972) demonstrated an increase in the amplitude of potentials evoked in secondary sites by single-pulse stimulation of the kindling site. It was proposed that kindling was accompanied by a strengthening of the connections mediating neural transmission. This strengthening, or potentiation, can be demonstrated using a variety of kindling paradigms (Goddard and Douglas, 1975; Racine, Newberry and Burnham, 1975; Racine, Tuff, and Zaide, 1975; Racine and Zaide, 1978) and bears a partial resemblance to the long-term post-activation potentiation (LTP) resulting from high frequency tetanus of certain forebrain pathways (e.g. Bliss and Lomo, 1973; Douglas and Goddard, 1975). However, it does not appear that LTP can account for all aspects of the kindling phenomenon (Racine, Kairiss, and Smith, 1981). Evoked potential studies have provided some indirect evidence that kindling produces a lasting increase in inhibition in the dentate gyrus (Goddard, 1982; Tuff, Racine and Adamec, 1983).

Spontaneous IISs result from kindling, but their temporal patterns and mechanisms of generation are controversial. Fitz and MacNamara (1979) reported the development of spontaneous IISs in all amygdala-kindled animals, and their subsequent decline over a 5-day period following the completion of kindling. These results are in general agreement with those of McIntyre and Goddard (1973), Racine (1972a), and Wada and Sato (1974). Rats which demonstrate spontaneous seizures as a result of long-term stimulation show a more persisting IIS activity (Pinel and Rovner, 1978).

While most studies claim that the IIS is absent in the unkindled animal, there have been observations of IIS-like activity in the normal hippocampus (Lopes da Silva, Wadman, Leung and Van Hulten, 1982; Suzuki, 1983; Vanderwolf et al., 1975; Wadman et al., 1983). Several lines of evidence suggest that the IIS in the hippocampus of kindled rats may be due to an inhibitory process (Engel and Ackermann, 1980; Engel et al., 1981b; Fujita, Harada, Takeuchi, Sato and Minami, 1983; Fujita, Sato, Takeuchi, Sato and Minami, 1983).

Compared to other models of epilepsy, there have been relatively few studies done at the single-cell level in kindled animals. Racine and Zaide (1978) and Racine et al., (1975) have studied the firing patterns of cells in the stimulated region, and in the regions to which it projects. The duration of evoked cell bursts and the action potential frequency during the bursts was increased by kindling, both in the stimulated site and to an even greater extent in projection sites.

Fujita and Sakarunaga (1981) recorded intracellularly from pyramidal cells of the kindled hippocampus in cat, and found that membrane hyperpolarizations accompanied the kindled IIS. These hyperpolarizations were larger in cells in kindled hippocampus than in unkindled hippocampus (Fujita et al., 1983a), and could be evoked by brain stem stimulation (Fujita et al., 1983b).

In a modification of the kindling procedure, Burchfiel, Duchowny and Duffy, (1979) investigated the response of hippocampal pyramidal cells to iontophoretically-applied acetylcholine (ACh) during the period following an evoked afterdischarge. They found that a

long-lasting supersensitivity to ACh developed after a latency of 40-60 minutes, and persisted for the duration of the recording.

Recently, several electrophysiological experiments on the in vitro hippocampus taken from kindled rats have been reported. Oliver, Hoffer and Wyatt (1980) found that hippocampal slices taken from pentylenetetrazole kindled guinea pigs could develop spontaneous IIS activity by exposing them to elevated levels of potassium. The presence of a convulsant was not required, Oliver and Miller (1982) reported unchanged IPSP characteristics in CA1 pyramidal neurones from commissural-kindled rats. However, Oliver and Miller (1983) have reported a prolonged inhibitory process in granule neurones of similarly-prepared rats.

1.3.3 Hypotheses

An hypothesis which can account for all of the observed features of kindling has not yet been put forward. The underlying mechanisms could possibly involve disinhibition, by means of either structural or biochemical degeneration. Observations of an apparent increase of certain inhibitory phenomena accompanying kindling speak against this as the sole responsible mechanism. Similarly, the enhancement of synaptic connectivity between regions of the brain participating in the seizure discharges cannot account for all of the electrographic observations, but could represent a necessary component for seizure spread and generalization. Corcoran (1980) has proposed a model of kindling based on potentiation combined with an erosion of inhibitory noradrenergic influences. Racine et al., (1981) have suggested that a chronic disinhibitory mechanism could provoke

paroxysmal bursting using mechanisms similar to those responsible for penicillin-induced bursts. Data at the cellular level is presently insufficient to provide conclusive support for these proposals.

1.3.4 Kindling As An Experimental Model of Human Epilepsy

The criteria for a good experimental model of epilepsy which were discussed in section 1.2, appear to be met by the kindling preparation. Wada (1977) has summarized the attractive features of electrical kindling as the following:

(1) The stimulated site may be chosen with stereotactic precision;

(2) No identifiable destructive pathology is introduced;

(3) The experimenter has control over the initiation and development of the seizures.

(4) Seizure stages can, in most cases, be readily identified;

(6) Spontaneous seizures may eventually develop;

(7) Remission of seizures may also take place.

No other laboratory model of epilepsy offers as many advantages to the experimenter.

Wada (1977) also argues that the kindling preparation is useful in the evaluation of antiepileptic drugs. Control over the development and stage of the epileptic state in kindling facilitates the screening of drugs for their prophylactic action against developing seizures, and their action against well-developed seizures. On the basis of dose-response studies of anticonvulsants on kindled rats, Albright and Burnham (1980) propose that the amygdala kindled focal seizure mimics closely the pharmacological behaviour of the complex partial seizure.

This form of seizure has in the past lacked a suitable pharmacological model for the study of anticonvulsant drug efficacy and mode of action.

The clinical relevance of kindling has several other aspects. An important issue is the progressive nature of the epilepsy, which seems to involve the maturation of a focus, the development of secondary foci, and the generalization of seizures. The "silent period" between a head injury and the onset of clinical seizure activity may be of several months' or longer duration. Pharmacological prophylaxis (Mutani, 1983) during this period may avert the onset of epilepsy. In cases of suspected complex partial epilepsy of non-traumatic etiology, early surgical intervention has been advocated to avoid an intensification of the seizures (Glaser, 1980; Olivier, 1983). These and other observations have led to the suggestion that a process similar to kindling may take place in human epileptic disorders (Adamec et al., 1981; Goddard, 1983; Perrin and Hoffman, 1979). Ongoing epileptiform activity due to trauma or other sources could serve as the kindling stimulus for other regions of the brain, and lead to a progressive intensification of the seizure activity. Kindling may thus provide useful hypotheses for the progressive development of clinical epilepsy.

1.4 The Hippocampus and Epilepsy

As outlined at the beginning of the chapter, the hippocampus possesses a number of unique properties with respect to seizures and epilepsy. This section outlines the physiology of the hippocampal formation and discusses its epileptic properties.

1.4.1 Anatomy and Physiology of the Hippocampus

The hippocampus is the most conspicuous structure of the limbic system. It is a long, horn-shaped body whose dorsal surface forms the curvature of the lateral ventricles. The hippocampal complex consists of the hippocampus proper and the dentate gyrus (or fascia dentata; see Figure 1-1). Both of these structures can be further subdivided into cytoarchitectonic strata and subfields. The hippocampus proper consists of four major subfields, CA1-4 (CA stands for Cornu Ammonis, derived from the gross resemblance of the hippocampus to the horns on the statue of Ammon; the term hippocampus reflects its similarity to a sea-horse). These subfields, first characterized by Lorente de No (1934), differ in certain details of neuronal morphology and connectivity. All are composed of pyramidal neurones whose cell bodies are localized to stratum pyramidale (refer to the cross-section of hippocampus in Figure 1-1), with their apical and basal dendrites extending into stratum radiatum and stratum oriens, respectively. Cell bodies range from 20-40 μm at their base and 40-60 μm in height (Shepherd, 1979), the larger ones being found in CA3. The principal neurone of the dentate gyrus is the granule cell.

The major fiber pathways of the hippocampus are a) the perforant pathway, which originates in the entorhinal cortex and terminates on granule cell dendrites; b) the mossy fibre system, consisting of the axons of granule cells and terminating on CA3 apical dendrites; and c) the schaffer collaterals (axon collaterals of CA3 neurones), which course through stratum radiatum to make en passage synaptic contact with

CA1 apical dendrites. Further details of anatomy are presented in Andersen (1975).

A remarkable feature of the hippocampus is its strikingly stereotyped internal organization. The synaptic and cellular organization outlined in Figure 1-1 is recapitulated throughout the length of the structure. The hippocampus may be pictured as consisting of a series of parallel lamellae, each of which is an elaboration of this basic circuit (Blackstad, Brink, Hem and Jeune, 1970; Hjorth-Simonsen and Jeune, 1972).

This structure has been one of the most extensively studied areas of the mammalian central nervous system (CNS), and various aspects of its physiology have been reviewed by Andersen (1975), Brodal (1947), Green (1964), Isaacson and Pribram (1975), Lopes Da Silva and Arnolds (1978), O'Keefe and Nadel (1978), and others. The characteristic response evoked by stimulation of an afferent pathway is referred to as a population field potential, which is the summated extracellular potential of all activated neurones (see Figure 2-2 of Chapter 2). This response contains components which represent the activity of presynaptic fibres, and the postsynaptic EPSP and action potential (Andersen, 1975; Lopes Da Silva and Arnolds, 1978). The latter responses are termed the "population EPSP" and "population spike", respectively.

The hippocampal pyramidal cell rivals the Mauthner cell and the spinal motoneurone as the most thoroughly investigated neurone in the vertebrate CNS. Intracellular studies of hippocampal neuronal properties have been reported in cat (Andersen and Lomo, 1966; Andersen

et al., 1964, 1969; Kandel and Spencer, 1961; Kandel, Spencer and Brinley, 1961; Purpura, McMurtry, Leonard, and Malliani, 1966; Spencer and Kandel, 1961a,b), rabbit (Fujita, 1975; Fujita and Iwasa, 1977; Fujita and Sato, 1964), and rat (Finch and Babb, 1977, 1980a,b). Since the development of the hippocampal slice preparation (Skrede and Westgaard, 1971; Yamamoto, 1972), numerous investigations of pyramidal cell membrane behaviour have been reported in vitro (e.g. reviews by Langmoen and Andersen, 1981; Lynch and Schubert, 1980).

1.4.2 Peculiar Epileptiform Properties of the Hippocampus

Comparisons of the seizure threshold of different brain areas have revealed the hippocampal formation to be one of the most seizure-prone areas of the brain (Andy and Akert, 1955; Correll and Ingram, 1956; Delgado and Sevillano, 1961; Elul, 1964; Gibbs and Gibbs, 1936; Green and Shimamato, 1953; Jung and Kornmuller, 1938; Kaada, 1951; Leclercq and Segal, 1965ab; Liberson and Akert, 1953; Niemer, Powell and Goodfellow, 1960; Segal and Leclercq, 1965). Seizure activity in the hippocampus can be triggered secondarily to stimulation of other brain regions (Elul, 1964; Von Euler and Green, 1960) without triggering an AD in the stimulated site. The hippocampus is also exquisitely sensitive to convulsant agents, such as penicillin (DeFeo et al., 1982; Gloor et al., 1966) and kainic acid (Lothman et al., 1981a,b; Nadler et al., 1978a,b; Nadler, Perry, Gentry and Cotman, 1980; Robinson and Deadwyler, 1981).

The normal hippocampus also displays some degree of spontaneous epileptiform activity. IIS-like activity has been reported to occur,

though at a low frequency (Hartse and Rechtschaffen, 1982; Hartse, Eisenhart, Bergmann and Rechtschaffen, 1977; Metz and Rechtschaffen, 1976; Suzuki, 1983; Vanderwolf et al., 1975; Wadman et al., 1983). Single pyramidal neurones, particularly those in the CA3 region, show epileptiform bursting characteristics. These cell bursts occur spontaneously or in response to afferent stimulation, and have been recorded under a variety of conditions: (1) in the intact, anesthetized preparation (Fujita, 1975; Fujita and Iwasa, 1977; Fujita and Sato, 1964; Kandel et al., 1961); (2) in the freely behaving animal (Ranck, 1973); and (3) in the in vitro hippocampal slice preparation (Schwartzkroin, 1977; 1978; Wong and Prince, 1978; 1981). Addition of convulsants to the slice preparation enhances the bursting and leads to the generation of spontaneous population IISs (Schwartzkroin and Prince, 1977, 1978, 1980).

1.4.3 Clinical Observations

Three families of clinical evidence implicate the hippocampus as playing a key role in epilepsy.

(1) Neuropathological Evidence: A consistent finding in the neuropathological study of temporal lobe tissue from epileptic patients is hippocampal sclerosis (Brown, 1973; Falconer, 1971; Falconer, Serepetinides and Corsellis, 1964; Margerison and Corsellis, 1966; Mathieson, 1975; Meldrum, 1981; Mouritzen Dam, 1982; Scheibel, 1980; Scheibel, Crandall and Scheibel, 1974). In addition, Scheibel (1980) and Scheibel et al., (1974) have reported loss of dendritic spines, and the development of nodulations along the dendritic shafts.

(2) Neurosurgical Evidence: When temporal lobectomy is indicated for treatment of medically intractable seizures, removal of the hippocampus has been considered essential for effective seizure control postoperatively (Falconer and Taylor, 1968; Glaser, 1980; Olivier, 1983). However, Rasmussen (1975, 1983) claims that residual spiking in unresected hippocampal tissue does not trigger clinical seizure activity.

(3) Localization Studies: In addition to the scalp-derived EEG, several other techniques are being used to assist in determination of the site of an epileptic focus. These include depth recording with stereotactically-implanted electrodes (stereoencephalography) (e.g. Crandall, Walter and Rand, 1963) and functional imaging of the brain (Engel, 1983). The value of these techniques in cases of partial complex seizures is still controversial (e.g. Rasmussen, 1975). However, individual patients sometimes show the onset of seizure activity to be localized to the hippocampus (e.g. Brazier, 1972; Lieb, Walsh, Babb, Walter and Crandall, 1976).

1.4.4 In Vitro Studies of Epileptic Phenomena in the Hippocampus

The in vitro slice preparation offers certain advantages for the study of normal as well as epileptiform cellular phenomena (Schwartzkroin, 1981): (1) technical simplicity; (2) control over the condition of the preparation; (3) mechanical stability; (4) improved visualization of tissue; (5) direct access to extracellular space due to the lack of a blood-brain barrier; (6) absence of distal brain effects

or neurohumoral influences; (7) maintenance of normal neuronal organization.

The ability of the preparation to sustain penicillin-induced epileptiform activity has led to its popularity as an experimental epilepsy model (Andersen, 1983; Prince, 1978; Schwartzkroin, 1980). Slice studies have shown that the mechanisms of PDS generation appear to involve several factors: (1) an antagonism of GABA-mediated inhibition (Dingledine and Gjerstad, 1979, 1980; Schwartzkroin and Prince, 1980); (2) an exaggeration of EPSP activity (Johnston and Brown, 1981); (3) an activation of dendritically-located voltage-dependent calcium influx (Schwartzkroin and Prince, 1977; 1978; Prince, 1978; Prince and Schwartzkroin, 1978).

The ability to manipulate the extracellular concentrations of drugs has led to studies on the potency of anticonvulsant agents against penicillin-induced paroxysmal activity (Mueller and Dunwiddie, 1983; Oliver, Hoffer and Wyatt, 1977). The mechanisms of synchronization of neuronal populations during penicillin-induced bursting (Traub and Wong, 1983a,b; Wong and Traub, 1983) have been studied by microsurgically isolating small fragments of the slice and by multi-electrode recording. These studies have revealed that the CA2 region of the slice is a trigger region, and that local synaptic integration processes serve to synchronize neuronal aggregates during the IIS. Recently, Miles and Wong (1983) have demonstrated that single neurones can initiate and synchronize paroxysmal activity.

Other in vitro studies have revealed that the hippocampal slice can sustain paroxysmal ADs even when synaptic transmission is blocked by

lowering the extracellular Ca^{++} concentrations (Jefferys and Haas, 1982; Taylor and Dudek, 1982). Ephaptic interactions among the tightly-packed pyramidal neurones, fluctuations in extracellular ions or electrotonic coupling between cells (MacVicar and Dudek, 1981) may underly this phenomenon.

1.5 Summary

Studies of the penicillin-induced IIS have provided insight into the cellular mechanisms of burst generation and hypersynchrony. There are two major drawbacks of this approach. First, it is limited to an analysis of the acute, focal IIS, which is only one component of epilepsy. It cannot address the questions of seizure spread, generalization, interictal-ictal transformation, etc. Second, it remains to be demonstrated that the penicillin-induced IIS is an accurate model of the IIS of chronic human epilepsy. The alumina model of primate focal motor epilepsy is a chronic preparation, and bears a stronger resemblance to certain forms of human epilepsy. However, it is not an advantageous neurophysiological preparation, and it is not a good model for the most common form of human epilepsy, that involving complex partial seizures of temporal lobe origin.

It was argued above that the kindling model overcomes many of these disadvantages. Extensive neurophysiological analyses of the kindled brain have not been carried out, and the brain-slice preparation offers an opportunity to perform experiments similar to those which have studied the mechanisms of the penicillin-induced IIS, but on tissue taken from an animal with a chronic form of epilepsy.

FIGURE 1-1: SCHEMATIC DIAGRAM OF HIPPOCAMPUS (from Shepherd, 1979, p. 309).

Inputs: right, fibers in the perforant (perf) and alvear (alv) pathways; left, fibers arriving through the fornix (f); below, mossy fibers (mf) from dentate fascia.

Principal neuron: pyramidal neuron (P); recurrent Schaffer collateral (Sc).

Intrinsic neuron: basket cell (B).

The hippocampal regions are indicated by CA1-4.

The layers are indicated on the right: SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; SL-M, stratum lacunosum-moleculare. Another layer, stratum lucidum, occurs at the level of mossy fibers (mf) in CA3.

Neuronal elements of the dentate fascia are shown at DF.

Inputs: from the right, perforant (perf) pathway; form the left fibers arriving through the fornix (f).

Principal neuron: granule cell (Gr) gives rise to mossy fibers (mf).

Intrinsic neuron: basket cell (B).

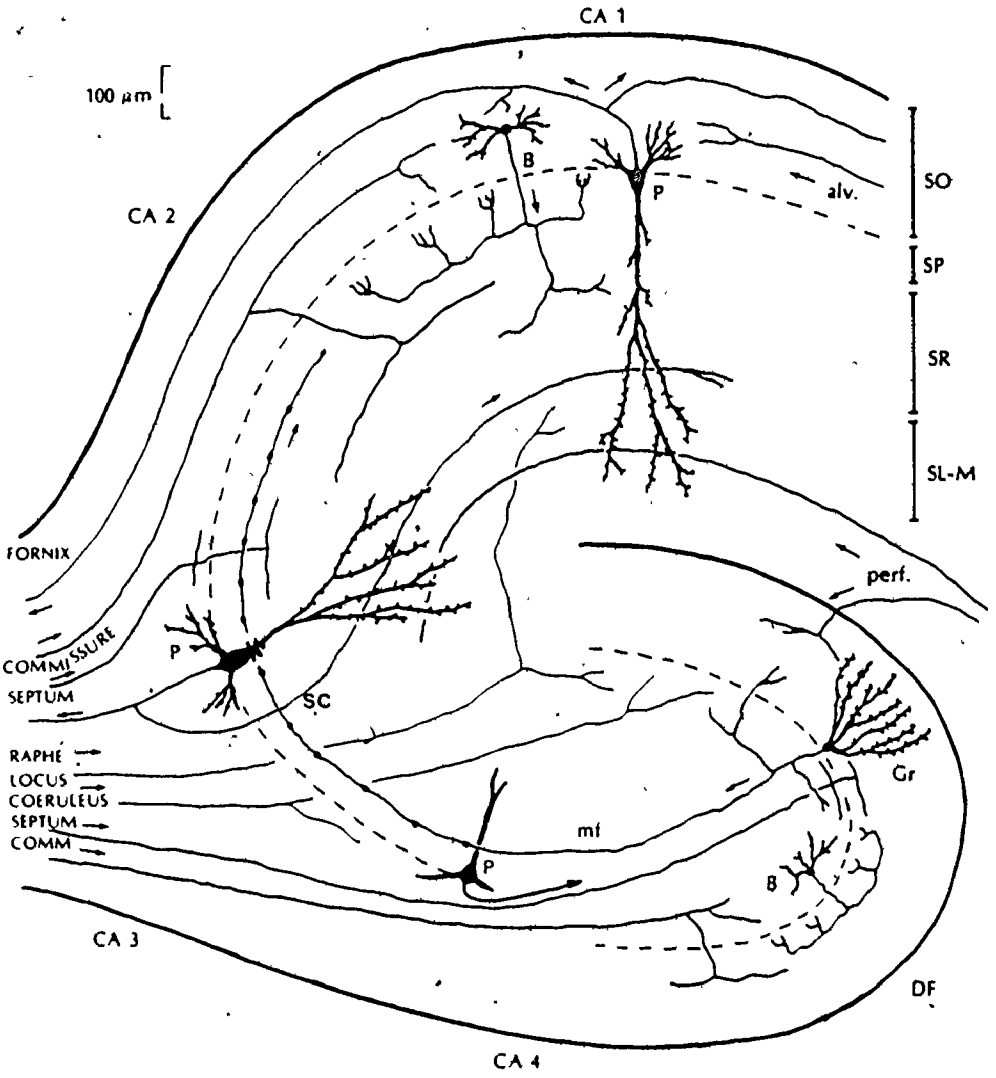

CA 1
100 µm
CA 2
B
P
alv.
SO
SP
SR
SL-M
FORNIX
perf.
COMMISSURE
SEPTUM
P
SC
RAPHÉ
LOCUS
COERULEUS
SEPTUM
COMM
Gr
mf
P
B
CA 3
DF
CA 4

FIGURE 1-1

CHAPTER 2: EXTRACELLULAR STUDIES

2.0 Introduction

As described in the previous chapter, an *in vitro* preparation of the hippocampus exposed to a low concentration of a convulsant drug will develop spontaneous bursts of multipeaked field potentials. The extracellularly-recorded evoked responses, which normally consist of a population EPSP and a single population spike, also develop paroxysmal characteristics. These events are believed to result largely from a reduction of GABA-mediated inhibition, although the convulsants which have been studied may also have other effects. If similar processes occur as long-lasting effects of kindling, then epileptiform activity may be detectable in slices of hippocampus taken from kindled animals.

In the experiments described below, we have examined tissue from animals which had been kindled by stimulation of the hippocampus. The properties of the hippocampal population responses evoked by afferent stimulation, as well as spontaneous activities, were studied and compared with those of control rats. Several other groups of rats were also examined, and these had received kindling stimulation either in a pathway projecting to the hippocampus, or in another region of the limbic system. For convenience, the term "kindled slice" will refer to a hippocampal slice taken from an animal which had received kindling stimulation in any of the brain regions described below. It does not imply that the tissue contained in the slice itself has been kindled, or is in a "kindled state". It is one of the goals of these experiments to

determine whether the slices taken from kindled animals possess epileptic properties.

2.1 General Methods

2.1.1 Kindling

The experimental animals used in the following study were kindled to the stage 5 seizure level (Racine, 1972b). The most recent stage 5 seizure was evoked 24 hours prior to sacrifice of the animal and preparation of the hippocampal slices. During the course of kindling, a variety of electrophysiological characteristics of kindling were examined in situ (Kairiss, Racine, and Smith, 1984). The experiments described below were concerned with the characteristics of the hippocampal slices taken from these and control animals.

The kindling stimulus was a one second train of biphasic, one millisecond duration pulses, with a peak to peak current intensity of 400 μA and a frequency of 60 Hz. Stimulations were applied daily. Four kindling sites were used in this study: the hippocampus, the perforant pathway, the fornix, and the amygdala. The stereotaxic coordinates for these sites are listed in Table 2-1. Four different groups of animals were studied, each stimulated in one of the above kindling sites until at least three stage 5 (evoked) seizures had developed. A fifth group of animals served as a control group and consisted of implanted (unstimulated) animals.

2.1.2 Preparation and Maintenance of Hippocampal Slices

The in vitro techniques employed in these experiments were similar to those reported by many laboratories (c.f. reviews in Kerkut and Wheal, 1981). Adult male rats, of the Long-Evans strain, weighing between 150 and 400 grams were used in these studies. They were either purchased from Quebec Breeding Farms Ltd. or raised from a breeding population maintained within the Department of Psychology at McMaster University.

The rat was anesthetized with ether (anesthesia grade, Mallinckrodt, Paris, Kentucky) and decapitated. If a skull cap connector was present, it was carefully pried off with a spatula assisted by bone scissors. The scalp was slit along the midline to expose the skull, which was removed with a pair of rongeurs. Care was taken to avoid excessive compression of the underlying brain. The dura usually came off with the bone, but if it remained a small scalpel was used to slit it at the margins of the exposure. The brain could then be lifted out of the skull cavity with a small periosteal elevator, which also cut the remaining cranial nerves. The brain thus freed was dropped immediately into a small beaker of ice cold oxygenated artificial cerebrospinal fluid (ACSF).

Following a brief (10 s) immersion in this bath, the brain was placed onto a block of frozen ACSF, with a small depression in its centre which served as a dissection chamber. With the brain lying on its ventral surface, the cerebellum and anterior third of the brain were cut off by a scissors-like motion of a pair of number 11 surgical scalpels. A small midline cut severed the callosal and commissural

fibres, and the right neocortex was peeled away with a spatula, exposing the dorsal portion of the hippocampus. Reflection of neocortex and entorhinal cortex continued until the entire right hippocampus was visible. The spatula was then used to cut the fimbrial and entorhinal connections, thereby freeing the hippocampus. It was lifted out and placed in a small dish of ice cold ACSF, and allowed to cool for approximately 30 seconds. The hippocampus ipsilateral to the stimulated region (usually the right lobe) was removed for study. In a few cases, both hippocampi were dissected.

The tissue chopper (based on the design of Duffy and Teyler (1976)) was prepared by gluing, with rubber cement, a disc of No. 1 filter paper onto the teflon cutting surface, and wetting it with a few drops of cold ACSF. The hippocampus was positioned, dorsal surface up, on this cutting stage and chopped into slices of 450 micrometres (μm) nominal thickness. The slices generally adhered to the cutting blade (Valet "Auto Strop") and were removed by a fine sable hair brush and transferred back to the cold ACSF. In most experiments, from 4-10 slices were cut from the middle third of the hippocampus. The tissue was transferred to the recording chamber (described below) using a wide-bore glass pipette. The entire procedure, from decapitation to placement of slices in the final incubation environment, occupied approximately 5 minutes.

Initial experiments were performed in a chamber constructed according to the description of Spencer et al., (1976). This was a static (non-perfusion) design and consisted of a thermostatically-controlled water bath and inner recording chamber. Later experiments

were performed in a perfusion chamber (purchased from West Coast Instrumentation, Moss Beach, California) similar to that described by Schwartzkroin (1981) (see Figure 2-1). It was modified to permit better control over the level of perfusate in the recording chamber, and a more convenient arrangement of reference electrodes for stimulation and recording. The slices rested on a nylon mesh covered with Kodak lens tissue. The ACSF flowed into the recording chamber from an opening below the mesh, flowed over the slices and drained into the surrounding ditch, from which it was removed by suction. The ACSF was prewarmed to 35°C and continuously bubbled with 95% O_2/5% CO^2; it was then gravity-fed to the chamber at a flow rate controlled either by a pinch clamp or a precision needle valve (Fisher). Flow rate was adjusted to approximately 2.5 mls/min for all experiments. The outer chamber of the apparatus consisted of a water bath which was heated by a coil of nichrome wire. A feedback circuit maintained the temperature of the ACSF in the recording chamber at 34.5°C ± 0.5°C directly below the slices. The gas mixture of 95% O_2/5% CO^2 was also bubbled through the outer water bath and the resulting warm, oxygen-enriched atmosphere was directed over the surface of the slices by means of a system of manifolds built into the cover of the chamber.

Reference electrodes for the recording chamber were Ag-AgCl sintered wires (A-M Systems, Penn.) sealed in place and led out to gold pin connectors. The slices were illuminated from above by a focussed microscope illuminator powered by a well-filtered 6VDC power supply. A similar supply powered the heating circuit, and appropriate shielding

and grounding techniques were employed to minimize noise pickup by the recording apparatus.

The ACSF was composed of (in mEq/l) the following ions: Na^+, 151.25; K^+, 3; $H_2PO_4^+$, 1.25; Mg^{++}, 2; Ca^{++}, 2; HCO_3^-, 26; Cl^-, 131; and 10 mM dextrose. When saturated with 95% O_2/5% CO_2 and warmed to 34.5°C, the pH of the ACSF was 7.2.

Slices were allowed to incubate in the recording chamber for approximately one hour before recording was begun. During this period, little electrophysiological responsiveness, apart from fibre potentials, could be detected. Synaptic responses generally appeared 30-45 minutes following dissection, and usually reached stable values within 60 minutes. Tissue prepared as described above maintained stable electrophysiological activity for periods of up to twelve hours. Less reliable activity could be detected up to 20 hours after dissection. Most data collection, however, took place within eight hours following preparation of the slices as this period seemed to represent the most stable phase of the preparation.

2.1.3 Electrophysiological Techniques

Recording electrodes were pulled from plain capillary glass, 1.5 mm OD, on a vertical puller (Model 900, David Kopf Instruments, Tujunga, California). The tips were broken back to 1.5-2.5 µm by percussion, and were backfilled under pressure with 4M NaCl. They were connected with a Ag-AgCl bridge to either an AC-coupled (P15) or a DC-coupled (P16, Grass Instruments, Massachusetts) amplifier. Recorded signals were displayed on an oscilloscope and photographed, or stored in the computer (PDP/8e,

Digital Equipment, Massachusetts) for further analysis and plotting. A schematic of the apparatus, including that used in the intracellular studies of the next chapter, appears in Figure 2-2.

Stimuli were delivered with a monopolar or bipolar arrangement of etched tungsten wire, tip diameter approximately 50 μm. Stimulus pulses were generated by digital stimulators (Series 830, WP Instruments, Connecticut) and coupled to the electrodes by photon coupled, constant current isolation units (WPI series 850). Typical stimulus parameters consisted of diphasic pulses, 100 μs in duration, of 200 μA peak to peak intensity. Stimulus current never exceeded 1000 μA.

The basic recording protocol consisted of the following: Upon completion of the 1 hour incubation period, a recording electrode was inserted into the slice in various cell body regions to detect the presence of spontaneous population potentials. The CA1 region was examined most thoroughly, but CA2, CA3, CA4, and the dentate gyrus were also studied. Single-pulse stimulation at increasing intensities was then applied to afferent fibres, i.e. the perforant pathway to activate the dentate granule cells, the mossy fibers to activate CA3 pyramidal cells, and stratum radiatum (Schaffer Collaterals) to activate CA1 pyramidal cells. Double-pulse stimulation consisted of a pair of pulses, with a varying inter-pulse interval (IPI). The characteristics of the test (second) response were studied as a function of those of the control (first) response characteristics and of the IPI. Finally, brief high frequency trains of stimuli were applied to the various afferents, and the responses before, during and after the trains were compared.

The above measurements were performed at three different levels of $[K^+]$ in the perfusate: 3mEq/l, 6mEq/l, and 9mEq/l. A few slices were also studied in 12 mEq/l $[K^+]$. ACSF containing 3mEq/l $[K^+]$ will be referred to as "3K" or "normal medium", since in situ measurements indicate this to be the physiological concentration of the ion in the extracellular space. ACSF containing 6mEq/l and 9mEq/l potassium levels will be referred to as "6K" and "9K", respectively.

2.1.4 Selection of Slices For Study

Initial experiments with the slice preparation demonstrated that trauma or anoxia to the slices during their dissection or study could produce either a severe depression of electrophysiological responsiveness, or in some cases, epileptiform activity (see Dingledine et al., (1980) and Langmoen and Andersen (1981)). These abnormal appearance of the slice. Unevenly cut and/or torn slices were often electrically depressed or hyperexcitable, as were slices whose surface appeared dry, probably due to inadequate humidity in the chamber. Occasionally, however, slices showed abnormal behaviour in the absence of such physical signs, and it must be assumed that some other factors are responsible. Possibilities include an excessive anoxia during dissection, and compression of the tissue during slicing. As experience increased, the number of depressed or hyperexcitable slices per dissection decreased. It was standard procedure however, to eliminate from study slices which failed to respond to maximal stimulation with a CA1 population spike of at least 6 mV, and the majority of slices

examined in these experiments had maximal population spikes of at least 10mV.

Another important methodological issue concerns the experimental protocol. From four to ten slices were usually prepared from each hippocampus and transferred to the recording chamber. Slices with obvious physical damage were immediately discarded. A preliminary electrophysiological survey of the remaining slices identified those which met the above-described electrophysiological criterion for viability. The number of slices which were studied in each experiment, then, was determined by the number of physically and electrophysiologically intact slices, and the particular experimental protocol. Ideally, this protocol should be identical for all experimental and control groups, but deviations from a standard protocol occurred for several reasons. Long-term electrophysiological stability of recordings could be insured by minimizing the number of repeated insertions of the electrodes into any particular slice. The most reliable observations were those in which the electrodes remained in one position for an extended period of time. On the other hand, slices taken from the same hippocampus could differ due either to differences in their preparation, or to an inhomogeneity of hippocampal properties (in unkindled as well as kindled rats). This fact demanded that as many slices as possible be examined. The standard protocol represents a compromise among these factors, subject to the constraints imposed by a limited useful lifetime of the preparation. Slices which displayed peculiar electrophysiological characteristics were studied more extensively and for a longer fraction of the experiment, sometimes at the expense of performing a thorough examination of the remaining

slices. Those slices which were not recorded from for a sufficient length of time to ensure the stability and reliability of the observed electrophysiological responses were excluded from the analysis. As a result, the number of slices comprising each experimental group varies.

2.2 Results

This section presents five groups of experiments: one control group and four experimental groups. The control group contains pooled data from implanted, unstimulated controls from each of the experimental groups, together with results from naive rats. Each experiment will be prefaced by an introduction, followed by a presentation of the data. The results for all groups will be discussed together in Section 2-3.

2.2.1 Slices From Unkindled Rats

Data for this group of experiments are summarized in Table 2-2. No spontaneous field potentials (in 49 slices) were observed in any slices perfused with normal (3mEq/l $[K^+]$) medium. Comparisons of the potential recorded by the extracellular electrode when immersed in the ACSF in the recording chamber and the potential when it was inserted into a slice revealed no differences greater than approximately 500 μV. Such small differences are likely to represent electrode tip potentials due to partial obstruction of the open tip by tissue. These potentials were usually quite steady and persisted even when the slices became unresponsive as a result of deterioration or prolonged anoxia.

Single-cell activity was sometimes encountered during advancement of the microelectrode through a cell-body-containing region of the slice. The extracellular action potentials of one or more neurones could be identified as brief (<1 ms) di- or triphasic due to a mechanical perturbation of a neuronal membrane by the advancing

microelectrode. Spontaneous single-cell activity which seemed to be less influenced by electrode movement was seen only when the ACSF contained elevated $[K^+]$.

Pyramidal neurones of the CA1 region could be activated by stimulation of stratum radiatum (SR) throughout the CA3, CA2, and CA1 regions. This stimulation activated several groups of fibres, including commissural and associational fibres, and the Schaffer collaterals of CA3 neurones. The characteristic CA1 field potentials to SR stimulation are illustrated in Figure 2-3A. Diphasic pulses of increasing current intensities were administered by the stimulating electrode, which was situated approximately 500 μm from the recording sites. The top set of responses were recorded from the cell body layer of CA1, and stratum pyramidale (SP). The lower set was recorded simultaneously by another microelectrode situated in the corresponding dendritic region in CA1. The smaller monophasic responses in each set are population EPSP's, and are generated by the summed extracellular currents of all neurones generating an EPSP. Note that this response is negative in the dendritic region, and positive in the cell body layer which is due to the dipole nature of the potential generated by the neurones. In this case, the negativity is associated with an active, inward flow of positive ions, while the positivity reflects passive outward flux. The strength of the stimulation activates a larger population of afferent fibres, and increases the size of the EPSP until some neurones are depolarized to the threshold for action potential (AP) generation. This appears in the field potential recordings as a negative deflection superimposed on the positive EPSP in the cell body layer and is referred

to as the population spike. The dipole reflection of this event appears as a positive-going notch on the dendritic population EPSP. Population spikes usually appeared at stimulus currents of 50-100 μA.

The amplitude and duration of the population spike are governed by the number of discharging neurones and their relative synchrony (Andersen et al., 1971a). Maximal population spikes of 20 mV in amplitude were routinely recorded in healthy preparations.

A similar pattern of responses was recorded in the granule cell region of the dentate gyrus. Stimulation of the perforant pathway, on either side of the hippocampal fissure produced responses like those shown in Figure 2-3B. The CA3 regions also generated a population EPSP-population spike pattern (Figure 2-3C), but the potentials were of a smaller peak amplitude than those seen in CA1. This is due to the lower neurone density of CA3 and the correspondingly lower current density resulting from its activation.

In summary, CA1 population potentials evoked by stimulation of afferent fibres in stratum radiatum consisted of graded population EPSPs upon which were superimposed single population spikes. The generation of a second population spike was very unusual (1/49 slices, cf. Table 2-2). No slices developed spontaneous population potentials.

Responses To Paired-Pulse Stimulation: A typical CA1 response to stimulation with a pair of stimulus pulses is shown in Figure 2-4A. Note that the test response is potentiated with respect to the conditioned response at all of the interstimulus (C-T) intervals shown. This facilitation can be on the order of several hundred percent, as seen in Figure 2-4B, where only a very small population spike appears in

response to the conditioning stimulus, but a much larger one is triggered by the test stimulus. The maximum C-T interval at which facilitation was still detectable varied considerably among slices, and ranged from 350 to 1000 ms. It was dependent on the location of the stimulation and recording sites, as well as on the magnitude of the conditioning response. The only consistent observation among slices, and among preparations, was that the maximal facilitation invariably occured within the 30-50 ms C-T interval.

Paired-pulse facilitation of the population EPSP was readily observed when both stimuli were subthreshold for a population spike. In most cases, however, the degree of facilitation was sufficient for the production of a population spike, and a pure EPSP response to the second stimulus could not be examined (Figure 2-5A).

Some slices displayed an inhibition of the population spike over the 10-30 ms C-T interval (Figure 2-5B). This inhibition could only be demonstrated with carefully selected recording and stimulation sites, and by suitable adjustment of the stimulus strength. The phenomenon could not be correlated either with any electrophysiological indicators of slice viability, or with slice thickness or appearance. In spite of the infrequent occurrence of paired-pulse inhibition, once detected, it was a robust effect; it could be observed to persist for the duration of an experiment.

Responses To High Frequency Tetanus: The application of a brief (e.g. 400 ms) train of high frequency stimuli (e.g. 100 Hz) often resulted in some degree of post-activation potentiation (Figure 2-4C, upper record). Note that the first population spike has approximately

doubled in size, and a second spike has appeared. This substantial degree of potentiation rapidly subsided over 10-15 seconds following the tetanus, and a longer-lasting potentiation remained (Figure 2-4C, lower trace). This effect was found, in several cases, to persist for several hours, and may represent the in vitro form of the long-term potentiation phenomenon seen in situ (Bliss and Lomo, 1973). Not all slices receiving high frequency stimulation displayed this effect, however, and it did not appear to be correlated consistently with slice viability or electrophysiological responsiveness.

High-frequency stimulation sometimes triggered an afterdischarge (AD) (Figure 2-5C). These were always quite brief (100-300 ms and were most commonly elicited in the CA1 and CA3 regions of the slice. The dentate gyrus never generated an AD when the perforant path fibers were stimulated with brief trains.

Effects of Elevated $[K^+]$ in Perfusate: Figure 2-6(A) illustrates the effect on evoked responses of raising the potassium concentration to 6 mEq/l and 9 mEq/l. The amplitude of the population spike increased, and the latency decreased, as the K^+ concentration increased. A second population spike appeared in 9K and sometimes appeared in 6K as well (Figure 2-6B; Table 2-2). Elevating the potassium concentration to 12 mEq/l inevitably resulted in multiple population spikes (lower trace in Figure 2-6B).

Spontaneous single-cell activity was more commonly encountered in 6K and 9K than in normal medium. Extensive extracellular observations of single-cell behaviour were not performed in these experiments, but the impression was gained that the sensitivity of

neurones to mechanical disturbance was increased during perfusion with elevated potassium levels.

Spontaneous epileptiform field potentials, which were never observed under normal conditions, appeared in a small number of slices in the elevated potassium experiments (10% of slices in 6K, 18% in 9K; see Table 2-2). They appeared only in those slices which also generated multi-peaked field potentials in response to stimulation. An example of this type of potential appears in Figure 2-14, where it is compared with that seen in a kindled preparation.

2.2.2 Hippocampal Kindling

This group of animals had received kindling stimulation to the CA3 region of the hippocampus, and all subjects had experienced several stage 5 motor seizures. It was expected that, in comparison with control tissue which did not generate spontaneous epileptiform activity under normal in vitro conditions, hippocampal slices from these rats would display some significant degree of abnormal behaviour.

A total of 56 hippocampal slices from 8 kindled rats were studied, and the results for this group are presented in Table 2-3. Slices from 3 other animals in this experiment displayed very weak electrophysiological responses and were not accepted for analysis.

Spontaneous Activity: Most preparations did not show any signs of spontaneous electrical activity, either from single neurones or from populations of neurones. A small number of slices (4/56 from 3 animals in 3K) showed spontaneous epileptiform field potentials (e.g. Figure 2-14). This value was found not to be significantly different from

controls (Fisher Exact Test, $p=0.05$). These spontaneous bursts occurred with a frequency of 0.07-0.5 Hz, and could also be elicited by electrical stimulation to a variety of areas within the slice. They appeared to originate in the CA3 region, and always resulted in the appearance of a multi-spiked field potential in CA1, presumably through activation of the Schaffer collaterals.

The slices which displayed these spontaneous discharges, as well as some which did not, also contained some spontaneously active single cells (Figure 2-15). Some of these could be synaptically driven (Figure 2-16) while others showed behaviour independent of stimulation. Cells were most easily isolated in the CA1 region, presumably due to the high packing density of somata in this region: CA3 neurones were not recorded, although their activity might be expected to show more intense paroxysmal characteristics.

Responses to Afferent Stimulation: The majority of slices (50/56 in 3mM $[K^+]$) responded to stimulation of afferent fibers in a fashion indistinguishable from controls. Figure 2-7 illustrates typical field potentials in response to single pulse activation of stratum radiatum and mossy fibers. High frequency tetanus often produced a transient potentiation of the responses, but never produced a prolonged afterdischarge (Figure 2-7D). Multiple population spikes were seen in the remaining 6/56 slices, a number not significantly different from control experiments (Table 2-3). The morphology of these atypical responses ranged from the appearance of a small 2nd population spike following the primary one (Figure 2-8A) to a long-lasting (> 200 ms) paroxysmal discharge (Figure 2-8C,D). In some slices, stimulation of

the CA1 region triggered, in addition to a primary CA1 population response, an all-or-none discharge in the CA3 region (Figure 2-9). A remarkable feature of this response was its variable latency following stimulation, ranging from approximately 21 ms to 60 ms. This CA3 activity appeared to reactivate the CA1 region, presumably via the Schaffer collateral system, resulting in an irregularly-shaped field potential in the cell body region. Dendritic recordings, however (Figure 2-10A,B,C) revealed a smaller field potential in the region of Schaffer collateral termination than that associated with the primary response. This all-or-none CA3 paroxysm could also be evoked by stimulation of stratum radiatum in the CA3 region and by stimulating the dentate hilus, thereby activating the mossy fiber afferents (Figure 2-11). A similar pattern of responses was seen in other slices (e.g. Figure 2-12).

Observations in Perfusate with Elevated $[K^+]$: The percentage of slices demonstrating multiple population spikes and spontaneous activities in 6K and 9K did not differ significantly ($p=0.05$, Fisher Exact Test and Chi-Square) from control experiments. The slices which had generated spontaneous discharges in normal medium maintained this activity in the elevated potassium levels, with an intensification in amplitude, frequency and number of spikes.

Summary: Slices taken from animals which had received kindling stimulation in the hippocampus did not show significant increases in the frequency of either multiple population spikes or spontaneous epileptiform bursting. Although not statistically significant, 4/56 slices did generate spontaneous epileptiform behaviour in normal medium;

no other experimental group (see below) displayed this behaviour under the low potassium condition.

2.2.3 Kindling of Hippocampal Inputs

The results of the previous experiment, in which electrodes had been implanted directly into the hippocampus for kindling stimulation, demonstrated no profound increase in epileptogenicity in hippocampal slices. It is possible that removal of the electrodes during dissection traumatized the hippocampus to a degree sufficient to depress epileptiform activity during the subsequent study. The following experiment attempts to circumvent this difficulty by examining slices derived from animals which had been stimulated in either of the two major input pathways to the hippocampus. The perforant pathway, originating in the entorhinal cortex, makes extensive en passage synaptic connections with granule cells of the dentate gyrus which, in turn activate the CA3 pyramidal neurones through the mossy fiber system. The CA1 region is also activated by stimulation of the perforant pathway, both monosynaptically by a component of the perforant pathway, and polysynaptically through the Schaffer collaterals. The fimbria/fornix system contains fibers from numerous regions and provides extensive activation of CA3 and CA1 regions. During kindling the hippocampus receives powerful synaptic input directly from the stimulated site, and indirectly from other areas participating in the seizure discharge. This experiment tests whether this activity has led to the production of epileptogenicity expressed in the slice

preparations, and eliminates the possibility of direct electrode-induced damage to the hippocampus.

Seventeen slices were studied from seven animals that received fimbria/fornix stimulation, and fifteen slices were studied from 5 animals that received perforant path stimulation. The results are summarized in Tables 2-4 (Fimbria-Fornix) and 2-5 (Perforant Path).

Spontaneous Activity: No slices from either of these groups displayed spontaneous field potentials, either in the CA regions or in the dentate gyrus. The spontaneous activity of single neurones, when detected, did not appear to be different from that seen in unkindled slices. These results are not statistically different from control values.

Responses to Afferent Stimulation: In the fornix/fimbria group 4/14 slices (29%) in normal medium responded to afferent stimulation with multiple population spikes. This value is significantly different from the control data at the 0.01 level (Fisher Exact Test). However, these slices were strongly clustered (3 slices from one animal, 1 slice from another). Only 1 slide from the perforant path group displayed a double population spike in CA1 (not significant, p=0.05). No multiple population spikes were ever evoked in the dentate granule cell region. High frequency tetanus to the Schaffer collaterals or to the perforant pathway did not induce sustained afterdischarges. Experiments in which double-pulse stimulation was applied revealed a pattern of facilitation and inhibition similar to that seen in unkindled tissue.

Responses in elevated $[K^+]$: The same slices of the fornix/fimbria group which displayed hyperexcitability in normal medium maintained multiple-spike evoked responses in 6K and 9K. An additional

slice (from another experiment) developed multiple population spikes in 6K. The number of slices in the perforant-path group generating multiple spike responses also showed an increase, but in neither of these groups were the results statistically different from the unkindled control values. The evoked responses of the dentate gyrus did not generate multiple population spikes at either level of elevated potassium.

An interesting feature of these experimental groups was the absence of spontaneous epileptiform field potentials in 6K and 9K. In control experiments, a small proportion (see Tables 2-3 and 2-4) of the slices which develop multi-spike responses also develop spontaneous IISs in 6K and 9K, but this was never seen in these experimental groups. Although the statistical comparisons show these differences to be insignificant, the trend may represent a suppression of underlying epileptogenicity.

Summary: Hippocampal slices taken from rats kindled in the fimbria/fornix or perforant pathway displayed no significant increases in epileptogenicity. The only exception to this was the finding, in the perforant path group, of a significant increase in the proportion of slices generating multiple population spikes in response to Schaffer collateral activation. This effect was observed only in normal (containing 3mEq/l of potassium) perfusate.

2.2.4 Kindling of the Amygdala

The amygdala is one of the most rapidly-kindling regions in the rat brain. Amygdaloid projections to the hippocampus are indirect, since it projects to the entorhinal cortex, which then acts as a relay to the

hippocampus by way of the perforant path (Price, 1981). The direct stimulation of the hippocampus or its inputs employed to kindle the rats in the previous experiments may have produced a suppression or inhibition of hippocampal epileptogenicity because of excessive activation. Stimulation of the amygdala may produce a different pattern of hippocampal activation during kindling, and trigger an epileptogenic mechanism. Twelve slices from four amygdala-kindled rats were studied in this group of experiments, and the results are summarized in Table 2-6.

Spontaneous activity: None of the slices studied generated IIS activity in normal medium. This result is similar to that found in the previous groups, and is not significantly different from the control behaviour.

Evoked Responses: Three out of twelve (25%) of the slices generated multiple population spikes in CA1 in response to Schaffer collateral stimulation. This differs significantly from the observed control value of 1/49 slices demonstrating multiple population spikes (Fisher Exact Test, $p=0.05$). The characteristics of paired pulse facilitation and inhibition were not different from those seen in unkindled slices.

Effects of Elevated $[K^+]$: Six out of twelve slices in 6K developed multi-spike responses, and the same six slices maintained this behaviour in 9K. These 6 hyperexcitable slices were distributed over 3 experiments. Only one slice (in 9K) developed spontaneous IIS activity. These results are not significantly different from controls.

Summary: Hippocampal slices derived from amygdala-kindled animals showed a significant (at the p=0.05 level) increase in multiple-spike population responses under conditions of normal perfusate. No significant differences were found at higher levels of potassium concentration, or in the development of spontaneous epileptiform activity.

2.3 Summary

The above experiments have examined several electrophysiological properties of hippocampal slices taken from kindled rats. Two types of electrophysiological features were studied. The first property was the appearance of spontaneous IIS activity in the CA1, CA3, or dentate gyrus regions of the slice. The other principle measurement involved the application of electrical stimulation to afferent fibre systems and study of the population EPSP and spike responses. These observations were performed while the slices were bathed in ACSF containing normal potassium ion concentrations (3 mEq/l) or elevated levels of this ion (6 and 9 mEq/l).

Hippocampal slices taken from unkindled rats, when bathed in normal ACSF, did not develop spontaneous epileptiform activity. Except for one group, none of the slices taken from kindled animals showed IIS activity. In the group kindled in the CA3 region, 7% of the slices did show spontaneous spikes, but this value is not significant at the 0.05 level. Interpreted as a trend, however, this observation suggests that kindling stimulation applied directly to the hippocampus may produce a small degree of hyperexcitability within hippocampal circuitry.

In ACSF containing elevated levels of potassium, some slices from unstimulated animals did generate spontaneous epileptiform spikes (10% of slices in 6K, 18% in 9K). Although the results from the kindled groups do not show a statistically significant difference from those of the control group, several interesting trends appear. The group which had received direct stimulation to the hippocampus, the CA3 kindled animals, showed a slightly higher occurrence of IIS activity than did controls (12% vs 10% in 6K, 34% vs 18% in 9K). With the exception of slices from amygdala-kindled animals in 9K, slices from the other kindled groups did not generate IIS activity under any of the conditions tested. Although the number of slices included in the analysis is rather small, these observations suggest that kindling of certain inputs to the hippocampus may in fact reduce the tendency of the hippocampus to generate IIS activity.

Application of electrical stimulation to the stratum radiatum region in the hippocampal slice resulted, in unkindled tissue, in the appearance of field potentials with graded characteristics in the CA1 cell body layer. When studied in normal medium, this response consisted of a population EPSP with a population spike superimposed at higher stimulation intensities. A second population spike was a rare event (1/49 slices). Exposure to elevated potassium ion concentration resulted in an increase in the occurrence of multiple population spikes (26% in 6K, 53% in 9K). Two groups of kindled slices departed from this behaviour. The fornix-fimbria kindled slices (in normal ACSF) and amygdala-kindled slices (also in normal ACSF) both demonstrated a significantly higher occurrence of multiple-population spike responses

than did control slices. Several interesting trends were present in other groups. The CA3-kindled slices, which had a tendency towards increased IIS activity when compared with controls, tended towards a lower occurrence of multiple population spikes at higher potassium levels. In contrast, the perforant-path group appeared to show a smaller frequency of multiple population spike occurrence as well as IIS activity.

TABLE 2-1
STEREOTAXIC COORDINATES (mm)

Brain Site	A-P (from Bregma)	M-L	D-V (from skull)
CA3	-2.6	4.25	4.25
Fornix/Fimbria	0.0	1.5	5.0
Perforant Path	-6.0	5.5	4.5
Amygdala	-1.0	5.5	8.5

Abbreviations:
A-P Anterior-Posterior
M-L Medial-Lateral
D-V Dorsal-Ventral

TABLE 2-2: CONTROL SLICES

	Normal	ACSF	6K	9K
Frequency of multi-spike evoked responses		1/49	13/49	18/34
Frequency of spontaneous epileptiform bursts		0/49	5/49	6/34

NOTE:
1. The fractions indicate the number of slices demonstrating either multiple population spikes or bursts out of the total number of slices studied.

TABLE 2-3: CA3 KINDLING

A. Frequency of Multiple Population Spike Evoked CA1 Responses

	3K	6K	9K
Kindled (N=8)	6/56	8/41	15/41
Control (N=12)	1/49	13/49	18/34

B. Frequency of Spontaneous Epileptiform Bursts

	3K	6K	9K
Kindled (N=8)	4/56	5/41	14/41
Control (N=12)	0/49	5/49	6/34

NOTES:
1. The fractions indicate the number of slices demonstrating either multiple population spikes or bursts out of the total number of slices studied.

2. Application of Fisher's Exact Probability test (for frequencies less than 5) or Chi-squared analysis to the 2X2 table formed at each potassium level reveals no significant differences between kindled and control frequencies.

3. The use of either Chi-squared or Fisher's Exact test in this and subsequent analyses assumes that all slices, including those derived from the same animal, are independent.

TABLE 2-4: FORNIX/FIMBRIA KINDLING

A. Frequency of Multiple Population Spike Evoked CA1 Responses

	3K	6K	9K
Kindled (N=7)	4/14 *	5/15	4/5
Control (N=12)	1/49	13/49	18/34

B. Frequency of Spontaneous Epileptiform Bursting

	3K	6K	9K
Kindled (N=7)	0/14	0/15	0/5
Control (N=12)	0/49	5/49	6/34

NOTE: * indicates a value significant at the 0.01 level (Fishers Exact Test).

TABLE 2-5: PERFORANT PATH KINDLING
CA1 Responses

	3K	6K	9K
Kindled (N=5)	1/15	2/15	5/12
Control (N=12)	1/49	13/49	18/34

B. Frequency of Spontaneous Epileptiform Bursting

	3K	6K	9K
Kindled (N=5)	0/15	0/15	0/12
Control (N=12)	0/49	5/49	6/34

NOTE: No significant effects were detected
(Fishers Exact test and Chi-square analysis).

TABLE 2-6: AMYGDALA KINDLING

A. Frequency of Multiple Population Spike Evoked

	3K	6K	9K
Kindled (N=4)	3/12 *	6/12	6/12
Control (N=12)	1/49	13/49	18/34

B. Frequency of Spontaneous Epileptiform Bursting

	3K	6K	9K
Kindled (N=4)	0/12	0/12	1/12
Control (N=12)	0/49	5/49	6/34

NOTE: * indicates a value significant at 0.05 level (Fishers Exact test).

FIGURE 2-1: PHOTOGRAPH OF SLICE CHAMBER.

Details of the chamber are described in the text. A glass recording pipette is positioned on the left, while a pair of tungsten stimulating electrodes is seen on the right.

FIGURE 2-2: SCHEMATIC DIAGRAM OF STIMULATING AND RECORDING APPARATUS

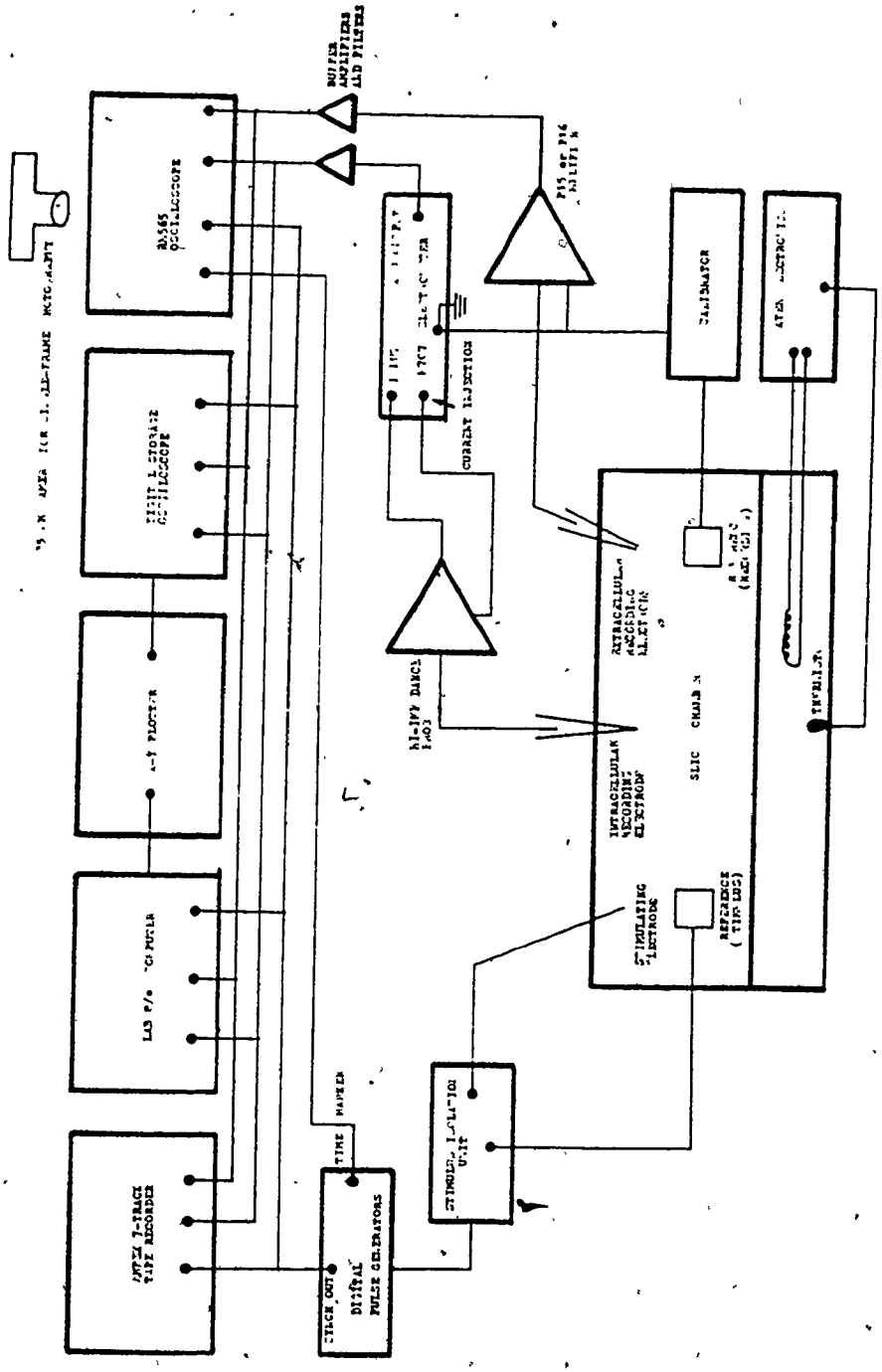

BUFFER AMPLIFIERS AND FILTERS
DIGITAL PULSE GENERATORS
STIMULATING ELECTRODE
REFERENCE (TIP LEAD)

FIGURE 2-2

FIGURE 2-3: RESPONSES OF NORMAL HIPPOCAMPAL SLICE.

Top trace of each pair is from cell body layer, bottom trace from dendritic layer. A. CA1 response to stimulation of stratum radiatum at increasing intensities. Both recording sites show a progressive increase in the response, characterized by an increasing population EPSP, with eventual superposition of a population spike. B. Dentate response to perforant path stimulation. The afferent volley, or fibre response, is clearly distinguishable in the dendritic recording. C. Response of CA3 region to a pair of stimulus pulses, applied to the mossy fibres at near-maximal stimulus strength. Note the smaller peak amplitude of the population spike, compared with CA1 response. Arrows mark stimulation. The calibration pulse in each figure is 2 ms and 2 mV.

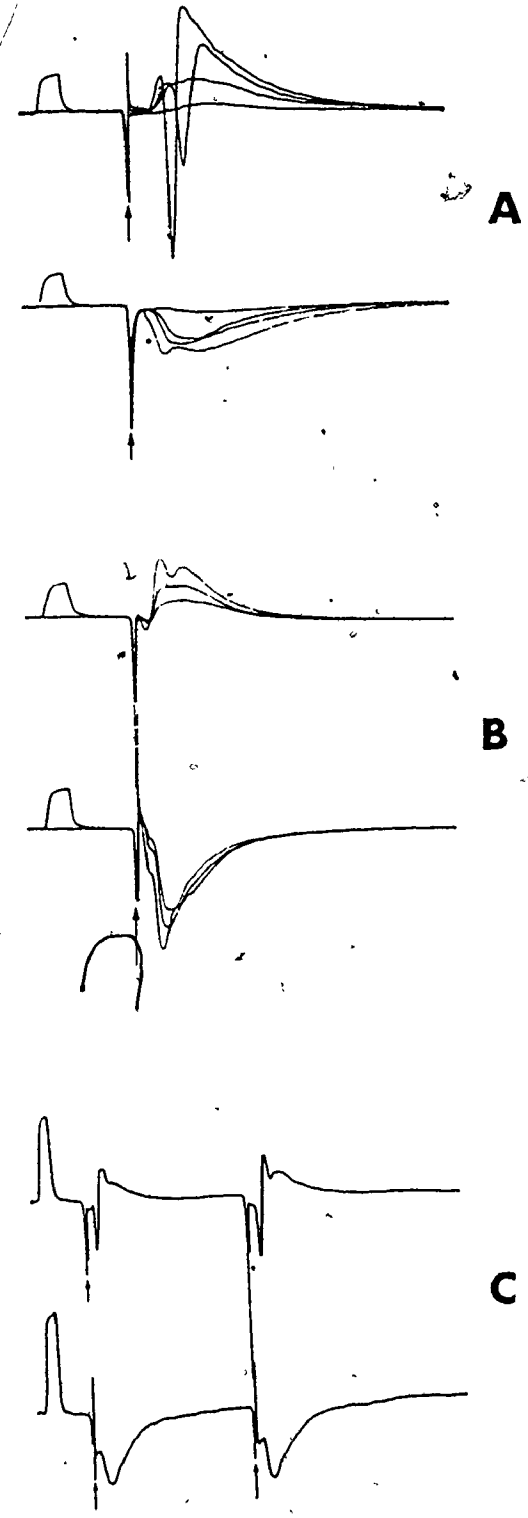


FIGURE 2-3

FIGURE 2-4: ADDITIONAL FEATURES OF CA1 RESPONSE.

A. Superimposed responses to stimulus pairs separated by 10, 30, and 60 ms. Note pronounced facilitation, and small degree of inhibition. B. Similar to A, but conditioning stimulus is of smaller amplitude, to exaggerate the degree of facilitation. C. Responses to high frequency tetanus. Top trace, control response (single small population spike) superimposed on the test response (double population spikes) evoked 5 seconds following a 400 ms train of 100 Hz stimulus pulses at the same strength as test pulses. Lower trace, the response 10 minutes later, demonstrating some degree of post-activation potentiation. The calibration pulse in each figure is 2 ms and 2 mV.

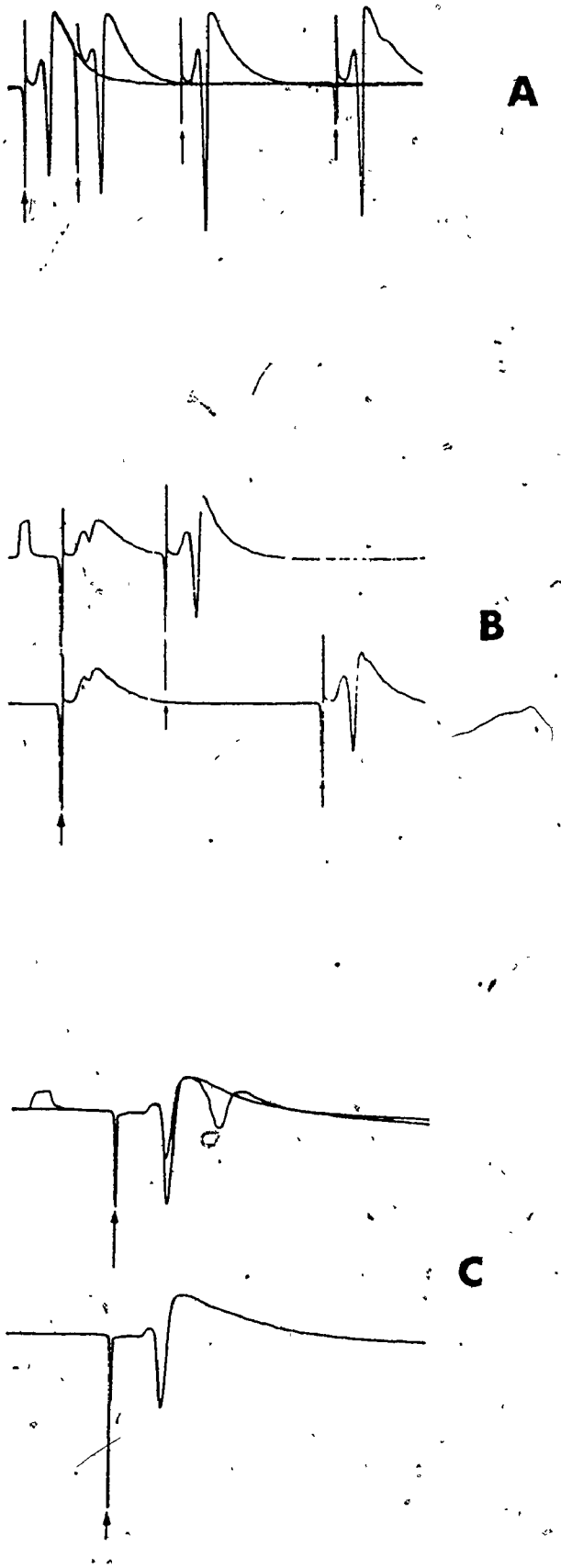


FIGURE 2-4

FIGURE 2-5: ADDITIONAL FEATURES OF CA1 RESPONSE.

A. Example of strong paired-pulse potentiation with the response to the first pulse consisting only of a population EPSP. B. When first response contained a population spike, some degree of inhibition of the second population spike could occur. C. Brief afterdischarge following a high-frequency stimulus train. The calibration pulse in each figure is 2 ms and 5 mV.

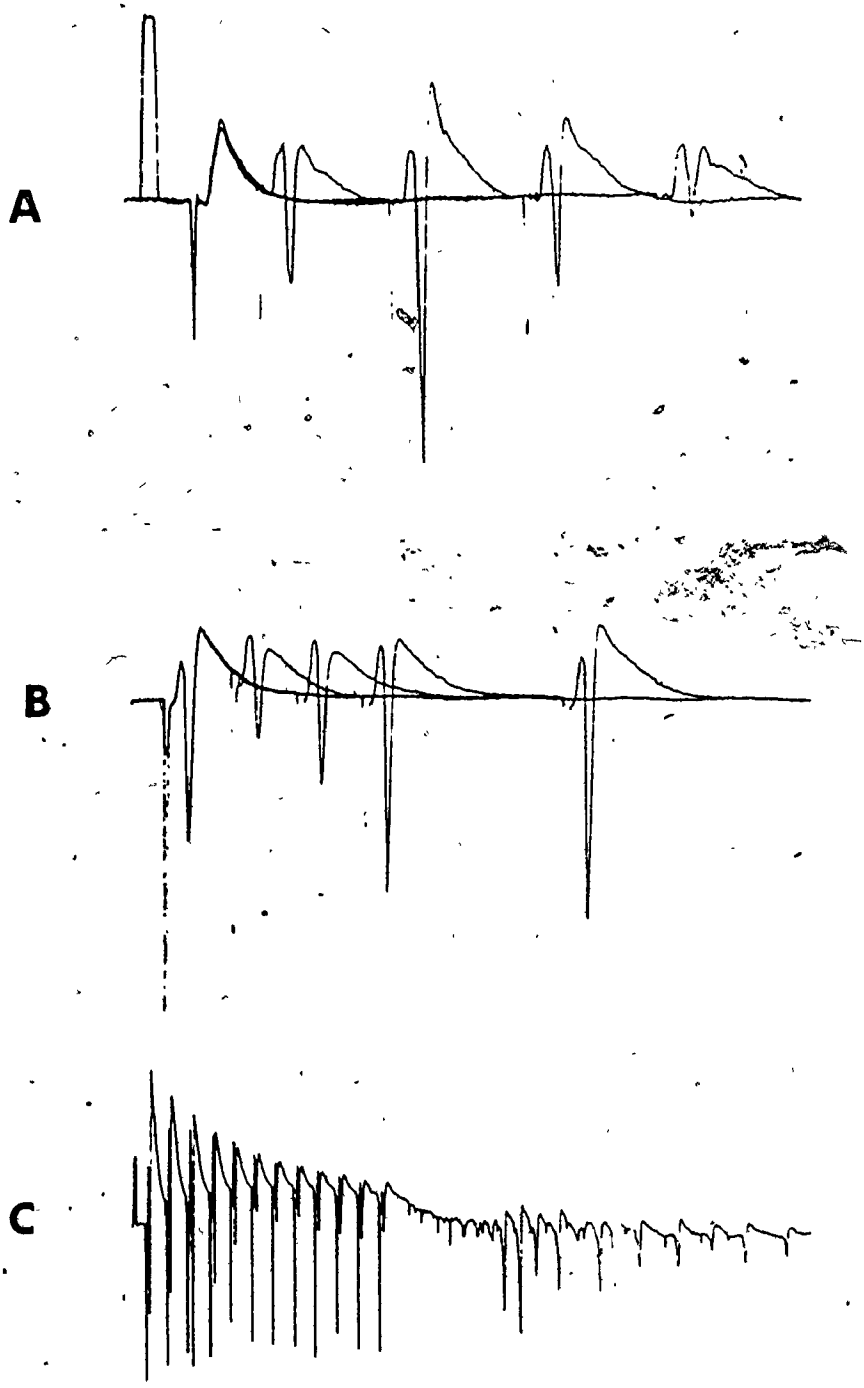


FIGURE 2-5

FIGURE 2-6: RESPONSES TO ELEVATED $[K^+]$ IN PERFUSATE.

A. CA1 cell body layer (top) and dendritic layer (bottom) responses to stratum radiatum stimulation, in 3,6, and 9 mEq/l potassium. The responses in 3K and 6K show single population spikes, that in 9K has a double spike. B. Top trace, cell body layer response in 6, 9, and 12 mEq/l $[K^+]$, in another preparation. Bottom trace, response in 12 mEq/l $[K^+]$, in another slice from same experiment. Calibration pulse is 2 mV, 2 ms.

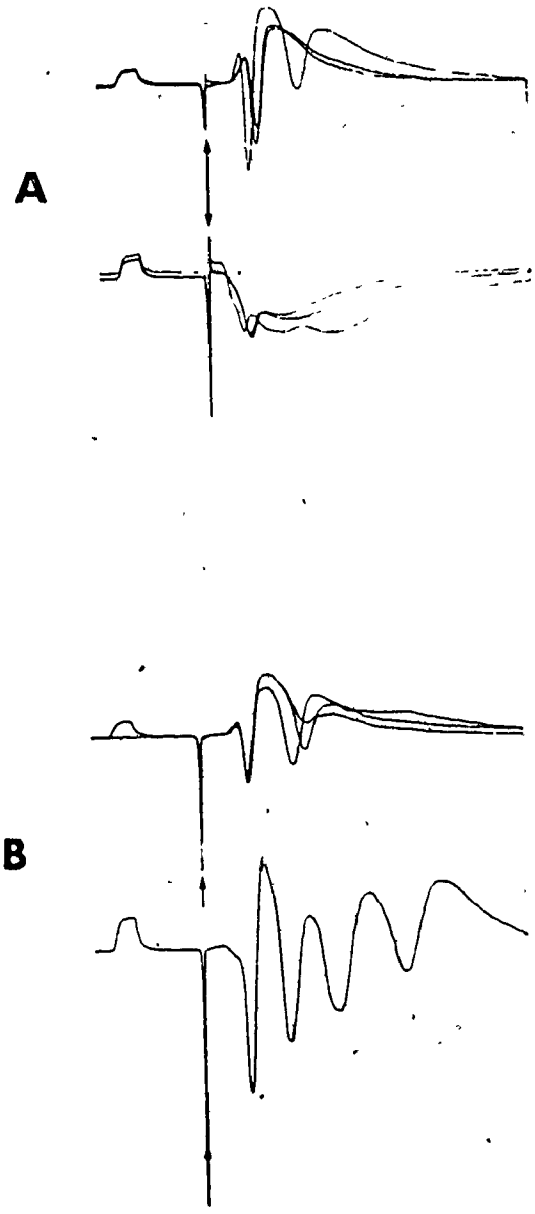


FIGURE 2-6

FIGURE 2-7: TYPICAL RESPONSES FROM HIPPOCAMPAL-KINDLED RAT.

A. CA1 response evoked by increasing stimulus current applied to stratum radiatum afferents. B. Population response of dentate granule cells to stimulation of perforant pathway. C. Response of CA3 region to stimulation of mossy fibers. D. Response of CA1 to a 50 ms train of 300 Hz pulses. Note lack of afterdischarge.

Calibration: horizontal = 8 ms (A,B,C,);
40 ms (D)
vertical = 2 mV (all)

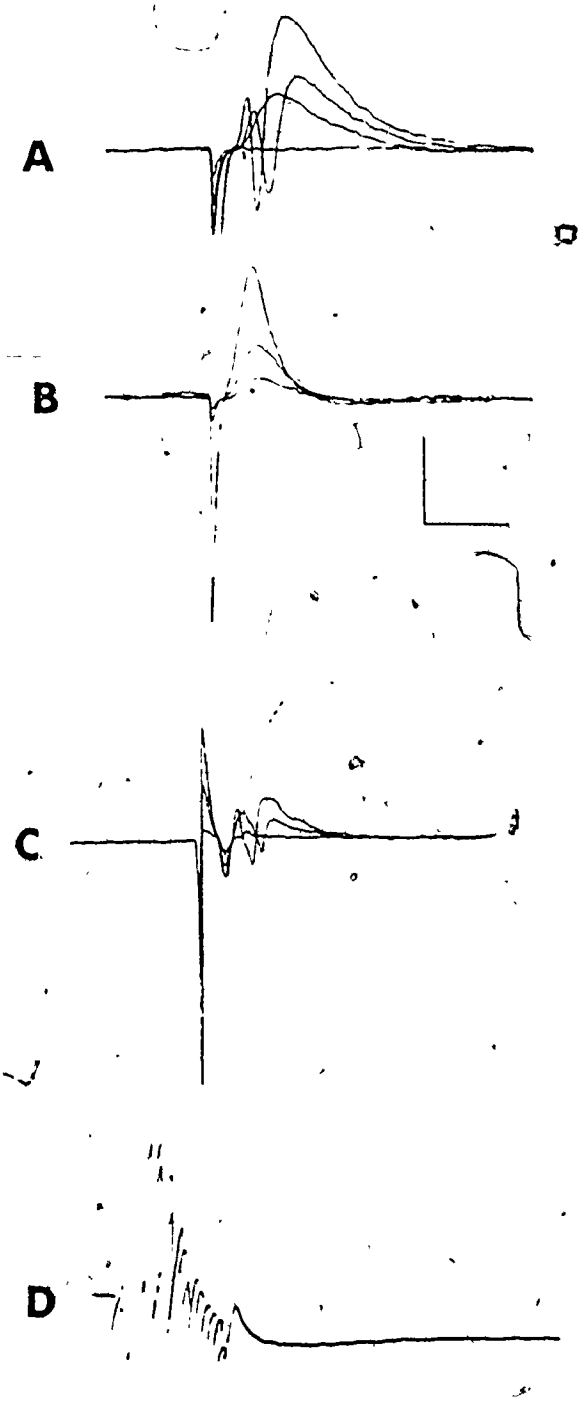


FIGURE 2-7

FIGURE 2-8: ATYPICAL CA1 FIELD POTENTIALS FROM HIPPOCAMPAL KINDLED RAT.

A and B are from different preparations; C and D are from the same slice, but different preparations from A or B. Note different time scales. Stimulation was a single pulse to stratum radiatum in all cases.

Calibration: horizontal= 8 ms (A);
16 ms (B);
40 ms (C&D).
vertical = 1 mV (all).

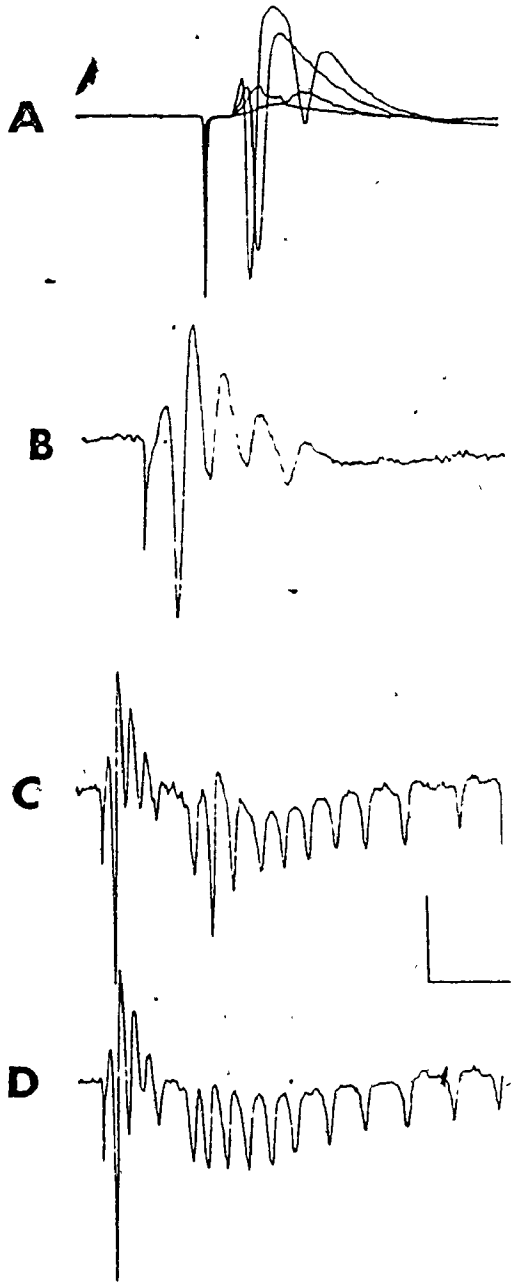


FIGURE 2-8

FIGURE 2-9: CA3 BURSTS TRANSMITTED TO CA1.

Top trace of each pair is from CA3; bottom trace from CA1. Single pulse stimulation applied to stratum radiatum in CA1. The CA3 potential possesses all-or-none characteristics, displays a wide range of latencies (from 21 ms to 60 ms in these examples), and always precedes the secondary response in CA1.

Calibration: horizontal = 10 ms

vertical = 2 mV

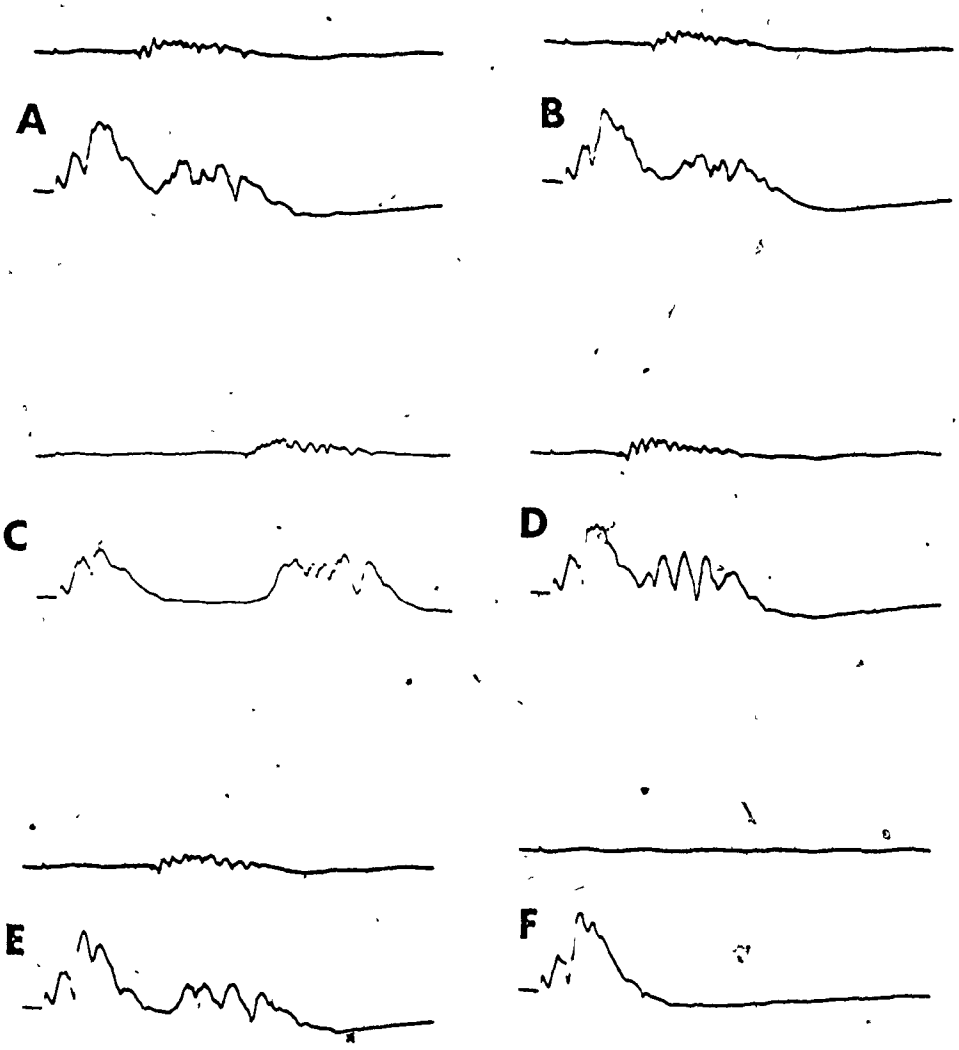


FIGURE 2-9

FIGURE 2-10: ADDITIONAL FEATURES OF CA1 FIELD POTENTIAL.

Responses are from same slice as FIG. 2-9. Stimulation applied to CA1 stratum rad. A and B. Top trace is the field potential recorded in stratum radiatum (dendritic layer) of CA1. Bottom trace is CA1 cell body layer. Note relatively small dendritic potential during CA3-triggered response. C. Same slice, approximately 30 minutes later. Top trace = CA3 cell body layer; bottom trace = CA1 cell body layer. Potentials are larger, but CA3 potential is still all-or-none and of variable latency.

Calibration: horizontal = 10 ms

vertical = 2 mV.

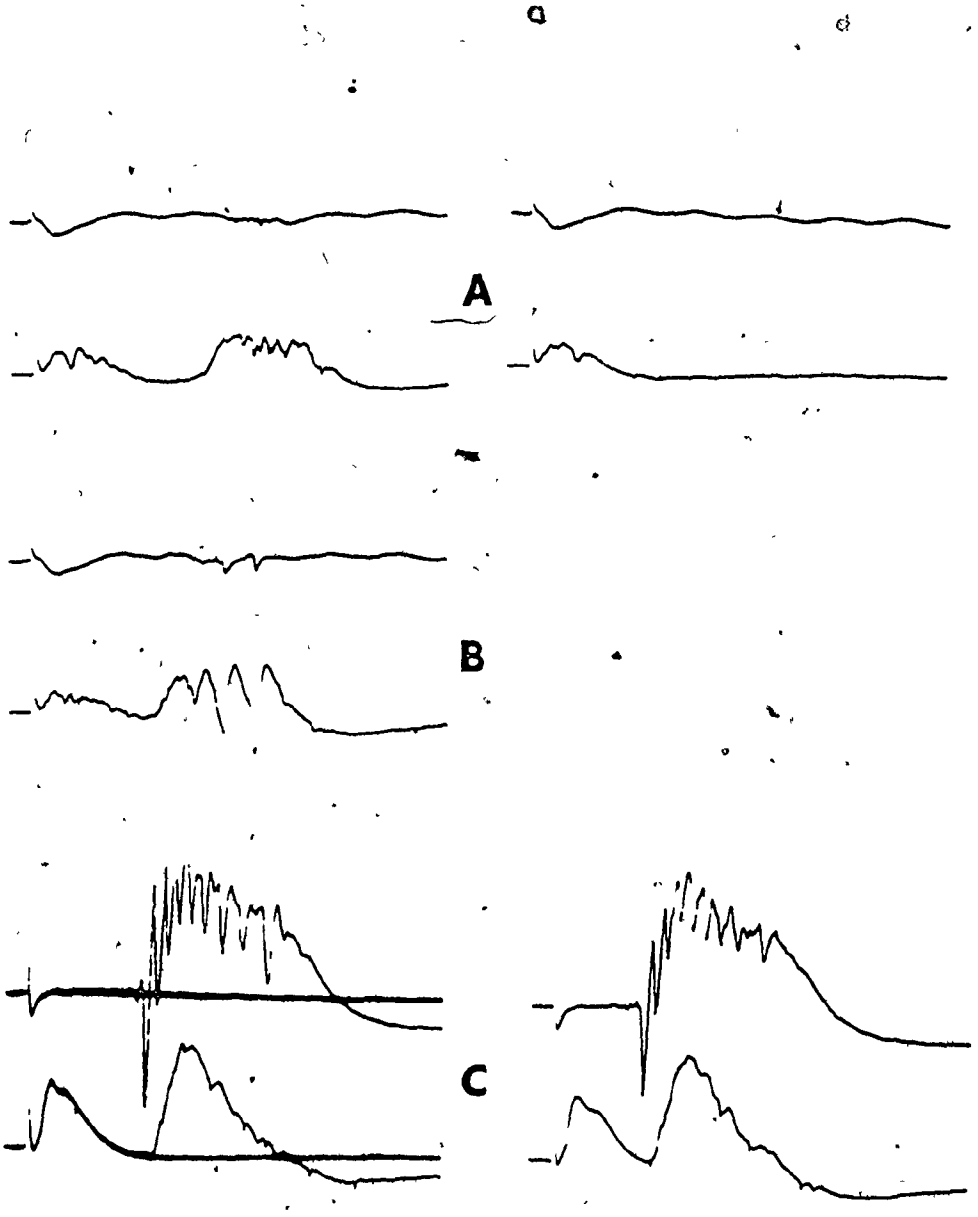


FIGURE 2-10

FIGURE 2-11: ADDITIONAL FEATURES OF CA3 BURSTS.

Top response in all figures is from CA3 cell body layer, bottom is CA1 cell body layer (except A2, where bottom response is from CA1 dendritic layer). A: Stimulation applied to CA3 stratum radiatum results in all-or-none burst in CA3 and "spiky" field potential in CA1. The CA1 response is associated with a large dendritic field potential (A3). B1: Similar to A1, but note different latency. B2,B3: Stimulation applied to dentate hilus. Note the longer latency of both responses, (B2), and the occasional failure (B3).
Calibration marker: 10 ms between pulses, large pulse is 2 mV.

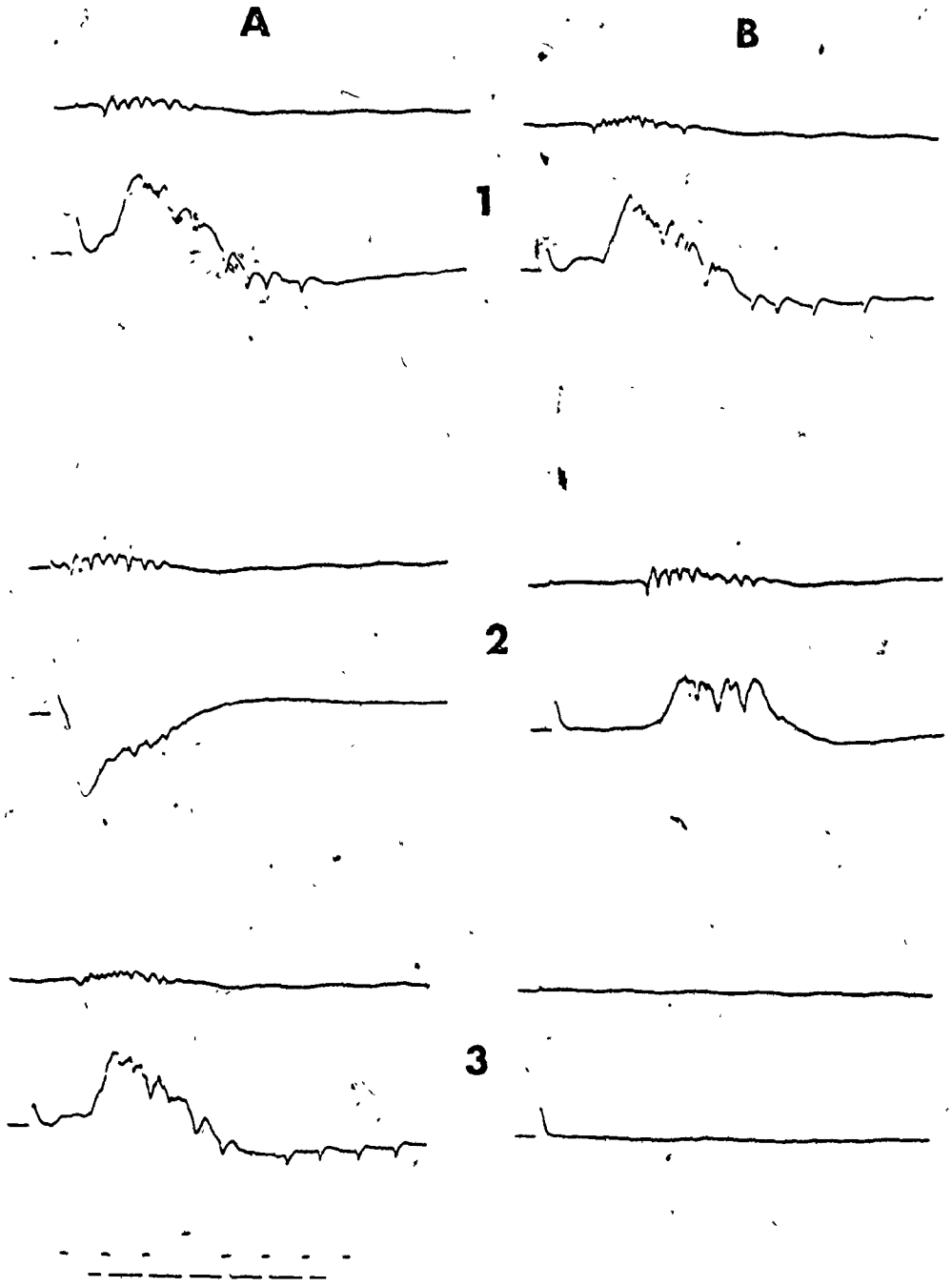


FIGURE 2-11

FIGURE 2-12: ADDITIONAL EXAMPLES OF CA3 BURSTS.

CA3 (top trace) and CA1 (bottom trace) responses from another slice in the same experiment as previous figure. Stimulation applied to stratum radiatum of CA3.

Calibration as in FIG. 2-11.

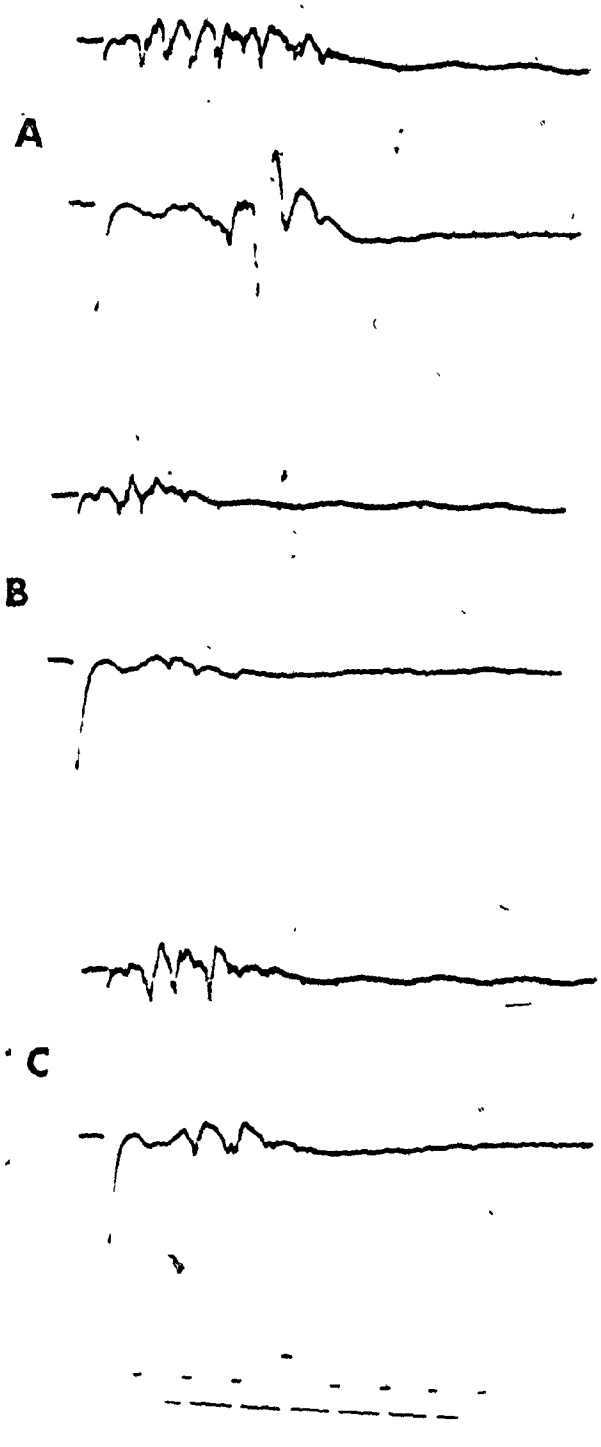


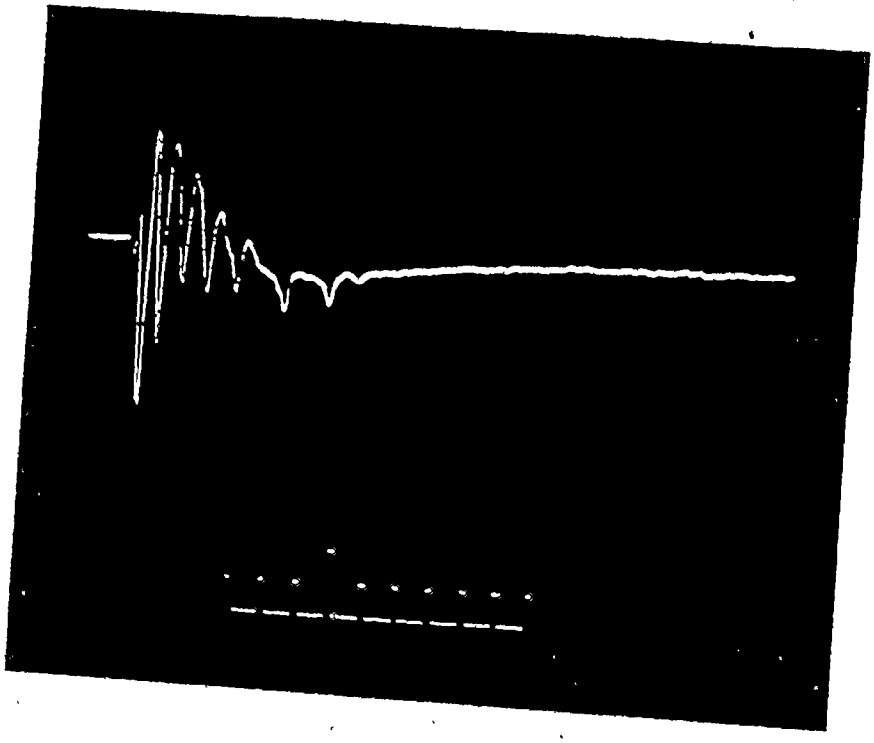
FIGURE 2-12

FIGURE 2-13: PENICILLIN EFFECTS.

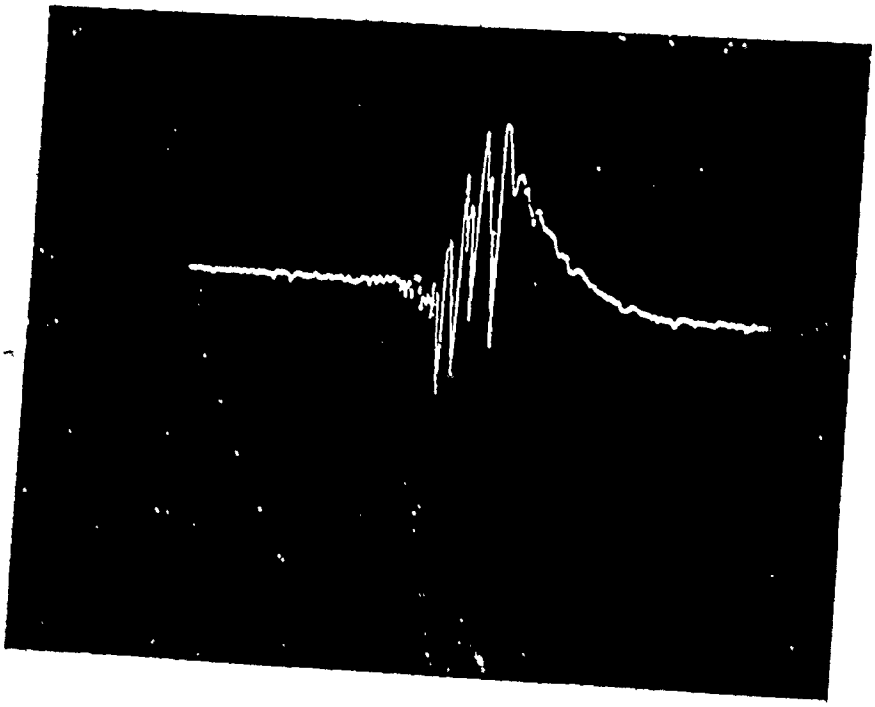
A. CA1 evoked potential 10 minutes following introduction of ACSF containing 100 µg/ml penicillin.

B. Spontaneous epileptiform potential, typical of that which appeared approximately 5 minutes after the above recording. The potassium concentration of the ACSF was 6 mEq/l. The large calibration pulse is 2 mV; each pulse is separated by 10 ms.

A



B



FIGURE

FIGURE 2-14: SPONTANEOUS BURSTS .

Top trace of each pair represents a spontaneous field potential. The bottom traces illustrate the corresponding evoked responses. The responses on the left are taken from a hippocampal-kindled preparation in 6K, while those on the right are from a control experiment. Both are representative of the IIS activity seen in all preparations. Calibration as in previous figure.

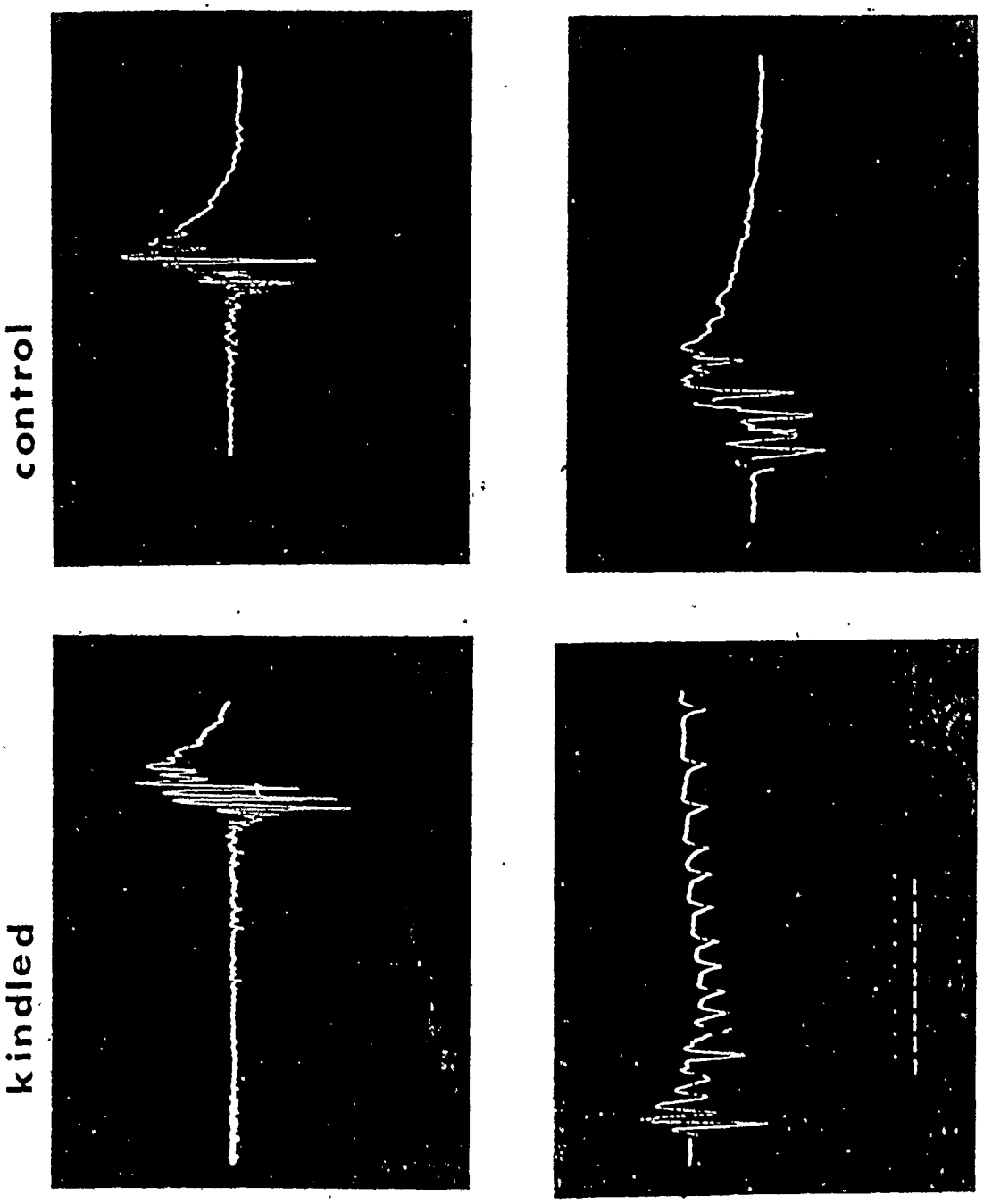

control
kindled

FIGURE 2-15: ISOLATED CA1 NEURONES.

Spontaneously active neurones in CA1 region. Top 3 tracings are from one experiment, bottom two from another. Spontaneous population potentials were not present in either preparation. Recording bandwidth was 300 Hz to 10 kHz.

Calibration: horizontal = 8 ms

vertical = 300 uV

FIGURE 2-16: ISOLATED CA1 NEURONES: RESPONSES TO STIMULATION.

Recordings taken from same experiment as those in lower records in Figure 2-15.

Calibration: same as in Figure 2-15.

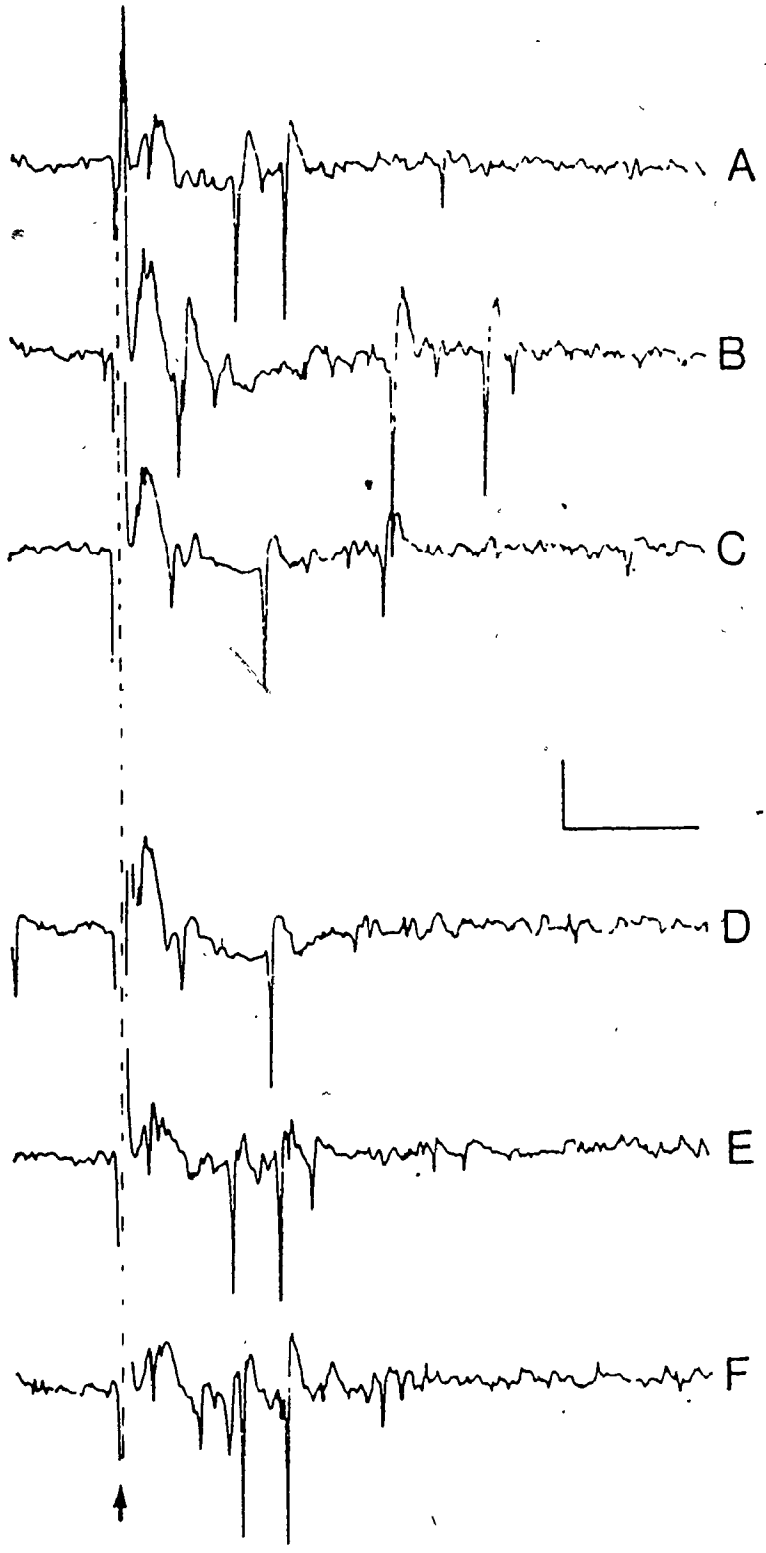


FIGURE 2-16

CHAPTER 3: INTRACELLULAR STUDIES

3.0 Introduction

Neither spontaneous nor evoked epileptiform activity was a consistent finding in the preceding extracellular studies of hippocampus from kindled rats. However, kindling could have altered the properties of individual pyramidal cells in a manner which was not readily detectable at the population level. Either the magnitude of the changes or the number of neurones affected may have been too small to produce measurable differences in the field potential generated by the population.

The observations described in this chapter were performed using intracellular microelectrodes to study the properties of individual pyramidal neurones. The number of cells studied is relatively small, due to unexpected difficulties in obtaining satisfactory impalements, and also because the criteria used for accepting impalements for analysis were quite strict. Nevertheless, these observations provide a preliminary indication of the degree of epileptiform behaviour seen in pyramidal neurones from the hippocampus of the kindled rat.

3.1 Methods

3.1.1 Subjects

Rats used in these studies were all adult males of the Long-Evans strain. Procedures for the kindling and preparation of

slices were identical to those described in the previous chapter. Slices were taken from 11 normal (unkindled) rats and and 13 amygdala-kindled rats. The latter group had been kindled to the stage 5 seizure level, and had experienced an evoked stage 5 seizure approximately 24 hours prior to the slice experiments. All studies were performed in ACSF containing 3 mEq/l K^+.

3.1.2 Apparatus for Intracellular Recording

The instruments specialized for the intracellular studies included an hydraulically-driven microdrive, an electrometer amplifier with a high impedance headstage, ultrafine microelectrodes, and a computer system tailored for intracellular data acquisition and analysis. The hydraulically-driven microdrive system was constructed in the laboratory and was designed to mechanically uncouple the manipulator which positioned and held the microelectrode from vibrations associated with advancement of the electrode. It consisted of 2 syringes (B-D Ultrafit) connected by 1.5 feet of teflon tubing. This system was sealed and filled with either hydraulic fluid or a light-weight machine oil. The slave cylinder was a 1 cc syringe cylinder which was mounted in a specially constructed holder together with the electrometer probe and clamped into a Narishige manipulator of conventional design. The plunger of this syringe was fitted with a lightweight assembly with facilities for the electrical connection of the microelectrode to the electrometer probe. The master cylinder consisted of a 2 cc syringe clamped into a supporting device. Its piston, which was fitted with a

return spring, could be advanced by a micrometer screw. The overall mechanical gain of the system was such that a 20 degree turn of the micrometer screw advanced the electrode by one micron. When situated remotely from the slice chamber and manipulators, this master cylinder could be activated with little concern for mechanical disturbance of the tissue slices or non-axial movements of the microelectrode.

The electrometer amplifier utilized in these experiments (WP Instruments, Model M4a or M707) possessed several important characteristics. The active headstage had an input resistance of 10^{11} ohms, which was necessary to satisfactorily couple electrodes with DC resistances on the order of 100 MOhms. Secondly, current could be injected through the microelectrode by means of an active bridge circuit. This permitted simultaneous recording of membrane potential and current induced depolarization or hyperpolarizations. Control signals in the form of steady voltages or pulses were generated by either manually operated pulse generators, or digital-to-analog converters on the laboratory computer (PDP8/e). Finally, neutralization of stray input capacitance (due to connections between the electrode and the amplifier input) was achieved using positive capacitative feedback. Although the output stage of the amplifier was unfiltered (bandwidth of DC to over 30 KHz), the overall frequency response of the recording system was limited by any uncompensated input capacitance.

Electrodes used in these studies were pulled from glass 1.0 mm O.D. with an internal capillary to assist in filling. The profiles were pulled on a Brown-Flaming design horizontal puller (Sutter Instruments,

San Francisco) using either a circular or trough-shaped filament. The electrodes were filled by capillary action with a solution of 4M potassium acetate and 0.01M potassium chloride. Bevelling was accomplished by introducing the electrode tip into a jet stream of 0.05 µm alumina particles suspended in saline (Ogden, Citron, and Pierantoni, 1979) while monitoring the fall in resistance. Electrodes were bevelled from initial resistances of over 200 MOhms to approximately 100 MOhms.

The apparatus for intracellular work was schematized in Figure 2-2. Action potential data could be recorded in several ways: photographed from the oscilloscope screen, recorded on magnetic tape, stored in the memory of the digital oscilloscope and plotted on an X-Y recorder, or sampled and stored in the memory of the computer. Control pulses of hyperpolarizing and depolarizing steps were generated by the computer and used to inject current into the neurone under study; the resulting membrane responses were sampled and on-line computation of input resistance, time constant, and interspike-intervals was performed. The responses, together with membrane potential, could be stored on mass storage for further analysis off-line.

3.1.3 Methodology of Impalement and Recording

The intracellular electrode was positioned, under visual guidance, onto the surface of the slice in the region of interest. Contact with the saline-moistened surface was signalled by: a) a brief transient in the audio monitor corresponding to closure of the recording circuit; b) recovery of the oscilloscope trace to the previously defined zero level; and c) a very small, but often detectable, meniscus formed

around the electrode tip. At this point, the manipulator Z-axis position was noted as a surface position reference. In addition, the DC offset control on the intracellular amplifier was adjusted to bring the amplifier output to zero. This manipulation resulted in the establishment of a zero reference potential in the extracellular space, and counterbalanced the presence of electrode tip potentials and asymetric junction potentials at the silver-chloride/electrolyte interfaces. The bridge circuit of the amplifier was balanced by passing a small amplitude depolarizing current pulse through the electrode and adjusting the bridge balance until only the onset and offset transients could be detected. The resistance and current-passing properties of the electrode were examined at this stage, and if determined to be unacceptable, the electrode was rejected. Generally, the most successful recordings were achieved with electrodes whose DC resistance fell in the range 60 to 130 MOhms, and which could pass up to 1.5 nA of current in both directions without either severe rectification or substantial increase in resistance. Potassium acetate-filled electrodes of this moderate resistance unavoidably rectify to a small extent. Consequently, we adopted the following 'rule of thumb' criterion that the passage of up to .5 nA in either direction should not result in bridge imbalance in excess of approximately 2 millivolts. In early experiments, less than 20% of the electrodes tested were acceptable. Electrodes which were fabricated on the Brown-Flaming puller and subsequently bevelled were found to be considerably superior, and the yield of acceptable electrodes increased to approximately 50%. As the electrode tip was advanced through the slice, spurious deviations from

zero (often in the order of 20-30 millivolts) of either polarity, could be observed. These presumably represent electrode tip potentials generated by charged particles which adhere to the glass near the tip of the electrode. Not uncommonly, these potentials were accompanied by a dramatic increase in electrode resistance, revealed as an imbalance of the bridge circuit. This was most likely due to partial obstruction of the electrode tip by either sub-micron particulates or a cell membrane. In most cases, where further manipulation of the electrode did not yield a neuronal impalement, the electrode could be 'cleaned' by the passage of high amplitude and high frequency current pulses (i.e. by briefly overadjusting the capacity compensation to produce oscillations). The DC offset and bridge balance were readjusted as required. If the electrode tip became severely obstructed as signalled by excessive resistance and severe rectification, it was discarded and replaced.

The electrode was advanced through the slice manually, by turning the micrometer attached to the master hydraulic cylinder. Initial efforts at facilitating cell penetration (by abrupt stepping of the electrode achieved by coupling the micrometer with an electronically controlled stepping motor) were unsuccessful, and the technique was abandoned. The step size achieved by manual advancement of the electrode was usually about 1 micron, and the maximum rate of advancement never exceeded one step per second.

Several electrical signs were used to anticipate neuronal impalement. Orthodromic stimulation of afferents excitory to the region under study produced characteristic extracellular field potentials, which, when recorded by the microelectrode, could be used as an

indication that the electrode tip was in the vicinity of neuronal somata. For example, the presence of a negative going population spike when recording from the CA1 area of the hippocampus signifies active, (i.e. spike) current inwardly directed at the soma-initial segment region. The electrode position was adjusted until this population spike was of maximal amplitude, and then impalements were sought. Increased electrode resistance, small positive-going action potentials (either spontaneous or superimposed on the population spike), and deviation of the recorded potential from zero were all reliable indications that the tip was very close to a neuronal membrane. Impalement could then occur spontaneously, or could be assisted by either gentle percussion of the manipulator, or, more commonly, a brief (< 50 ms) oscillation of the amplifier. Penetration of the membrane was accompanied by the sudden appearance of a negative potential and, usually, by a brief high frequency discharge of action potentials. In most cases, the recording deteriorated within several seconds and the recorded potential fell back to zero. However, where the penetration produced minimal damage to the membrane (and other conditions were favourable), the impalement was maintained, and often improved and stabilized over the subsequent four or five minutes. It was found helpful to apply a small level (up to 0.5nA) of hyperpolarizing current continuously for the first several minutes following penetration of the membrane. This procedure of applying a 'holding' current hyperpolarized the membrane, quelled spontaneous activity, and assisted the 'sealing' of the membrane around the electrode as indicated by the gradual rise in input resistance during its application. The mechanism for this process is unclear, but

may involve a voltage-dependent stimulation of a membrane pump. Holding current was never maintained during the study of neuronal properties unless a particular measurement called for it, and these instances will be clearly indicated.

Criteria for an acceptable impalement are discussed below. The neurones described in these experiments could be held for periods ranging from several minutes to over 3 hours, often with little or no signs of deterioration. Following the withdrawal (intentional or unintentional) of the electrode from the neurone, the extracellular potential was often observed not to be zero, but some residual value. This probably resulted from 1) incomplete withdrawal from the impaled neurone, with the resulting potential representing a severely deteriorated membrane potential; 2) an artifactual tip potential due to plugging of the tip during withdrawal; or 3) the DC offset was improperly adjusted prior to penetration, and the post-withdrawal potential reflects the true extracellular potential. Possibilities 1 and 2 could be verified by further movement and cleaning of the electrode. If the potential still did not return completely to the previously established zero, then the intracellular potential measured during the penetration was corrected by adding (or subtracting) one half of the residual value. This correction rarely amounted to more than a few millivolts.

3.1.4 General Criteria for Acceptable Penetrations

Four basic criteria were used as initial indications of a successful penetration of a neurone. 1) A resting membrane potential of

at least -55mV (measured with reference to a potential of 0 mV immediately prior to penetrating the membrane), 2) Resting input resistance, measured with a 0.5nA, 100 ms hyperpolarizing pulse, of at least 20 MOhms; 3) a spike overshoot of at least 10 mV; 4) a low frequency of spontaneous spiking (less than approximately 1/sec).

The above criteria are, to some extent, arbitrary and their strict observance could introduce a serious bias into the data, particularly since these experiments were attempting to detect abnormal cellular behaviours. This difficulty, and other issues related to the intracellular technique will be dealt with more extensively in the discussion chapter. The basic procedure for intracellular data collection, then, was to continue observation of a penetrated neurone if several, but not necessarily all, of the above conditions were met. In some cases, for example, a neurone with a satisfactory membrane potential, spike overshoot, and response to direct stimulation could be encountered, but its input resistance measured below 20 MOhms. Data collection on a cell with these properties would not be terminated because a) as the penetration 'matured', the input resistance could eventually increase (presumably due to an improvement of membrane sealing around the electrode) or b) the measured resistance may reflect the real, albeit anomolous, resistance of the neurone. For similar reasons, a recording which met all of the other criteria but had a high spontaneous rate of firing would not necessarily be dismissed as injured.

3.1.5 Recording Protocol

Following impalement of the neurone, the tests described in the preceding section were applied to determine if the criteria for an acceptable penetration were met. Manipulations of the electrode position and holding current were made if necessary. Resting membrane properties were then measured, including membrane potential, input resistance, and the frequency of spontaneous spiking, if present. Input resistance was measured by injecting hyperpolarizing current pulses and measuring the resulting voltage deflection. Input resistance was then computed by application of Ohm's law, R=V/I to the measurements obtained at several levels of current injection (see Figure 3-1). Stimulation to stratum radiatum was applied, and measurements of the amplitude of the EPSP peak were made. The EPSP recorded intracellularly is usually followed by a long hyperpolarization, and its peak amplitude and duration were also measured. By increasing the stimulus strength, the cell discharged an action potential, and its threshold, overshoot, and width at the base was measured. The threshold injected current for action potential discharge (rheobase) was determined, and the pattern of firing with increasing current injection was observed. These measurements were repeated as many times as the maintenance of the penetration permitted.

3.2 Results

A total of 24 neurones (from 11 experiments) from normal rats and 20 neurones (from 13 experiments) from amygdala-kindled rats were studied. A summary and comparison of these measurements is presented in Table 3-1. Resistance of neurones from the two groups of studies did not differ significantly (Table 3-1). Three neurones from each group displayed some spontaneous activity, which in all cases could be eliminated by the passage of no more than 0.1 nA of hyperpolarizing current. Measurements of input resistance in these cases were performed without this steady current, but during periods of low spontaneous firing. No instances of spontaneous burst-firing were encountered in either group of neurones.

3.2.1 Properties of Subthreshold Synaptic Responses.

All of the recorded neurones responded to stratum radiatum stimulation with a sequence of depolarization followed by a hyperpolarization (Figure 3-2), representing a superposition of feedforward and feedback EPSPs and IPSPs. Some components of this response may also be due to voltage-dependent ionic fluxes. Measurements were made of the peak depolarization which was just subthreshold for action potential discharge. The range of values for this parameter was quite large (1-11mV in control neurones), and the mean values did not differ significantly between the two groups ($p=0.05$, Students t-test).

3.2.2 Properties of the Action Potential.

With stimulation adjusted to be slightly above threshold, neurones generated a single action potential (Figure 3-3A). The amplitude and base-width (at threshold) were not significantly different between groups. The AP appeared to be triggered at the peak of the EPSP, and its latency relative to onset of the EPSP decreased with further increases in stimulus strength. Multiple spike discharges were seen only with very intense stimulation or when paired-pulse potentiation produced a large EPSP on the second response. One neurone (from the kindled group) did generate a double spike with only moderate stimulation applied to afferents (Figure 3-3C and 3-3D), but the typical response resembled that in Figure 3-3A and 3-3B.

3.2.3 Hyperpolarizing Potentials.

The depolarization and action potential produced by synaptic activation was invariably followed by a hyperpolarization (HP) of the membrane (Figure 3-3). Nicoll and Alger (1981) have identified the first phase of this potential as a GABA-mediated IPSP, and it has a maximum duration of approximately 100 ms (Thalmann and Ayala, 1982). A second, long-latency, hyperpolarizing potential (not mediated by GABA) is sometimes seen in CA1 neurones (Alger, 1984; Nicoll and Alger, 1981; Lancaster and Wheal, 1984). The peak of this late hyperpolarization has a latency of approximately 135ms (Lancaster and Wheal, 1984). Since our measurements of peak-amplitude and paired-pulse facilitation were performed within an 80 ms interval following stimulation, they are likely to represent only the GABA-mediated potential. The mean peak

amplitude of this response was 10.2 mV in kindled slices, and 7.4 mV in controls (Table 3-1). These are the only measurements which differ significantly between the two groups of neurones ($p=0.05$, Students t-test, two tailed).

The hyperpolarization following the EPSP-action potential response was quite variable in its ability to inhibit subsequent action potentials. Paired-pulse potentiation of the EPSP was quite evident in all neurones (Figure 3-3B,D), but the second stimulus did not invariably result in action potential discharge. Figure 3-3C (control) represents an example where the hyperpolarization is rather small but nevertheless effective in inhibiting the action potential. In contrast, the hyperpolarization seen in Figure 3-3D (control) is much larger but is ineffective in inhibiting the spike. Both types of behaviour were commonly observed in neurones of control and kindled slices, even in the same neurone at different times during the impalement.

3.2.4 Effects of Depolarizing Current.

Injection of depolarizing currents resulted in depolarization of the membrane and cell discharge (Figure 3-4). The rheobasic current did not differ between groups, and the pooled mean value was 0.12 nA. The pattern of cell discharge was similar for all neurones when the current injected was at a low level. One or two spikes appeared at a long latency after current onset (Figure 3-4A,B). The latency of the first spike rapidly decreased as the injected current increased, and the frequency of cell discharge increased. The train of spikes elicited by the current persisted for the duration of the current pulse; no spikes related to the applied current were triggered during the recovery of the membrane towards resting potential.

With increasing current levels, neurones responded with an intense spike train (Figure 3-5B,C). Some neurones developed a short (<2 ms) 1st, and sometimes 2nd, interspike interval (ISI) (Figure 3-5A,B,C) but higher order ISIs were longer. An interesting feature unique to the responses of cells in kindled slices was a paroxysmal burst of spikes at the onset of the pulse (Figure 3-4D, 3-5A thru D). This type of response was seen in 12 of the 21 cells recorded in kindled slices. These bursts consisted of 3 or 4 high frequency spikes, and were followed by a relatively long interval before the next spike. These bursts never appeared later during the current-induced depolarization, although pulses longer than 1000 ms were not tested. They could be triggered with pulses as short as 50 ms but only one neurone demonstrated some degree of all-or-none characteristics (Figure 3-5D). In this particular example, the action potential discharge and the depolarization outlasted the period of current injection. Smaller levels of injected current did not produce this effect.

The presence of this form of bursting in a given neurone did not appear to be correlated with other neuronal properties. The bursting cells had input resistances ranging from 21 MOhms to 38 MOhms, and membrane potentials from -67mV to -80mV. Bursts could not be evoked by synaptic activation of these neurones.

3.3 Summary

A total of 21 neurones from normal rats and 20 neurons from amygdala-kindled rats were studied in the hippocampal slice preparation. Resting membrane properties and properties of the action potential did

not differ between groups. The mean value of the hyperpolarizing phase of the synaptically-induced responses was larger in neurons from the kindled slices, although this difference was significant only at the 0.05 level. Finally, a qualitative difference in the firing patterns to depolarizing current injection was observed. Twelve of 21 neurones in the kindled group responded to moderate levels of depolarization with a brief high-frequency burst of action potentials, a property not observed in control slices.

TABLE 3-1: NEURONAL PROPERTIES

Parameter	Group	N	X	SD	Range	Units
Resting Membrane Potential	K	19	66.3	6.3	60-80	mV
	C	24	65.8	8.7	56-84	
Resting Input Resistance	K	19	31.0	7.0	21-40	MOhms
	C	24	31.5	8.4	20-48	
Peak Subthreshold EPSP	K	11	9.4	5.0	1-17	mV
	C	13	6.8	3.6	1-11	
Action Potential Amplitude	K	19	83.2	6.5	72-93	mV
	C	20	83.0	8.9	68-100	
Action Potential Width	K	19	1.62	.14	1.4-1.8	ms
	C	17	1.67	.09	1.5-1.8	
Peak Afterhyper-polarization	K	15	10.2*	4.8	2-18	mV
	C	14	7.4	3.0	2-12	
Rheobase	K	12	0.2	.13	.05-.53	nA
	C	20	0.2	.12	.03-.35	

* these values differ significantly (p=0.05, two-tailed t-test).

Abbreviations for Units:

mV	millivolts
MOhms	megohms
ms	milliseconds
nA	nanoamps

FIGURE 3-1: INPUT RESISTANCE MEASUREMENT

Responses of the membrane to the injection of pulses of hyperpolarizing current. Top trace: current monitor. Bottom trace: membrane potential recorded by the microelectrode. Input resistance in this case is 25 MOhms. Calibration: the vertical bar represents 0.5 nA and 20 mV, the horizontal bar represents 100 ms.

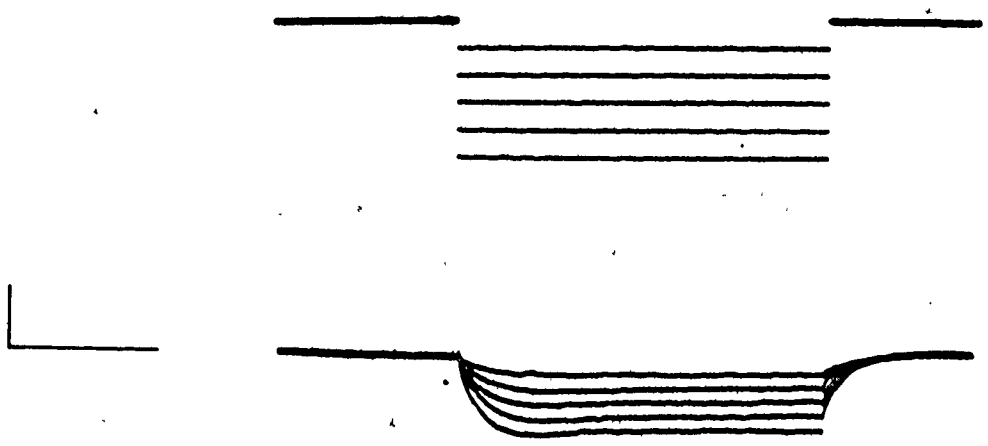


FIGURE 3-1

FIGURE 3-2: SYNAPTIC RESPONSES

The left column contains examples from normal neurones, the right from kindled slices. Calibration signal: the bottom trace is a calibration signal which also appears in subsequent figures. The large vertical pulses represent 20 mV (for voltage signals) and 0.5 nA (for current monitor signals). The time between small pulses (overlapped in this figure) is 10 ms while the large pulses are separated by 100 ms.
A: Upper trace is extracellularly-recorded field potential, demonstrating a population EPSP subthreshold for a population spike. Lower trace is the simultaneously-recorded intracellular potential from a CA1 pyramidal neurone. The two recording electrodes were in the same region of CA1, separated by no more than 50 µm. Both kindled and normal examples show the depolarizing-hyperpolarizing sequence typical of responses elicited by stimulation of stratum radiatum. Calibration for extracellular recording is 1mV/small pulse on calibration trace.
B; Similar to A, but with a slower time base. Note that in the kindled case, a second stimulus pulse was applied during the hyperpolarization, and an EPSP appeared. In contrast, the initial response consisted of only a very small depolarization followed by the hyperpolarization.
C: Similar to A, but from two other neurones.
D: Responses of two different neurones, displayed at a slower time base. Note that the hyperpolarizing phase of the response may extend for several hundred milliseconds.

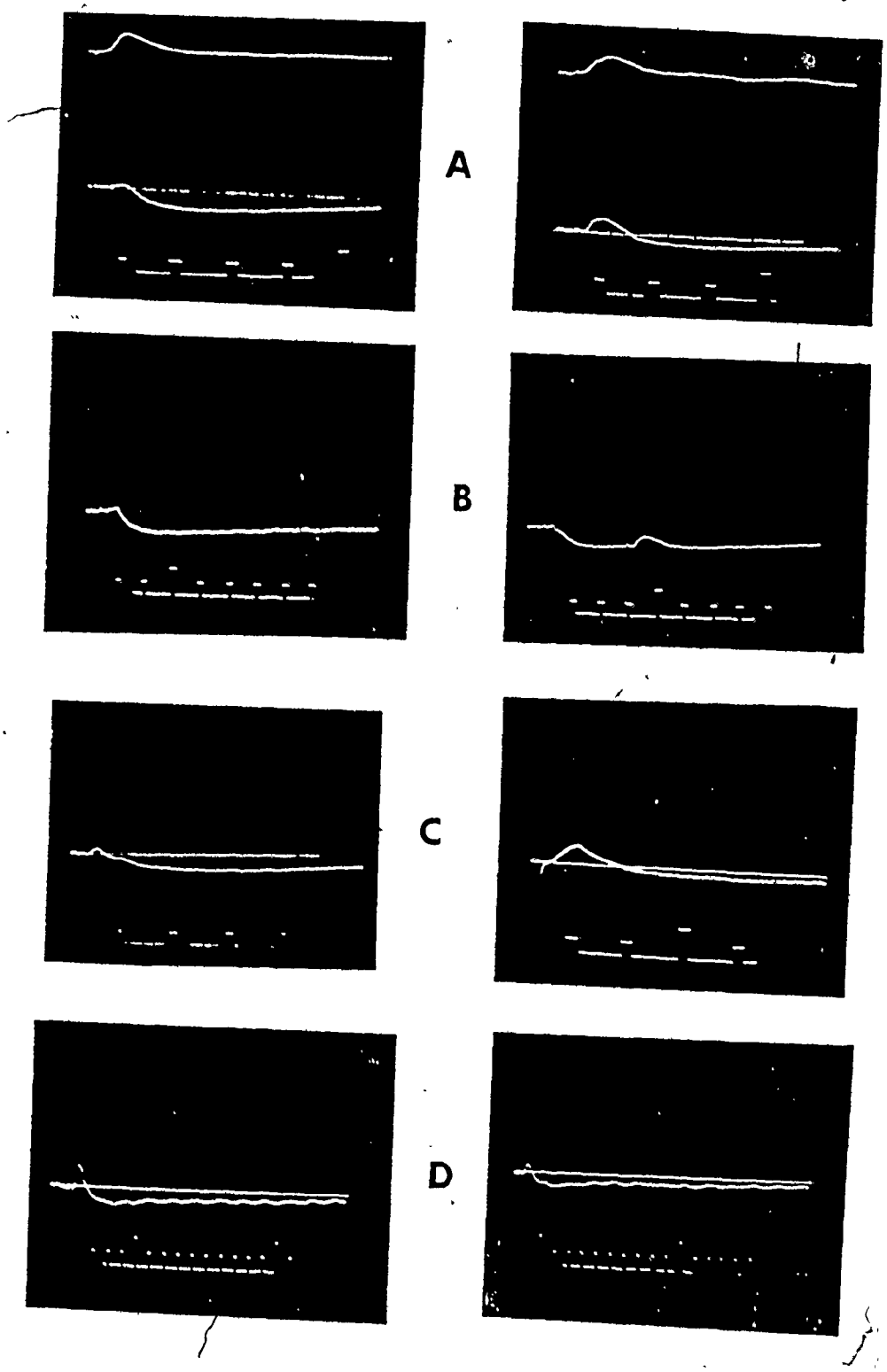


FIGURE 3-2

FIGURE 3-3: ACTION POTENTIALS

The left column contains examples from normal neurones, the right from kindled slices. Calibration signal as described in Figure 3-2.

A: Action potentials superimposed on graded synaptic potentials. The example from normal tissue contains several superimposed tracings with increasing levels of stimulus current.

B: Activation by a pair of stimulus pulses. In these examples, the single-cell potentials (middle traces) discharge action potentials in direct correspondence with the population responses (top traces). In other cases, a neurone could discharge an action potential even when the population response did not display a population spike. The response of the kindled neurone demonstrates paired pulse-potentiation with the first response consisting only of an EPSP. Calibration for the extracellular signal is 2 mV/small pulse.

C: Control response demonstrates a degree of inhibition of action potential discharge in a paired-pulse stimulation. The kindled example is from a neurone (the only one encountered in this study) which discharged double action potentials in response to a single stimulus pulse.

D: Further examples of paired-pulse stimulation. Note the robust potentiation in both examples. The kindled response is from the same cell as above, and the double-spike discharge matches the pattern seen in the extracellular recording.

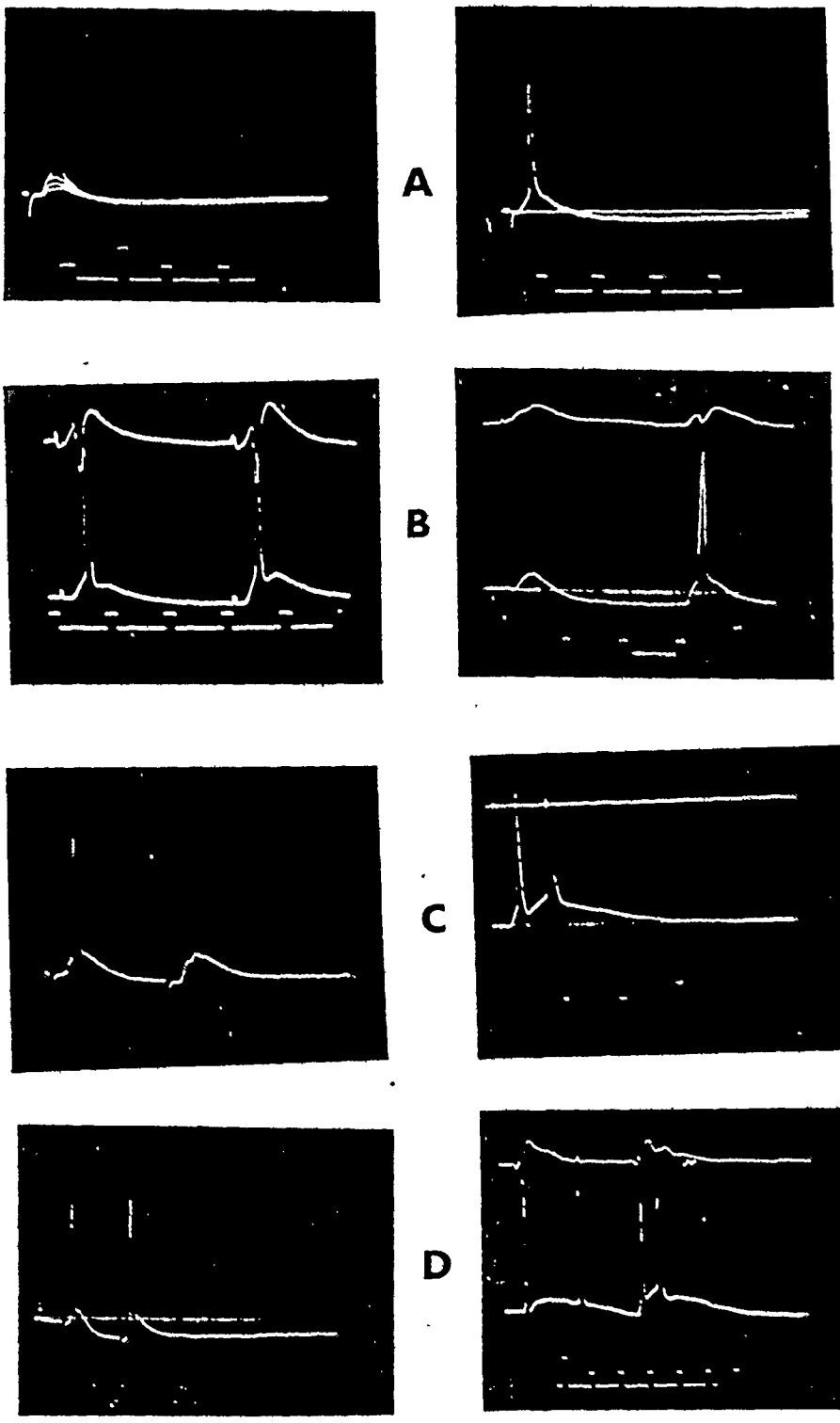


FIGURE 3-3

FIGURE 3-4: RESPONSES TO DIRECT ACTIVATION

The left column contains examples from normal neurones, the right from kindled slices. Calibration signal as described in Figure 3-2. A,B,C and D represent the responses of neurones to increasing levels of current injected directly into the neurone through the bridge circuit of the recording system. In this and the following figure, spikes have been retouched for clarity. Each frame is taken from a different neurone, except C and D from the kindled group, which are from the same neurone. Note the high frequency discharge apparent at the onset of current injection.

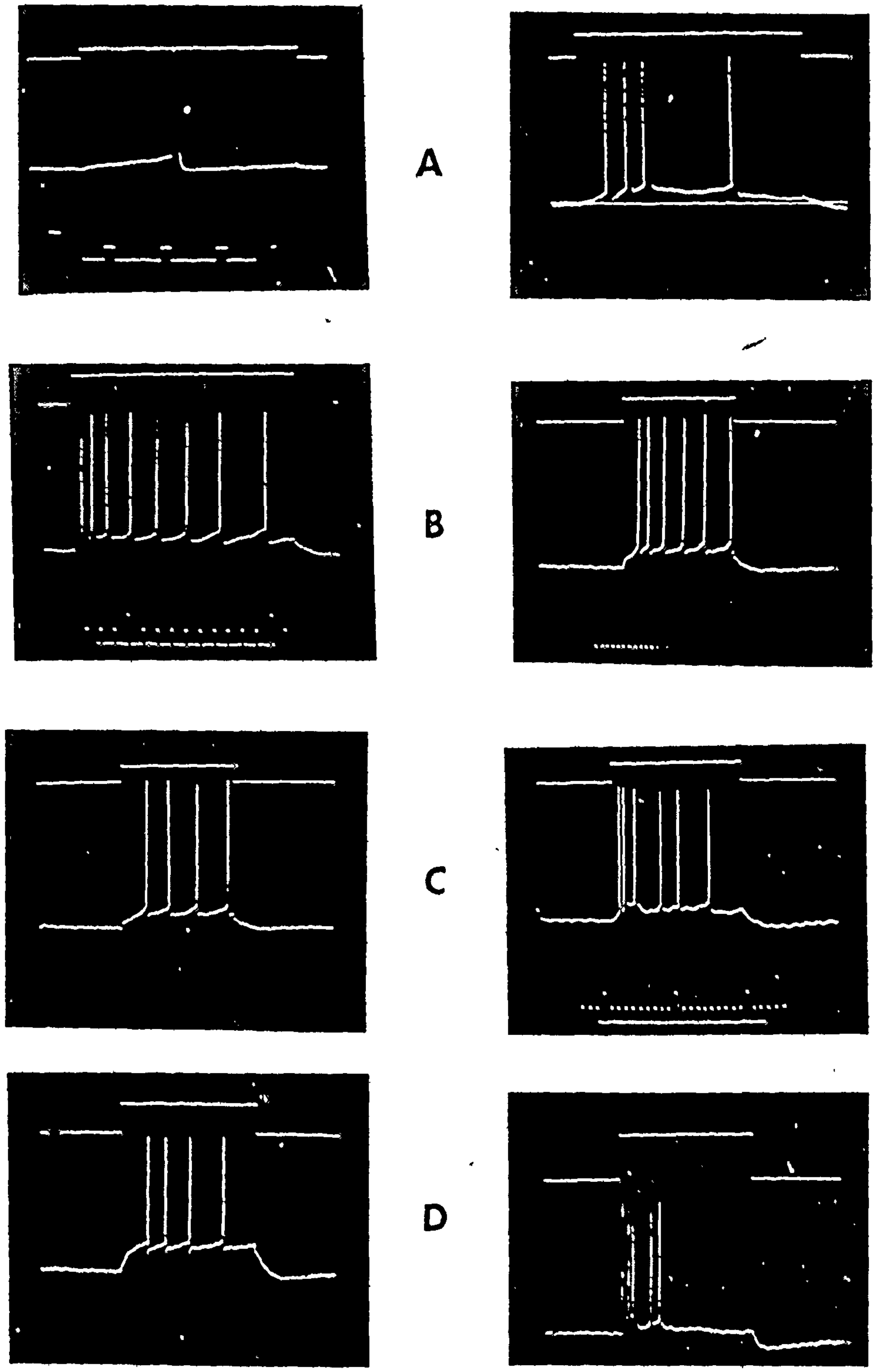


FIGURE [illegible]

FIGURE 3-5: RESPONSES TO DIRECT ACTIVATION

The left column contains examples from normal neurones, the right from kindled slices. Calibration signal as described in Figure 3-2. Similar to the previous figure. B and C of the kindled group are from the same neurone. Note that the discharge of action potentials outlasts the current injection in D.

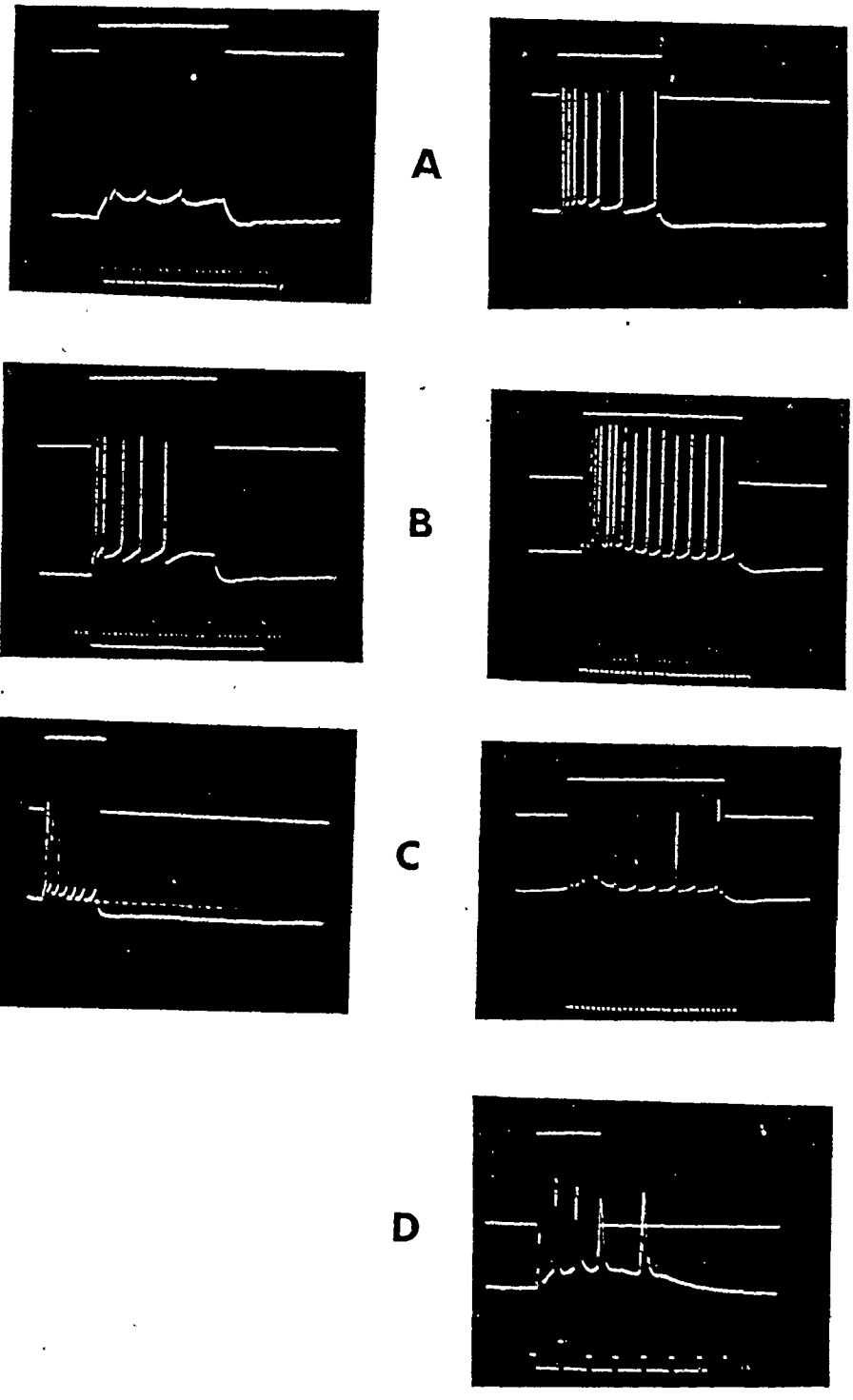


FIGURE 3-5

CHAPTER 4: GENERAL DISCUSSION

4.0 Summary of Findings

The experiments reported in this thesis demonstrate that the electrophysiological properties of hippocampal tissue from kindled rats, when studied under *in vitro* conditions, do not differ dramatically from those of control tissue. No differences in the pattern of appearance, or $[K^+]$ sensitivity, of spontaneous IISs was observed. The properties of CA1 evoked potentials were generally similar between the control and experimental groups, although slices from two kindled groups showed a significantly higher occurrence of multiple-population spike responses than did control slices. Intracellular recordings from CA1 neurones revealed no differences in resting membrane properties. The hyperpolarizing phase of the membrane response to stratum radiatum stimulation had a larger mean peak amplitude in neurones of kindled slices. Intracellular injection of depolarizing current produced, in some kindled neurones, a pattern of action potential discharge that was qualitatively different from that of controls. Other neuronal parameters were unchanged.

4.1 Extracellular Studies

4.1.1 Multiple Population Spikes in Hippocampal Slices

Our extracellular studies were concerned with the measurement of two types of "abnormal" electrophysiological activities in the CA1

region of the hippocampal slice: multiple population spikes in the field response evoked by stimulation of afferents, and spontaneous IIS discharges. What are the cellular mechanisms underlying these activities?

A complete answer to this question is not available, but the most tenable explanation at the present involves the integrity of inhibitory synaptic influences. Intracellular recordings from CA1 pyramidal neurones have revealed that the normal membrane response to synaptic activation consists of a depolarizing-hyperpolarizing sequence (e.g. Schwartzkroin, 1975). The depolarization is most likely an EPSP resulting from the action of excitatory neurotransmitters released from afferent fibres onto the dendrites of pyramidal neurones. The source of the hyperpolarization is less clear. A feedforward inhibitory pathway has been suggested by Alger and Nicoll (1982) and Ashwood, Lancaster and Wheal (1984) and may involve direct synaptic inhibition of somatodendritic membrane by afferent fibres, or the action of an inhibitory interneurone. A recurrent inhibitory pathway, activated by pyramidal neurone action potential discharge, is also present (Andersen,1975; Knowles and Schwartzkroin, 1981). Another component of the hyperpolarizing phase may involve a calcium-activated potassium conductance. Studies by Nicoll and Alger (1981) have demonstrated that the postsynaptic influx of Ca^{++} during synaptic activity and action potential discharge activates a potassium conductance, the overall effect of which is inhibitory to further action potential generation (but see Lancaster and Wheal (1984) who demonstrate that this potential is resistant to calcium buffers injected intracellularly). In summary,

GABA-mediated IPSPs and the calcium-activated potassium conductance combine to control EPSP-driven spike generation.

In the simplest conceptualization of this scheme, multiple spikes are generated when the normal balance between excitation and inhibition is upset in favour of excitation. This could result from an increase in the net excitatory influences, and/or a decrease in the inhibitory ones. The majority of evidence in support of the existence of such a mechanism comes from studies in which the slice has been exposed to agents which selectively block GABA-mediated inhibition. Penicillin, for example, has little or no effect on presynaptic fibre activity, EPSP properties, passive membrane properties, or action potential mechanisms in the hippocampal slice (Andersen, 1983). However, it appears to reduce postsynaptic inhibition in pyramidal cells (Dingledine and Gjerstad, 1979) concomitant with the appearance of multiple action potential discharges in response to synaptic activation. Other GABA antagonists, such as picrotoxin and bicuculline (Schwartzkroin and Prince, 1980), have similar effects. Interference with the calcium-activated potassium conductance also produces multiple spiking. Johnston, Hablitz, and Wilson (1980) have shown that exposure of slices to barium, which enters the cell through calcium channels but does not activate calcium-activated potassium conductance, results in cell bursting.

In our experiments, multiple population spikes were observed under conditions of elevated $[K^+]$ in the perfusing medium. The mechanisms by which this occurs are likely to involve at least three

factors. (1) Elevating extracellular potassium has a depolarizing effect on neural membranes, as predicted by the role of K^+ conductance on the regulation of resting membrane potential (e.g. Huxley and Stampfli, 1951). Although this reduces the driving force for the EPSP, less membrane depolarization is required to attain action potential threshold. (2) Similar effects on nerve terminals may produce a reduction of transmitter release Erulkar and Weight (1977), but this may not hold for all excitatory and inhibitory synapses (Kriz, Sykova, Uzic and Vyklicky, 1974; Singer and Lux, 1973). (3) A reduction in the driving force for K^+ would result, and the net hyperpolarization due to calcium-activated potassium conductance activation would decrease. The overall effect of these mechanisms is an excitatory one, which may account, at least in part, for the observed increase in the appearance of multiple population spikes with elevated extracellular $[K^+]$.

Slices from two experimental groups of kindled rats showed a significant increase in the appearance of multiple population spikes when compared with controls. This effect was seen in slices from rats kindled in the fimbria-fornix and in the amygdala, but not in those from rats kindled directly in the hippocampus or the perforant pathway. This increase may involve mechanisms similar to those which increase multiple spiking under conditions of elevated extracellular potassium, although no attempt was made in these studies to identify these mechanisms. For example, the neurones may have been depolarized, excitatory transmitter release may have been increased, or inhibitory mechanisms may have been compromised. Results from our intracellular

studies (discussed below) suggest that the first two are unlikely, but that a reduction in inhibitory potentials may be a possibility.

Alternatively, the increase in multiple spiking may involve mechanisms quite different from those discussed above. For example, an increase in the density of dendritic calcium conductances would result in abnormally large dendritic calcium currents when triggered by synaptic potentials, and additional somatic spikes would then be triggered. This possibility remains to be investigated.

4.1.2 Spontaneous IIS Discharges in Hippocampal Slices

The mechanisms by which spontaneous IIS activity is generated in slices are less clear than those responsible for the multiple action potentials seen during disinhibition. The IIS activity consists of rhythmic, high-amplitude spikes which are clustered in bursts of varying duration. In the penicillin-treated slice, IISs appear at rates varying from 1/minute (or even lower), to as high as 0.5/second (Schwartzkroin and Prince, 1977a). The same manipulations which result in multiple-population spike generation (convulsant agents, elevated K^+), can also generate IIS discharges. Schwartzkroin and Prince (1977b) have demonstrated that when slices are perfused with penicillin, multiple population spikes appear first, while the tissue concentration of the drug is still relatively low. As the concentration increases, the multiple population spikes increase in number and intensity until paroxysmal discharges are observed. Eventually these occur spontaneously in the absence of applied synaptic activation. A similar pattern is seen in preparations exposed to elevated extracellular

$[K^+]$ (Hablitz and Lundervold, 1981; Ogata, 1975; Ogata, Hori and Katsuda, 1976; Chapter 2). As discussed at length in Chapter 1, intracellular studies by a number of laboratories have demonstrated that the intracellular correlate of the IIS is a large amplitude depolarization of the membrane, termed the paroxysmal depolarization shift (PDS), but details of the ionic mechanisms underlying PDS generation remain controversial.

4.1.3 Comparison With Other Studies

Oliver, Hoffer and Wyatt (1980) kindled guinea pigs with daily intraperitoneal injections of pentylenetetrazol (PTZ). Hippocampal slices were prepared from these animals 3 to 20 days after the last injection. When exposed to ACSF containing 8 mEq/l K^+, some of the slices developed spontaneous IIS discharges. These discharges did not appear in their "normal" ACSF, which contained 6 mEq/l K^+. The probability of abnormal discharge depended on the number of PTZ-induced seizures, and ranged from less than 10% (in animals with 2 seizures) to approximately 90% (in animals with 5 seizures). The effect persisted for at least 20 days following the last seizure. A previous study from the same laboratory (Oliver et al., 1978) found that elevating K^+ alone did not induce epileptiform activity in slices from unkindled animals; the addition of a convulsant agent such as penicillin was necessary. They concluded, therefore, that kindling with PTZ produced long-term changes in hippocampal excitability.

There are several important differences between the the study of Oliver et al., (1980) and the experiments reported here: (1) Systemic

convulsants may produce a qualitatively different pattern of seizure activity than focal electrical stimulation and thus the degree of hippocampal involvement could have been different in our studies from those of Oliver et al., (1980). Our kindled rats had experienced at least two stage 5 convulsions, and had progressed through preliminary stages of kindling over an extended period of daily stimulation. In contrast, Oliver et al. triggered daily motor seizures for, at the most, 5 days in succession. Thus, the total afterdischarge activity during kindling was very different in the two studies. (2) In Vitro hippocampal properties: In our preparation, a small percentage (10%) of slices from normal rats display spontaneous IISs when exposed to ACSF containing 6 mEq/l $[K^+]$. Oliver et al., (1978) report that "very few" slices in 6.2 mEq/l $[K^+]$ show spontaneous discharges. In a study specifically examining the K^+ threshold for IIS activity, Rutecki and Johnston (1983) report that 6.0 mEq/l $[K^+]$ is sufficient to induce epileptiform discharges in hippocampal slices, in the absence of a convulsant. Oliver et al., (1978) reported no IIS activity even when the K^+ was increased to 8 mM. One might expect, ceteris paribus, that their kindled slices would show a lower degree of epileptiform behaviour than our preparations, but this was not the case. (3) Oliver et al., (1980) performed their studies at least 3 days following the last PTZ injection. In contrast, our experiments were performed 24 hours after the last stage 5 convulsion triggered by electrical stimulation. Our studies may have been more sensitive to short-term post-ictal events than those of Oliver et al. This point is discussed in greater detail in a subsequent section.

In summary, while the study of Oliver et al. bears a close similarity to ours in terms of hypothesis and overall design, the above differences make a direct comparison rather difficult.

4.2 Intracellular Studies

4.2.1 Resting Membrane Properties and Action Potentials

Our measured values for resting membrane potential, input resistance, and action potential amplitude and width are within the range reported in other studies using similar techniques (e.g. Schwartzkroin, 1975). Our intracellular recordings presumably involved somatic impalements, since electrodes were visually guided into the cell body layer of the CA1 region of the slice. As a result, these measurements are less sensitive to dendritic membrane phenomena than to those involving the cell body. The fact that the parameters listed above did not differ between neurones from control and kindled rats suggests that kindling may not produce long-lasting alterations in the mechanisms underlying the control of resting membrane potential. These include resting sodium and potassium conductances and ionic concentration gradients. A similar integrity of action potential-generating mechanisms, including voltage-dependent sodium and potassium channels and the corresponding ionic gradients, is implied. Neurones did not display spontaneous burst firing, which, in the convulsant-treated slice, reflects epileptogenic alterations in synaptic and non-synaptic factors.

4.2.2 Synaptically Activated Potentials

Some of the mechanisms involved in the depolarizing-hyperpolarizing sequences seen in response to stimulation of stratum radiatum have been discussed above. Our measurements indicated a small but significant difference in the the peak amplitude of the hyperpolarizing phase of the synaptically-triggered response. Our studies did not attempt to differentiate between the various components of this response. These components include GABA mediated IPSPs (Alger and Nicoll, 1982a,b), located on somatic and dendritic membrane (Andersen, Dingledine, Gjerstad, Langmoen and Mosfeldt Laursen (1980)) and intrinsically-generated currents such as those mediated by calcium-activated potassium conductance (Nicoll and Alger, 1981). The peak value of this hyperpolarization was increased in neurones from kindled rats. This increase may be a response to the epileptiform activity experienced during kindling, and may represent a mechanism by which any kindling-induced hyperexcitability or tendency towards epileptiform bursting is maintained under control.

Another possible mechanism for the apparent increase of the hyperpolarizing phase of the response would involve an effective decrease in excitatory (depolarizing) potentials. When summated with a hyperpolarizing potential of normal amplitude, the net hyperpolarization would appear to be decreased. This would appear to be unlikely in these recordings, as the peak subthreshold EPSP appeared to be unaltered. A more sensitive test of the changes underlying these potentials could be made by measuring membrane conductance during synaptic activity.

4.2.3 Current-Induced Bursting

Several neurones from the kindled group demonstrated a high frequency discharge in response to direct current injection. This pattern differed from the normal one in the shorter intervals between action potentials and in the appearance of increasingly lower amplitude spikes as the burst progressed. Studies by Wong and Prince (1979) and Masukawa, Benardo and Prince (1982) have suggested that dendritic calcium channels are in involved in the mechanisms by which pyramidal neurones fire bursts in response to direct stimulation by current injection. Critical depolarization of the dendritic membrane activates voltage-sensitive calcium influx which contributes to soma depolarization and high-frequency firing. The reduction in action potential amplitude as the burst progresses presumably reflects a significant conductance shunt of the membrane in the vicinity of spike generation.

Control over the frequency of discharge is exerted by several factors, including calcium and sodium channel density, relative distribution over somato-dendritic membrane, and the balancing effect of calcium-activated potassium conductance. The modelling studies of Traub and Llinas (1979) have demonstrated that relatively subtle alterations in the distributions of these conductance channels over the pyramidal cell membrane can dramatically alter the cell's response to injected current.

4.2.4 Comparison With Other Studies

The results of our intracellular experiments may be compared with several related findings. Oliver and Miller (1982) reported that CA1 IPSPs were unchanged following commissural kindling. Our experiments, however, revealed a small decrease in the afterhyperpolarization, only one component of which may be an IPSP.

The other comparable study is that of Schwartzkroin, Turner, Knowles and Wyler (1983). They measured the membrane properties of neurones from two kinds of chronic epileptic foci using the in vitro slice preparation to maintain human biopsy material and tissue from the cortex of the chronic alumina gel monkey model. Intracellular recordings were performed on neurones from the presumably focal regions and compared with those from more peripherally involved sites (human) or from normal cortex (monkey). Their results were equivocal and inconclusive. They saw no major differences in cell properties between epilepic tissue and normal cortex. Spontaneous epileptiform activity was never observed, and bursting neurones were encountered in both types of tissue. These bursts, however, did not resemble those seen in penicillin-treated preparations, as they were graded with stimulus intensity and did not possess the all-or-none characteristics of the PDS. Schwartzkroin et al. were forced to conclude that (a) neuronal chronic epileptic focus, and (b) the in vitro preparation may be of little value or relevance in the study of chronically epileptic tissue.

4.3 Implications for Role of Hippocampus in Epilepsy

The failure of these experiments to detect a significant degree of epileptiform behaviour was unexpected. In applying these data towards a rejection of the hypothesis that the hippocampus plays a significant pacemaker role in kindling-induced epilepsy, four possibilities must be considered.

1. Hippocampal immunity to kindling-induced hyperexcitability: The hippocampus does not develop epileptic properties during kindling, and the atypical epileptiform responses are artifactual.

2. Artifacts of the *in vitro* technique: The hippocampus does develop epileptic properties, but they are masked or suppressed in the slice because of specific unphysiological properties of the preparation.

3. Insensitivity of the technique: The hippocampus does develop epileptic properties, but they are very subtle and were not detected, or triggered, by the experimental procedures that were used.

4. Lack of critical neuronal aggregate: The hippocampus does develop epileptic properties, but these are highly localized and anatomically discrete. The examples of atypical behaviour seen here represent "true" epileptic modifications resulting from kindling. Interictal spiking and ictal discharges are not strongly expressed in the slice because more intact tissue and extra-hippocampal input is required.

The four possible explanations are not mutually exclusive, but will be given separate discussion.

4.3.1 Hippocampal Immunity to Kindling-Induced Hyperexcitability

The first interpretation, that the hippocampus is resistant to kindling-induced alterations in neuronal properties, is the strongest that can be drawn from the present data. The majority of slices did not display any spontaneous epileptiform behaviours, and their response to challenge with K^+-elevated perfusate was similar to that of control slices. Field potential recordings revealed little evidence of stimulus-evoked paroxysmal activity, and paired-pulse tests indicated inhibitory function to be similar to that of controls. At the neuronal level, intracellular recordings showed that the characteristics of the resting membrane and synaptic potentials are indistinguishable from control values. Exceptions to these similarities between control and kindled slices did occur, however, and are not easily explained by this interpretation. Other studies lend some support to the interpretation.

Oliver and Miller (1982) measured the duration and inhibitory efficiency of IPSPs in CA1 pyramidal cells of the commissurally-kindled hippocampal slice. They found no difference in the properties of IPSPs between kindled and control rats. A subsequent report (Oliver and Miller, 1983) indicated that there was an increase in a non-GABA inhibitory process in the dentate gyrus of kindled rats. Several other *in vivo* studies also indicate that hippocampal inhibitory systems are not eroded by kindling stimulation. Goddard (1982) examined the time course and strength of dentate inhibition in kindled rats using the paired pulse paradigm, and found a small strengthening of inhibition. Tuff *et al.*, (1983a) have reported a similar increase in paired pulse depression. Studies of the neurotransmitter receptor systems in the

hippocampus of kindled rats have, in general, failed to reveal modifications which may reflect an increased epileptic tendency. Tuff, Racine and Mishra, (1983) found no change in labelled muscimol GABA binding in the hippocampus ipsilateral to perforant path-applied kindling stimulation. Receptors for benzodiazepines were found to increase as a result of kindling. McNamara et al., (1980, 1981) also found an increase in hippocampal benzodiazepine (BDZ) receptors following a large number of seizures, and Valdes, Dasheiff, Birmingham, Crutcher and McNamara (1982) found this increase to be localized to granule cells. Liebowitz et al., (1978) found an increase in the potassium-stimulated release of GABA from hippocampal slices of entorhinal-kindled rats.

These studies indicate that inhibitory processes remain intact, or may even be augmented in the hippocampus of kindled animals. However, an augmentation of excitatory mechanisms, or the enhancement of "intrinsic hyperexcitability" could still provide the basis for hippocampal epileptic behaviour. The present experiments, however, showed little evidence for such processes. Excitatory field potentials were reliably triggered by afferent stimulation, but were not larger than those seen in unkindled tissue. Intracellularly-recorded EPSPs possessed similar characteristics in kindled and unkindled slices, and no instances of PDS activity were recorded. No other studies are available to directly support these findings, but a number of indirect observations are consistent with a lack of increased hippocampal epileptogenicity. Acetylcholine (ACh) has been demonstrated to be a powerful excitatory agent in the hippocampus (Benardo and Prince, 1982;

Dodd et al., 1981), and produces disinhibition (Ben Ari et al., 1981) and seizures (Vosu and Wise, 1975) when applied to hippocampal neurones. An increase in hippocampal excitability could result if kindling produces an increase in cholinergic activity in the hippocampus. A variety of studies, however, have demonstrated either a transient decrease (Byrne et al., 1980; Dasheiff and McNamara, 1980; Savage et al., 1983) or no change (Blackwood 1982; Noda et al., 1982) in hippocampal ACh receptors following kindling.

In summary, the evidence from a variety of sources and from these experiments supports the hypothesis that the hippocampus is resistant to kindling-induced epileptic changes.

4.3.2 Artifacts of the In Vitro Technique

A second possible interpretation of these results is that the hippocampus in kindled rats does develop epileptic properties, but these are suppressed due to the nature of the slice preparation. The trauma of decapitation, dissection, slicing, etc. could produce a long-lasting (for the duration of the experiment) depression of epileptogenic mechanisms. For example, excitatory transmitter release could be partially blocked due to terminal depolarization in decentralized axons. Neuronal depolarization could inactivate intrinsic epileptogenic processes, such as Ca^{++} influx. However, the application of penicillin to the slices (when bathed in 6 mEq/l K^{+}) invariably led to epileptiform behaviour, suggesting that at least one set of epileptogenic substrates is not suppressed by the preparation of the slices. The cellular elements involved in kindling, however, could be different from those

involved in penicillin-induced epilepsy. Since the cellular and field properties of the slices studied in these experiments closely resembled those reported by other investigators, including in situ experiments, it is unlikely that trauma alone suppressed any kindling-induced hyperexcitability.

Another possibility is that the perfusing medium may be lacking critical levels of some ion or other substance which is necessary for kindled epileptiform activity. The perfusate used in most slice experiments is not identical to extracellular fluid or cerebrospinal fluid (CSF). Amino acids and CSF proteins are absent, and the levels of Ca^{++}, Mg^{++} and K^{+} are somewhat higher than those measured under physiological conditions. These differences do not seem to influence the responses of the hippocampal slice, although exhaustive comparisons with in vivo properties have not been carried out. There is a lack of information concerning the influence of such factors on the in vivo properties of kindled hippocampus, and therefore it is impossible to speculate what elements in the perfusate might influence kindled responses.

In summary, the in vitro technique does introduce certain artifacts into the electrophysiological properties of hippocampal tissue, but insufficient evidence exists to pinpoint any possible sources of suppression of epileptiform activity.

4.3.3 Insensitivity of the Technique

Kindling-induced alterations in hippocampal electrophysiology may have escaped detection in these experiments because of the

particular experimental procedures used. This is a limitation in any scientific experiment, but several points deserve emphasis with respect to the present experiments. Spontaneous IIS were readily detected, since they possessed all-or-none characteristics and were large in amplitude. The observation period in any given slice varied from a minimum of 5 minutes to several hours. Usually, electrodes were reinserted into all slices several times during the course of an experiment. Very low frequency IISs could have been missed, but they would be very rare events.

4.3.4 Lack of Critical Neuronal Aggregate

The hippocampal slice may not contain the necessary cellular substrates for the expression of kindling-induced epileptiform activity. If, as mentioned above, the mechanisms of kindling-induced hyperexcitability differ from those involved in the penicillin-treated slice, then our experiments may not disclose these phenomena. For example, the expression of kindled epileptiform activity in vitro may require a larger mass of neuronal elements, perhaps that resulting from a thicker slice. The only evidence speaking against this possibility is the observation that the electrophysiological properties of the hippocampal slice are relatively insensitive to slice thickness, at least over the range where slice viability is not limited by metabolic and oxygen demands. Nevertheless, a "critical mass" of tissue may be required, and it may exceed that which can be successfully maintained under in vitro conditions.

4.4 Further Studies

The experiments in this thesis generate a number of questions amenable to further study. The principal one involves the mechanism responsible for the increases in multiple-spike field potentials seen in the extracellular experiments. Intracellular recordings during these events should be carried out in order to determine whether they are accompanied, at the cellular level, by PDS activity. Similarly, the ionic basis for the decrease in afterhyperpolarization seen in neurones from kindled slices should be examined. Ionic substitution experiments may be carried out to assess the relative contributions of chloride-mediated IPSPs and calcium-activated potassium currents to this potential. The high-frequency discharge seen in kindled neurones when depolarized by direct current injection also merits further study. In particular, it may be possible to determine whether a dendritic calcium current underlies this behaviour by means of intracellular calcium buffer injection and extracellular ionic manipulations.

Another important question to be addressed involves the long-term changes associated with kindling. Since the present experiments were performed within 24 hours of the last kindled seizure, some of the observations may reflect post-ictal changes rather than the permanent modifications underlying kindling. Similar measurements should be performed several weeks following the last triggered seizure to distinguish post-ictal phenomena from those directly associated with the kindled state.

Finally, an attempt should be made to replicate these observations <u>in situ</u>. Technical difficulties notwithstanding, questions

concerning a critical neuronal aggregate and triggering inputs can be directly attacked in the intact preparation.

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