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DISSERTATION

ASSESSMENT OF SPONTANEOUS SEIZURE ACTIVITY AND EVOKED HIPPOCAMPAL RESPONSES IN FREELY-BEHAVING RATS TREATED WITH LOW-DOSE SYSTEMIC INJECTIONS OF KAINATE

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

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COLORADO STATE UNIVERSITY

19 March 1999

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JENNIFER LEE HELLIER ENTITLED ASSESSMENT OF SPONTANEOUS SEIZURE ACTIVITY AND EVOKED HIPPOCAMPAL RESPONSES IN FREELY-BEHAVING RATS TREATED WITH LOW-DOSE SYSTEMIC INJECTIONS OF KAINATE BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Adviser

Department Head/Director

ABSTRACT OF DISSERTATION

ASSESSMENT OF SPONTANEOUS SEIZURE ACTIVITY AND EVOKED HIPPOCAMPAL RESPONSES IN FREELY-BEHAVING RATS TREATED WITH LOW-DOSE SYSTEMIC INJECTIONS OF KAINATE

Human temporal lobe epilepsy is associated with complex partial seizures that can produce secondarily generalized seizures and motor convulsions. This condition is also coupled with hippocampal sclerosis and mossy fiber sprouting in the inner molecular layer of the dentate gyrus. In many patients with temporal lobe epilepsy, the seizures and convulsions occur following a latent period after an initial injury and may progressively increase in frequency for much of the patient's life. However, the mechanisms and time course that cause epileptogenesis are not well defined. In this study, we used the kainate-treated rat as an animal model for temporal lobe epilepsy to test the following hypotheses:

1) rats treated with multiple, low-dose intraperitoneal injections of kainate will develop a chronic epileptic state following a latent period after the initial treatment;
2) like some humans with epilepsy, rats with kainate-induced epilepsy will exhibit more spontaneous motor seizures during inactivity than during activity;

3) kainate treatment will induce both electrographic status epilepticus and permanent abnormal electrographic events; and
4) hippocampal inhibition will be restored within the first week after kainate treatment, when minimal, if any, mossy fiber sprouting has occurred in the inner molecular layer of the dentate gyrus.

Our results show that rats treated with multiple low-dose injections of kainate developed a chronic epileptic state characterized by a latent period before the onset of chronic motor seizures. We also found that the frequency of spontaneous motor seizures depends primarily on activity state (i.e., inactivity) rather than time of day, which is similar to some humans with epilepsy. Electrographic status epilepticus was recorded in all rats during kainate treatment, and interictal spikes were observed as early as one day after kainate treatment. Finally, chronic electrophysiological recordings from the dentate gyrus suggest that inhibition may be reduced in some rats treated with kainate; but when inhibition was reduced, partial to full recovery occurred within one week, when little or no mossy fiber sprouting had developed. Thus, kainate-treated rats have similar behavioral, anatomical, and electrophysiological characteristics to those seen in humans with temporal lobe epilepsy.

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DEDICATION

This dissertation is dedicated

to the most important people in my life,

my loving and generous husband, Chuck,

who I cannot live without,

my supportive family and friends,

and to all the animals used for experiments.

TABLE OF CONTENTS

INTRODUCTION

History of epilepsy from Antiquity through the Middle Ages

> Jet too he flees, which through ascending vapours
> All mortals makes to suffer with its pungency.
> Smoke-hued and flat, not large to look upon,
> It flames up brightly like some dried up fir,
> Yet to the nostrils brings destructive power; and men
> Will not escape the test thou settest
> To prove them sufferers from the sacred ill.
> For quickly will they bend and forwards tilt,
> As to the earth it draws them. Smeared by froth
> From their own mouths, hither and thither will they turn,
> And wallow on the ground. For filled with anger toward
> them
> She laughs to see their woe, Mene, the horrid and swift!
>
> Quote from Orpheus, *Lithica*, 474-84

The word "epilepsy" is of Greek origin (*epilepsia*) and has the same root as *epilambanein*, meaning to seize or to attack. Etymologists hypothesize that "epilepsy" has its name because it "attacks" both senses and mind or because it "seizes" the senses of its victim (Temkin, 1971). As early as Babylonian times, physicians, magicians, and scientists documented seizures and epilepsy. They believed that epilepsy was a demonic disease and had "seized" or "attacked" a man. Over the centuries, epilepsy has been called the "sacred disease," "falling sickness," or "falling evil".

Before Christ was born, the term "sacred disease" was used because it was thought that the epileptic caused a great sin against a deity, in particular, the moon goddess Mene. The above poem from Orpheus, describes how epileptics are punished by Mene. Later, during the first century A.D., it was believed that evil spirits or demons possessed epileptics. In fact, the Gospel of Mark describes Jesus driving out an unclean spirit within a man, "...the evil spirit shook the man violently and came out of him with a shriek" (Mark 1:26; also see Mark 9:14-29). Victims of epilepsy were considered unclean, and their possession was contagious. People tried to keep the demon away to escape this infection by spitting at the epileptic.

The terms "falling sickness" and "falling evil" were used during the Middle Ages to describe the sudden fall of the epileptic. Although the common person still believed that sins and demonic possessions caused epilepsy, physicians were beginning to think that epilepsy was also a natural disease (e.g., excess phlegm in the brain). The doctors, though, did not dismiss the idea of a demonic possession. Near the end of the 15th century, however, physicians moved away from the hypothesis that epilepsy was a natural disease, and began to discuss the possibilities of magic and witchcraft, in addition to possession. Medical authors of the 16th and 17th centuries believed more strongly in the power of magic and witchcraft than had the average medieval doctor (Temkin, 1971; Gross, 1992).

Superstitious cures for epilepsy were described as early as the 4th century B.C. Physicians and scientists recommended that epileptics eat camel's hair, the

gall and rennet of the seal, and the heart and genitals of the hare. The use of plants (e.g., mistletoe and peony root) and human blood, bones, and organs were also recorded as remedies for epilepsy. Doctors would attempt to release the demon or evil spirit by making a hole in the skull or try to pin the evil by driving an iron nail into the first vertebra of the victim. These and similar remedies are described in medical literature from before the birth of Christ and throughout the Middle Ages.

The epilepsies in modern neurology

During the Age of Enlightenment (late 17th century through the 18th century), physicians began to disregard the idea that epilepsy was caused by witchcraft, supernatural powers, and demonic possession. The advancements of anatomy and pathology, as well as the development of the sciences (e.g., chemistry, pharmacy, and physiology) promoted this change in ideology (Gross, 1992). At the beginning of the 19th century, the new concept that epilepsy was a physical illness of the brain (e.g., excess phlegm) began to be accepted (Eadie, 1992). Eventually, Marshall Hall (1836) described epilepsy as an "irritable" reflex arc at the level of the spinal cord. Brown-Sequard (1858) refined this hypothesis, suggesting that the irritated reflex involved the medulla (Eadie, 1994). John Hughlings Jackson near the end of the 19th century rejected these ideas of exaggerated reflex activity as the mechanism of epilepsy. Hughlings Jackson demonstrated that epilepsy began in the cerebrum, and not from altered functions at lower levels of the nervous system (i.e., brainstem and spinal cord).

Today, epilepsy is divided into three categories: 1) idiopathic – genetic origin, 2) symptomatic – pathological origin, and 3) cryptogenic – unknown origin. Because there are so many different types of epilepsy, it is more correct to refer to "the epilepsies" (Engel, 1989). Clinically, the epilepsies are generally defined by seizure type: convulsive or non-convulsive. Epileptic seizures are caused by partial or generalized paroxysmal discharges in the cerebral cortex. Partial seizures involve one cerebral hemisphere and may be localized to a specific region. Generalized seizures have a simultaneous onset involving both cerebral hemispheres. Partial and generalized seizures are further divided by additional characteristics (Engel, 1989; Löscher, 1997). There are three types of partial seizures: 1) simple partial – no loss of consciousness; 2) complex partial – impaired consciousness; and 3) secondarily generalization of simple and/or complex partial seizures – overt convulsions. Generalized seizures are sub-classified by the presence or absence of convulsive activity (e.g., absence, tonic-clonic, etc.). Complex partial seizures are the most common seizure type in all humans with epilepsy, and these seizures primarily originate in temporal lobe structures (Löscher, 1997).

Characteristics of temporal lobe epilepsy

Temporal lobe epilepsy is characterized by complex partial seizures originating in or involving the mesial temporal limbic structures. These seizures will frequently evolve into secondarily generalized seizures (Engel, 1989; French et al., 1993, Löscher, 1997). Most patients with temporal lobe epilepsy have had

some prior cerebral injury (e.g., febrile convulsions or status epilepticus), and the subsequent onset of recurrent seizures usually follows a latent period that can be as long as several years (Spencer and Spencer, 1994; Babb et al., 1991). Temporal lobe epilepsy is usually a permanent condition, with variable seizure frequency observed within a population of afflicted individuals.

In many cases, complex partial seizures in temporal lobe epilepsy are refractory to conventional anticonvulsant drug treatment, and surgical resection of the hippocampus and closely associated limbic structures (e.g., amygdala and entorhinal cortex) is required to eliminate seizures (Falconer and Taylor, 1968; Engel and Shewmon, 1993; Spencer and Spencer, 1994; Noachter et al., 1996). Examination of resected tissue from patients in which surgical intervention was successful in curtailing seizures has shown that the most common pathology is mesial temporal sclerosis (Falconer and Taylor, 1968). In particular, there is a profound loss of neurons in the hilus of the dentate gyrus (Margerison and Corsellis, 1966; de Lanerolle et al., 1989; Mathern et al., 1995b). Loss of hippocampal CA3 and CA1 pyramidal cells can also occur, but this is more variable. Investigators have also demonstrated that the axons of granule cells (i.e., the mossy fibers) make new synaptic connections with the inner molecular layer of the dentate gyrus (de Lanerolle et al., 1989; Sutula et al., 1989; Houser et al., 1990; Babb et al., 1991). These new connections are not usually present in "normal" human hippocampi. Reorganization of other neurotransmitter and neuromodulator circuits has also been reported (de Lanerolle et al., 1992, 1994).

If we are to understand the underlying mechanisms of seizures in temporal lobe epilepsy, appropriate animal models – in addition to human studies – are essential. Human based studies cannot be as rigorous in experimental design as animal models because of ethical considerations. Therefore, an appropriate animal model must share similar behavioral, electrographic, and anatomical changes with human temporal lobe epilepsy.

Animal models of temporal lobe epilepsy

The criteria for an animal model of temporal lobe epilepsy are 1) an initial cerebral injury, 2) a latent period between the injury and the first spontaneous seizure, 3) chronic seizures, and 4) appropriate histological changes. Generally, two types of treatments are used to generate such models: electrical stimulation of limbic structures and injection of chemical convulsants. Both methods are artificial and produce unwanted effects. Electrical stimulation causes an intense and synchronous activation of neurons that is unnatural, and can also produce secondary lesions. Similarly, it is difficult to separate the secondary effects of chemical treatments (e.g., penicillin, strychnine, kainic acid, and pilocarpine) from their initial neurotoxic effects.

The pilocarpine-treated rat (Turski et al., 1989; Cavalheiro et al., 1991; Mello et al., 1993) and the self-sustained limbic-status-epilepticus model (i.e., constant hippocampal stimulation $\geq$ 30 min; Lothman et al., 1990; Bertram and Cornett, 1993, 1994; Lothman and Bertram, 1993) both use status epilepticus (i.e., electrographic seizures lasting $\geq$ 30 min) as the precipitating

injury. These two models, which involve injection of an excitotoxin or intense electrical stimulation respectively, are associated with prolonged and repetitive seizure activity within the limbic circuit. In both models, spontaneous recurrent generalized seizures arise following a latent period, and histological changes resembling those seen in humans with temporal sclerosis have been observed in the hippocampus following the initial treatment (e.g., Lothman et al., 1990; Mello et al., 1993; Bertram and Cornett, 1994). However, pilocarpine treatment has a high mortality rate (i.e., 30-41%; Mello et al., 1993; Cavalheiro et al., 1991), although nearly all of the surviving animals become epileptic (Cavalheiro et al., 1991). In contrast, the self-sustained limbic-status-epilepticus model is associated with lower mortality, but yields a smaller proportion (50-73%) of rats with spontaneous recurrent seizures (e.g., Lothman et al., 1990; Bertram and Cornett, 1993).

Two other commonly used models of temporal lobe epilepsy are the kindling model (McNamara, 1986; Cavazos et al., 1994) and the repetitive-perforant-path-stimulation model (Sloviter, 1994; Wasterlain et al., 1996). These models are based upon the idea that "seizures beget seizures" (Lösher, 1997). It is hypothesized that through a positive feedback mechanism, a localized epileptiform discharge will spread to other cortical regions. However, kindled animals generally do not have spontaneous seizures, although there are some anecdotal reports of an occasional spontaneous seizure after many stimulated seizures (i.e., 'over kindling'; McNamara, 1986). Similarly, some anecdotal reports are available to suggest that rats treated with the repetitive-perforant-path

stimulation paradigm develop spontaneous generalized seizures. Overall, these two models produce rats with very few spontaneous motor seizures, and may not be good models for temporal lobe epilepsy.

Kainate-treated rats

The kainate-treated rat is one of several animal models of temporal lobe epilepsy. Histologic examination of the dentate gyrus from kainate-treated rats revealed a pattern of neurodegeneration in the hippocampus very similar to that observed in human temporal lobe epilepsy (e.g., Nadler et al., 1980; Mathern et al., 1992; Meier et al., 1992; Meier and Dudek, 1996; Buckmaster and Dudek, 1997a,b). Mossy fiber sprouting into the inner molecular layer also occurs in kainate-treated rats (e.g., Nadler et al., 1980; Cronin and Dudek, 1988; Buckmaster and Dudek, 1997a,b). However, other behavioral and electrographic characteristics of temporal lobe epilepsy have not been studied as extensively in kainate-treated rats as have histologic changes. Prior behavioral studies verified that kainate-treated rats and cats have spontaneous motor seizures (Cavalheiro et al., 1982; Tanaka et al., 1982; Cronin and Dudek, 1988; Tanaka et al., 1992; Mathern et al., 1993), but this condition may be transient (Cavalheiro et al., 1982). In our laboratory, we have observed that rats treated with multiple low-dose injections of kainate have spontaneous motor seizures that persist until euthanasia. Based on these observations, we have performed a series of experiments to test the hypothesis that rats receiving multiple kainate injections

share similar behavioral, electrographic, and anatomical characteristics with humans with temporal lobe epilepsy.

Statement of the problem

Chapter One: Recurrent spontaneous motor seizures after repeated low-dose systemic treatment with kainate: assessment of a rat model of temporal lobe epilepsy.

In this first chapter we asked, "Do kainate-treated rats have behavioral events similar to those seen in humans with temporal lobe epilepsy?" Although there are several animal models for temporal lobe epilepsy, relatively little work has sought to define behavioral characteristics exhibited in these models. The primary hypothesis driving this work is that rats treated with multiple, low-doses of kainate have behavioral characteristics that are similar to those seen in patients with temporal lobe epilepsy. Using the rat with kainate-induced epilepsy as an animal model, we compared motor seizure activity of these rats to seizure characteristics of temporal lobe epilepsy. To accomplish this goal, we monitored rats for 6-8 hr/week and recorded their seizures according to the scale developed by Racine (1972). We also video monitored a subset of the treated rats to determine if the frequency of spontaneous motor seizures increased during the first four months after treatment. These experiments used behavioral observations and statistical models to evaluate spontaneous seizures in chronically epileptic rats until euthanasia (i.e., 5-22 months after kainate treatment).

Chapter Two: Spontaneous motor seizures of rats with kainate-induced epilepsy: effect of time of day and activity state.

The primary questions we dealt with in this research project were: 1) "Do rats with kainate-induced epilepsy have more spontaneous motor seizures during a specific time of day?" and 2) "Do more motor seizures occur during inactivity than activity in kainate-treated rats?" The overall hypothesis driving this work is that rats with kainate-induced epilepsy have more spontaneous seizures during inactivity, a characteristic that has been reported in many patients with epilepsy. To accomplish this goal, we video monitored 32 rats (3 and 4 months after kainate treatment) for six consecutive days. We viewed all videotapes and recorded motor seizure activity and behavior. Motor seizures were determined from analysis of behavioral postures (e.g., lordosis, straight tail, forelimb clonus, and/or rearing). This project combined observational techniques and statistical modeling (i.e., mixed model ANOVA) to determine the relationship between spontaneous seizures and time of day and/or activity state of rats with kainate-induced epilepsy.

Chapter Three: Assessment of hippocampal inhibition and epileptiform seizure activity in freely-behaving rats during the first week after kainate treatment.

The fundamental questions we addressed in this study were: 1) "Is inhibition reduced after a kainate treatment protocol that will induce epileptogenesis?" and 2) "If inhibition is reduced, does it undergo substantial recovery during the first week when little or no mossy fiber sprouting has

occurred?" Our hypothesis is that inhibition is not necessarily reduced, and if it is reduced, substantial recovery occurs independent of mossy fiber sprouting (i.e., within the first week after kainate treatment). We used chronic in vivo recordings to characterize the electrographic events during the kainate-induced status epilepticus. We also evaluated progressive changes in inhibition during the first week after kainate treatment using single- and paired-pulse protocols in vivo. Concomitantly, 24-h video monitoring of the treated rats was performed to better determine the latent period between the injury (kainate-treatment) and the first spontaneous seizure. Half of the rats were used for histological studies to quantify mossy fiber sprouting and hilar neuron loss at 7-8 days after treatment. The remaining animals were allowed to survive to confirm that they would become epileptic. These experiments combined direct observations and in vivo electrophysiological techniques to evaluate the relationship between anatomical characteristics and spontaneous and evoked electrographic activity in freely-behaving rats during the first week after kainate treatment.

CHAPTER ONE

Recurrent spontaneous motor seizures after repeated low-dose systemic treatment with kainate: assessment of a rat model of temporal lobe epilepsy.

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ABSTRACT

Human temporal lobe epilepsy is associated with complex partial seizures that can produce secondarily generalized seizures and motor convulsions. In some patients with temporal lobe epilepsy, the seizures and convulsions occur following a latent period after an initial injury and may progressively increase in frequency for much of the patient's life. Available animal models of temporal lobe epilepsy are produced by acute treatments that often have high mortality rates and/or are associated with a low proportion of animals developing spontaneous chronic motor seizures. In this study, rats were given *multiple*, low-dose intraperitoneal (IP) injections of kainate in order to minimize the mortality rate usually associated with single high-dose injections. We tested the hypothesis that these kainate-treated rats consistently develop a chronic epileptic state (i.e., long-term occurrence of spontaneous, generalized seizures and motor convulsions) following a latent period after the initial treatment. Kainate (5 mg/kg per h, IP) was administered to rats every hour for several hours so that class III-V seizures were elicited for $\geq$3 h, while control rats were treated similarly with saline. This treatment protocol had a relatively low mortality rate (15%). After acute treatment, rats were observed for the occurrence of motor seizures for 6-8 h/week. Nearly all of the kainate-treated rats (97%) had two or more spontaneous motor seizures months after treatment. With this observation protocol, the average latency for the first spontaneous motor seizure was 77±38 (±S.D.) days after treatment. Although variability was observed between rats, seizure frequency initially increased with time after treatment, and nearly all

of the kainate-treated rats (91%) had spontaneous motor seizures until the time of euthanasia (i.e., 5-22 months after treatment). Therefore, multiple low-dose injections of kainate, which cause recurrent motor seizures for $\geq$3 h, lead to the development of a chronic epileptic state that is characterized by (i) a latent period before the onset of chronic motor seizures, and (ii) a high but variable seizure frequency that initially increases with time after the first chronic seizure. This modification of the kainate-treatment protocol is efficient and relatively simple, and the properties of the chronic epileptic state appear similar to severe human temporal lobe epilepsy. Furthermore, the observation that seizure frequency initially increased as a function of time after kainate treatment supports the hypothesis that temporal lobe epilepsy can be a progressive syndrome.

INTRODUCTION

Temporal lobe epilepsy is characterized by complex partial seizures that involve and apparently originate in the mesial temporal structures of the limbic system. These complex partial seizures can evolve into secondarily generalized, tonic-clonic seizures (Engel, 1989; French et al., 1993). Temporal lobe epilepsy often appears to be due to a prior cerebral injury (e.g., febrile convulsions, trauma, status epilepticus, etc.), and the subsequent onset of recurrent seizures occurs after a latent period that can be as long as 5-10 years (French et al., 1993; Glaser, 1993; Spencer and Spencer, 1994; Mathern et al., 1995). The seizures characteristic of temporal lobe epilepsy occur randomly or in clusters at variable intervals (French et al., 1993; Commission on Classification and

Terminology of the International League Against Epilepsy, 1995). In some patients with temporal lobe epilepsy, a progressive increase in seizure frequency may occur as part of the chronic epileptic state (Hopkins and Shorvon, 1995) that can become refractory to anticonvulsant drug treatment (Engel, 1989). Surgical resection of the hippocampus and closely associated limbic structures (i.e., amygdala and entorhinal cortex) is often the necessary mode of treatment for chronic intractable epilepsy (Falconer and Taylor, 1968; Engel and Shewmon, 1993; Spencer and Spencer, 1994; Noachtar et al., 1996).

The ethical and experimental limitations of human studies make appropriate animal models of epilepsy essential. Animal models that develop a chronic epileptic state are necessary for rigorously testing hypotheses concerning the characteristics of the epileptic process and the cellular mechanism(s) of chronic epileptogenesis. Questions such as: 'Is epilepsy progressive?' are difficult if not impossible to address directly in human clinical studies because of ethical considerations. A similar problem exists with experimental studies on human tissue, because appropriate control tissue is not available. The optimum animal model would be easily and efficiently produced (i.e., low mortality rate and high percentage of animals with recurrent spontaneous seizures), and would have the behavioral, electrographic, and anatomical characteristics of human temporal lobe epilepsy.

The kainate-treated rat is one of several animal models used to study temporal lobe epilepsy. Examination of the hippocampus and dentate gyrus from kainate-treated rats has revealed a similar pattern of neurodegeneration in the

hippocampus (Nadler et al., 1980; Mathern et al., 1992; Meier et al., 1992; Meier and Dudek, 1996; Buckmaster and Dudek, 1997a,b) and the presence of mossy fiber sprouting in the inner molecular layer of the dentate gyrus (Nadler et al., 1980; Cronin and Dudek, 1988; Meier et al., 1992; Mathern et al., 1993; Meier and Dudek, 1996; Buckmaster and Dudek, 1997a,b). Whether the occurrence of seizures in kainate-treated rats is similar to human temporal lobe epilepsy has not been studied as extensively. Previous studies have demonstrated that kainate-treated rats and cats can have spontaneous motor seizures (Cavalheiro et al., 1982; Tanaka et al., 1982; Cronin and Dudek, 1988; Tanaka et al., 1992; Mathern et al., 1993), however, this may only be a transient condition (Cavalheiro et al., 1982). Many animal models have high rates of mortality during (Turski et al., 1989; Cavalheiro et al., 1991) or after the treatment (Cavalheiro, 1995, 1996) and yield only a small proportion of rats with spontaneous recurrent seizures (Bertram and Cornett, 1993). In this study, we test the hypothesis that rats receiving multiple low-dose systemic injections of kainate (i.e., 5 mg/kg per h, IP) chronically develop seizure characteristics similar to those of human temporal lobe epilepsy. We examined whether: (i) there is a latent period between the initial insult (kainate treatment) and the appearance of chronic motor seizures; (ii) the frequency of seizures increases with time; and (iii) the epileptic condition is long-lasting or chronic. This paper also reports the seizure history of animals used in recently published in vivo and in vitro electrophysiological and anatomical studies (Wuarin and Dudek, 1996; Buckmaster and Dudek, 1997a,b; Smith and Dudek, 1997; Patrylo and Dudek, 1998).

MATERIALS AND METHODS

Kainate treatment

Sprague-Dawley rats (150-250 g; Harlan) were used for kainate (n=139 males and 18 females) and control (n=67 males) treatments. Rats were injected with kainate (5 mg/kg, IP) or saline every hour. By the third or fourth injection, most male kainate-treated rats began to have obvious motor seizures. Most female rats began having seizures after the fourth or fifth kainate injection. Seizures were scored according to a modified Racine's scale (Racine, 1972; Ben-Ari, 1985), and only motor seizures were considered (i.e., Class I and II seizures were not scored). Briefly, motor seizure severity was characterized as follows: class III, animals displayed forelimb clonus with a lordotic posture; class IV, animals reared with concomitant forelimb clonus; and class V, animals had a class IV seizure and fell over. Once an animal began showing excessive inactivity or excessive activity (i.e., exaggerated running or jumping), or had $\geq$10 class IV/V seizures/h, subsequent injections were delayed or reduced to 2.5 mg/kg per 30 min. The injections were altered because we have observed that these behaviors frequently precede death if treatment continues at the same rate. We defined excessive inactivity to be when the rat was barely moving and was lying in one location of the cage. The animal did not appear to be asleep (eyes were open). When the rat did move, it appeared to move very 'laboriously'. We defined excessive activity to be when the rat continually ran in circles, 'paced' the cage, jumped, and/or stood in the corner of the cage sniffing excessively. Treatment was continued until class IV/V seizures were elicited for $\geq$3 h, yielding

a total dose of 20-50 mg/kg. We defined motor seizures for ≥3 h to be when treated rats had at least one convulsive seizure during each, consecutive 1-h period, although most rats had many more seizures during these 1-h periods (e.g., 7-22 seizures). All rats received lactated Ringer's (approximately 1-4 ml subcutaneously, directly after treatment) and moistened rat chow for the first week after treatment.

Chronic seizures

Both kainate- and saline-treated rats were viewed directly during random 1-2 h intervals, totaling 6-8 h/wk. These observational periods occurred during the 12-h interval when lights were on. Behavioral observations of seizure activity were recorded, applying the same modified Racine scale used during kainate treatment. Behavioral monitoring of seizure activity was initiated either 1 day (n=98) or approximately 3 months after treatment (n=22) until euthanasia. Control rats (n=52 rats monitored 1 day after treatment, n=15 rats monitored 3 months after treatment) were similarly observed until euthanasia (e.g., 2-22 months). Animals in the first group were used to determine the time between kainate treatment and the onset of chronic motor seizures (i.e., latent period to seizure onset) and the effect of time after kainate treatment on seizure frequency. Both groups of animals were used to determine if the epileptic state was chronic.

Video-monitoring and seizure analysis

Exactly 90 and 120 days after kainate treatment, 32 rats (16 male and 16 female) were placed in labeled kennels and video-monitored for six consecutive 24-h periods (12/12 h light cycle). The behavior and seizures were recorded on 8-h videotapes from a MTI 65 Silicon Intensified Target camera. Three trained technicians viewed all videotapes and rated seizure activity with the same modified Racine scale used during kainate injections. Seizures were determined by looking for behavioral postures (i.e., lordosis, straight tail, jumping/running, forelimb clonus, and/or rearing) during the fast-forward speed of the videotapes. Once a behavioral posture was seen, the videotape was rewound to the beginning of the behavior and examined at real-time speed. Two male and one female kainate-treated rats were observed to have periods of behavioral status epilepticus. Since this abnormal behavior is manifest by a motor seizure every 1-2 min, these seizures were eliminated from the data. None of these animals died during their spontaneous status epilepticus.

RESULTS

Mortality and percent of kainate-treated rats that became epileptic

Of the 157 rats treated with kainate, 133 survived (15% mortality rate during treatment). During the initial treatment, 120 rats (111 males and nine females) had $\geq$3 h of recurring seizures, while 13 (6 males and 7 females) had <3 h of seizures. An animal was considered to be epileptic if $\geq$2 spontaneous motor seizures were observed, although nearly all kainate-treated animals were

observed to have many more seizures. Of the rats that underwent <3 h of status epilepticus during treatment, 85% eventually developed spontaneous motor seizures (i.e., became epileptic), while 116 of 120 rats (97%) with ≥3 h of kainate-induced seizures later showed ≥2 spontaneous motor seizures. Of these rats, 96% of the males and 100% of the females developed epilepsy when ≥3 h of kainate seizures were induced. Ninety-six percent of the males and 94% of the females developed epilepsy when all surviving rats were analyzed. The results described in the remainder of the manuscript are from those rats in which we observed ≥3 h of status epilepticus during kainate treatment (n=120).

Latency of spontaneous seizures after kainate treatment

Ninety-eight kainate-treated and 52 control rats were monitored 6-8 h per week from the day immediately following kainate treatment until euthanasia. The mean latency between kainate treatment and the onset of the first motor seizure was 77±38 (±S.D.) days in male rats (n=87, two male rats were never observed to have spontaneous seizures) and 78±42 (±S.D.) days for females (n=9) (Fig. 1.1). One rat was observed to have a spontaneous seizure 2 days following kainate treatment, but no additional seizures were observed in this animal for another 80 days. In two other treated rats, their first spontaneous motor seizure was observed 3 weeks after kainate treatment. Six weeks after treatment only 20% of the rats (19 of 98) were observed to have had their first spontaneous motor seizure. Not until 10 weeks following treatment did over 50% of the rats (51 of 98) have their first spontaneous motor seizure. These observations

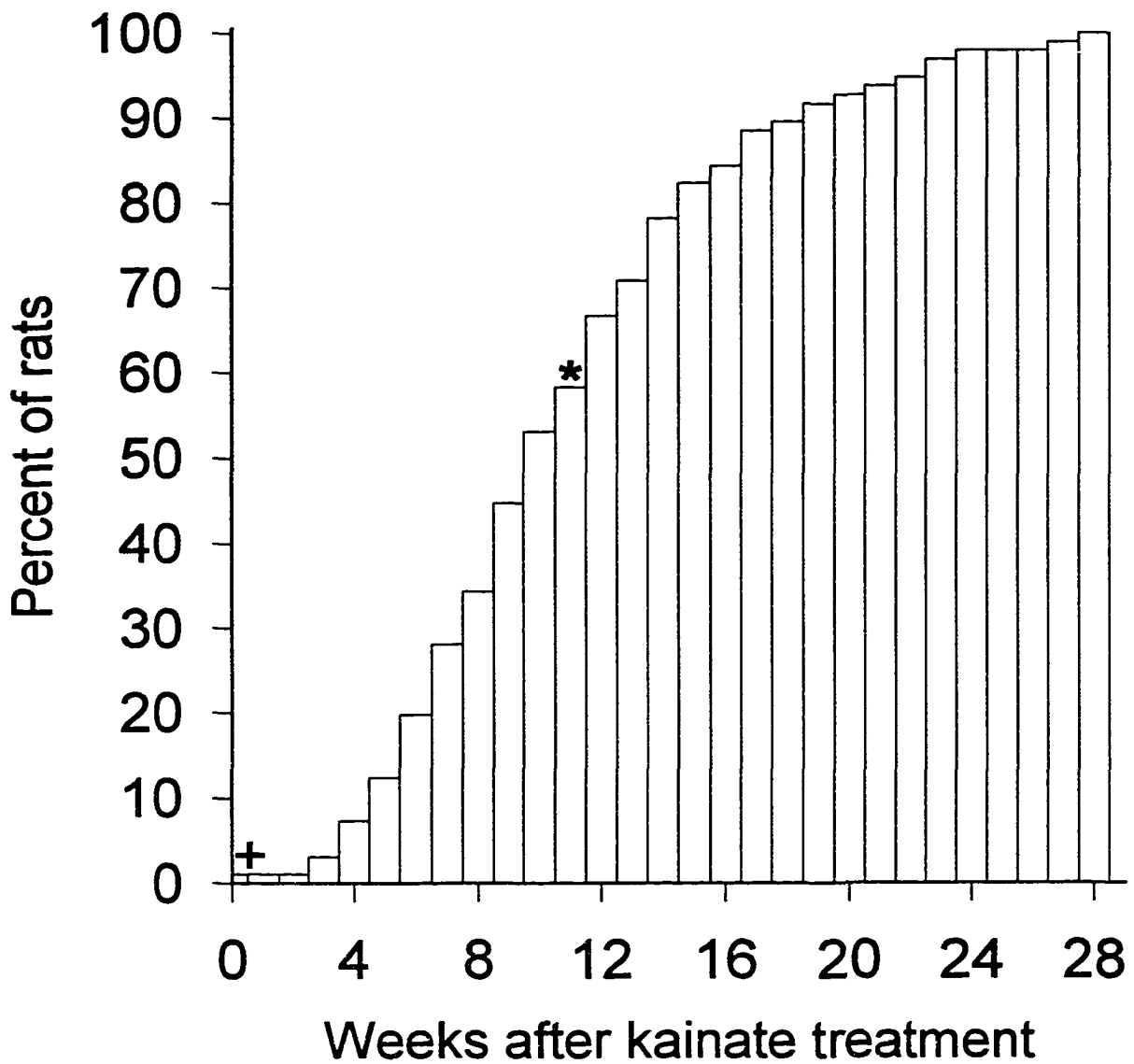

Figure 1.1: Latent period for chronic motor seizures in kainate-treated rats. The mean time interval from 1 day after treatment until the first observed spontaneous seizure was 11 weeks (asterisk) in both male (n=87) and female rats (n=9). The plus symbol represents the rat which was observed to have a seizure 2 days after treatment, but no additional seizures were seen in this individual for 80 days. A latency of $\geq$3 weeks was observed in 99% of kainate-treated rats.

support the hypothesis that there is a latent period of weeks between the initial neuronal injury associated with kainate treatment and the occurrence of the first seizure associated with chronic epileptogenesis.

Seizure frequency and permanence

The mean number of seizures observed in the kainate-treated rats was 45.3±4.3 (±S.E.M.). Overall, 83% (99 of 120) of all kainate-treated rats were observed to have ≥10 motor seizures (Fig. 1.2). Most animals were seen to have many more seizures (e.g., ≥100 seizures for 13% of the kainate-treated rats). Those rats with a large number of seizures (e.g., ≥100) were not necessarily those rats that were monitored for the longest duration after kainate treatment (e.g., 20-22 months). The 16 kainate-treated rats with the most observed motor seizures (i.e., 100-226 seizures) were monitored for 214-475 days after treatment (i.e., 7-16 months). Thus, the total number of observed motor seizures from kainate-treated rats was not directly a function of the length of monitoring (e.g., 2-22 months). Only 3% (two of 67) of the saline-treated male rats were observed to have a spontaneous motor seizure (one rat had a single seizure, and three seizures occurred over 3 consecutive days in another rat, but no other seizures were observed in these two animals).

The frequency of spontaneous motor seizures initially increased as a function of time after kainate treatment. Only kainate-treated rats with a seizure frequency ≥0.03 seizures/h per month, once they began having spontaneous seizures, were included in this analysis. This criterion was chosen because it is

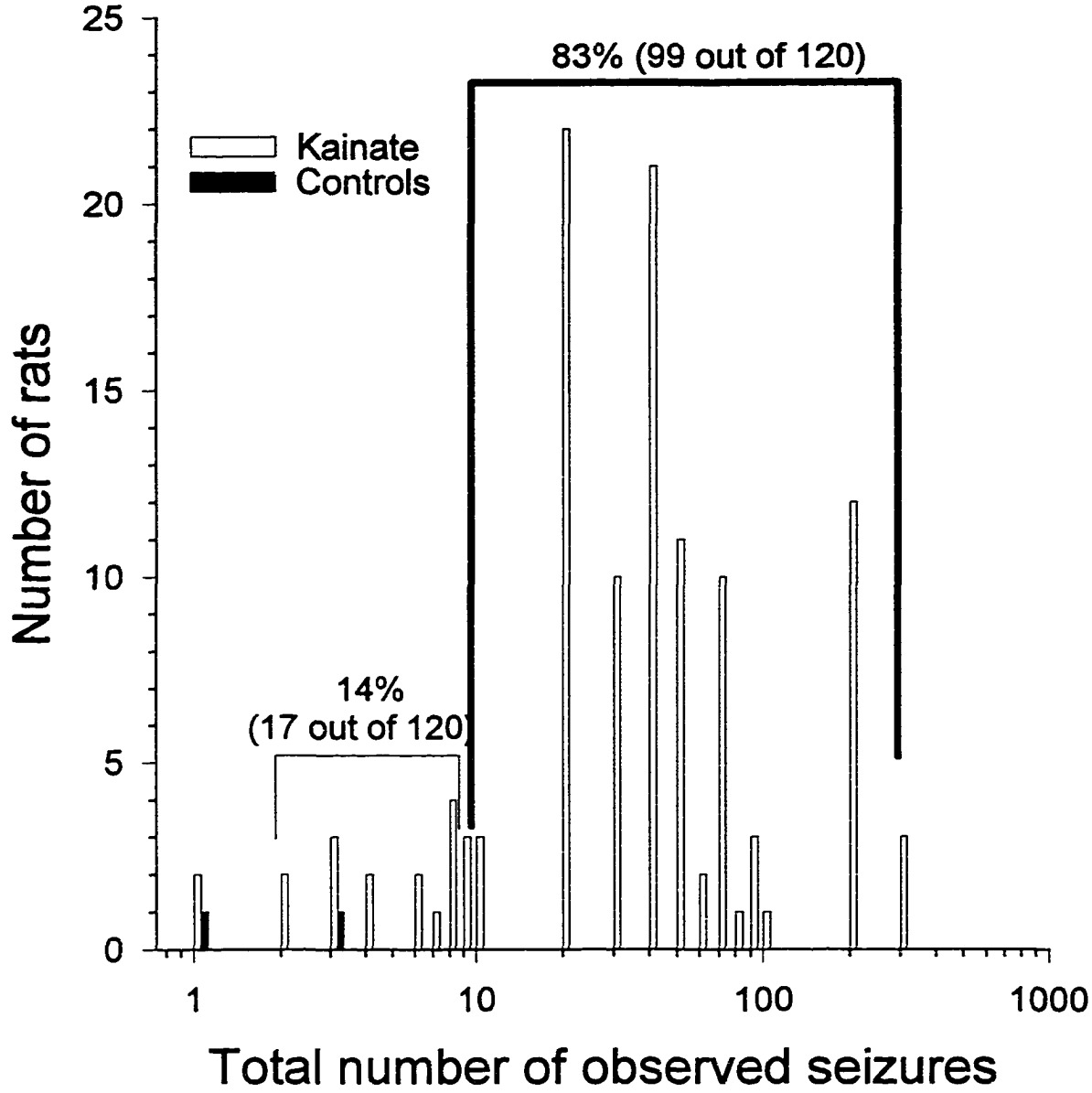

Figure 1.2: Total number of seizures observed in control and kainate-treated rats. Two of the 67 control rats (black bars) were observed to have seizures, whereas 116 of 120 kainate-treated rats had at least two spontaneous recurrent seizures (open bars). Eighty-three percent of kainate-treated rats were observed to have from 10 to >200 spontaneous seizures (the group under the thick bracket), 14% had 2-9 seizures (the group under the thin bracket), and the remaining 3% were observed to have 0-1 seizures. Thirty-eight percent of the rats with <10 seizures were euthanized <4 months after treatment, and 95% of the rats with >11 seizures were euthanized or died >4 months after treatment.

equivalent to an animal having one seizure during approximately 30 h of monitoring, and each animal was monitored for 24-32 h per month. Seizure frequency across all animals meeting this criterion (n=47) increased from 2 to 6 months after kainate treatment (Fig. 1.3A). To account for the variable latent periods, seizure frequency was plotted against time after the onset of chronic seizures (Fig. 1.3B); this graph also shows a gradual increase in seizure frequency for the first 4 months. Figures 1.3A,B suggest that seizure frequency levels off or decreases, but the later time points in Fig. 1.3A,B could be underestimated due to a methodological error (i.e., the number of rats was lower at later times because those with a high seizure frequency were used in experiments). Therefore, seizure frequency was plotted against normalized points during the epileptic period (Fig. 1.3C), which also shows that seizure frequency increased with time. To show further that the apparent increase in seizure frequency as a function of time after kainate treatment was not an artifact of rats with a high seizure frequency being removed from the pool early (i.e., euthanized $\leq$4 months after the onset of seizures), in Fig. 1.3D we analyzed a particular group of seven kainate-treated rats from treatment to euthanasia (i.e., n=7 rats for each month after the onset of seizures). When a square root transformation of the data was used to produce more homoscedastic variances, seizure frequencies at 4-7 months after the onset of seizures were significantly higher than the seizure frequency at 1 month after the onset of seizures ($P<0.05$, Student-Newman-Keuls test). Seizure frequency appeared to plateau at

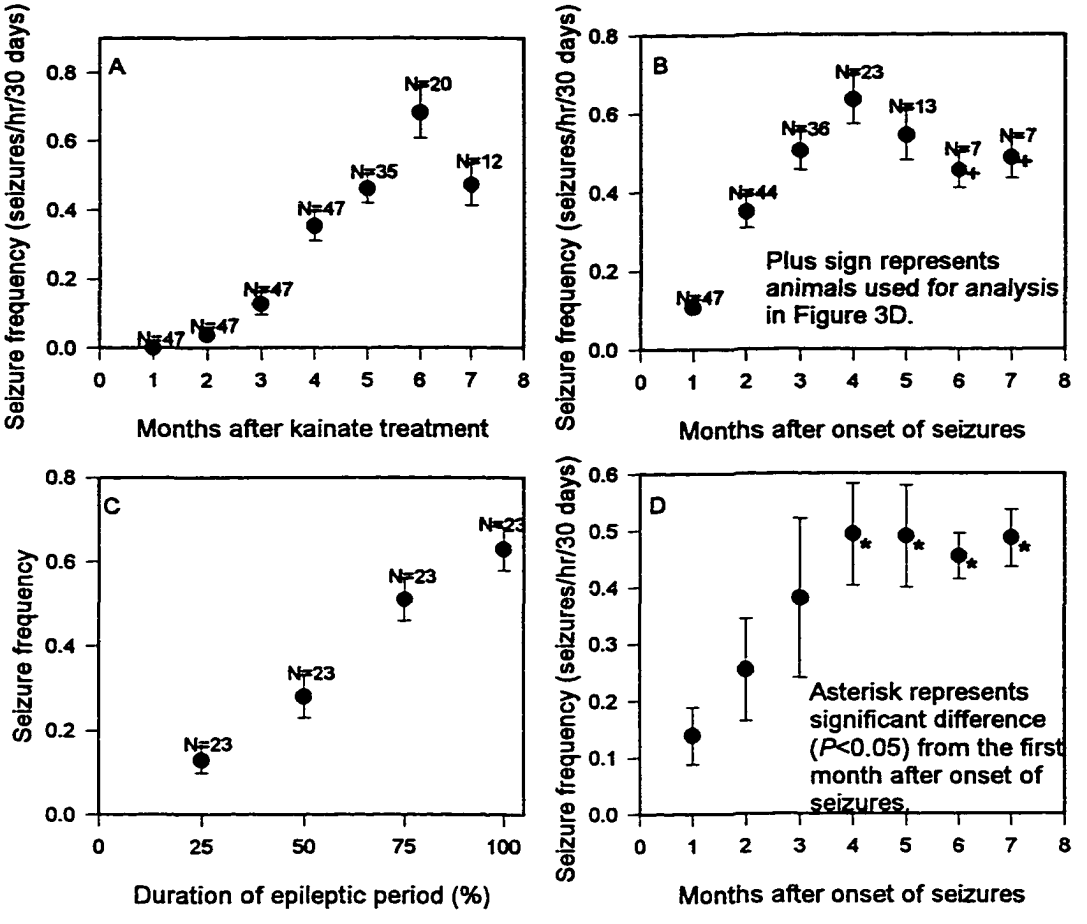

Figure 1.3: Mean seizure frequency as a function of time following kainate treatment. These results (mean and standard error of the mean) are from kainate-treated rats (n=47) that had at least one seizure every 30 days after their initial seizure and that were euthanized >4 months after treatment. (A) Seizure frequency as a function of time following kainate treatment. The first 3 months reflect the variable latent period for different rats. Less kainate-treated rats were available at the later time periods, because animals were either euthanized for electrophysiological and anatomical experiments or died. (B) Seizure frequency as a function of time after the onset of seizures. This graph compensates for the variable latent periods, and illustrates the increase in seizure frequency independent of the variable latent period. (C) Seizure frequency as a function of normalized epileptic period. The epileptic period is defined as the time (i.e., weeks) between the onset of chronic seizures and death or euthanasia. This graph therefore compensates for the effect of preferentially using animals with a high seizure frequency and for the observation that animals with high seizure frequency were more likely to die. (D) Seizure frequency as a function of time after the onset of seizures for a particular group of rats (n=7 at each point). A square root transformation of the data shows significantly higher seizure frequencies ($P<0.05$, Student-Newman-Keuls test) at 4-7 months compared to 1 month after the onset of seizures (asterisks). Data include animals with 4-8 months of chronic seizures.

4-7 months, but earlier it increased as a function of time. These data suggest that the epileptic state was chronic and initially progressive.

In another assessment of whether seizure frequency increased as a function of time after kainate treatment, we video-monitored a subset of treated rats for 6 consecutive days (24 h/day) at 3 and 4 months after kainate treatment (n=21). Fifteen of 21 treated rats were observed to have at least one spontaneous motor seizure (seizure frequency = 0.0-0.06 seizure/h) 3 months after treatment, while all 21 rats had at least one seizure 4 months after treatment (seizure frequency = 0.007-0.89 seizure/h). Of these 21 treated rats, 71% (15 of 21) had significantly more spontaneous motor seizures at 4 months compared to 3 months after treatment ($P<0.005$, Wilcoxon signed-rank test), further suggesting that seizure frequency initially increased as a function of time after kainate treatment (data not shown).

To further test for chronicity of the epileptic state, 17 animals were observed for $\geq$1 year after kainate treatment. Most of these rats (82%) displayed higher seizure frequencies at the longer versus shorter time periods after treatment. These data indicate that seizure frequency continued to increase for 1 year after kainate treatment, and the presence of recurring seizures was a chronic condition in most kainate-treated rats.

To assess the severity of the epileptic condition, we calculated the seizure frequency for each rat during each month after treatment (i.e., seizures per hour monitored per month). Monthly seizure frequency was calculated for each kainate-treated rat, and the month with the maximal frequency was then

determined. A maximal seizure frequency of one to two spontaneous motor seizures per 2 h of observation (i.e., 0.5-1.0 seizure/h) was found in 53% of the kainate-treated rats (Fig. 1.4). Although some animals had only one spontaneous motor seizure every 10-30 h of observation (i.e., 0.03-0.1 seizure/h), 47% of these animals (i.e., eight of 17 rats) were euthanized <4 months after kainate treatment; therefore, these particular rats may not have achieved their maximal seizure frequency, which was usually seen many months after kainate treatment (i.e., 4-12 months). These data indicate that most kainate-treated rats had a high seizure frequency, particularly if a period of many months was allowed for epileptogenesis to occur.

DISCUSSION

The principal finding of this study is that systemic treatment with multiple low doses of kainate reliably and efficiently produces chronically epileptic rats. Treatment with this multiple-kainate-injection protocol resulted in a low mortality rate (15%) and a high percentage (97%) of epileptic rats. This definition of epilepsy only required two or more spontaneous seizures, but 88 of the 98 rats monitored from treatment to euthanasia had ≥6 seizures (three of the rats with <6 seizures were euthanized for experiments after 2-3 months, and seven were euthanized after 4-8 months). Most rats were kept for >4 months after treatment, and most of them had many more than 10 seizures (Fig. 1.2). With 6-8 h/week of monitoring, the average latent period for spontaneous seizures was >2 months (i.e., 77±38 days). Even after accounting for the variability in latent period,

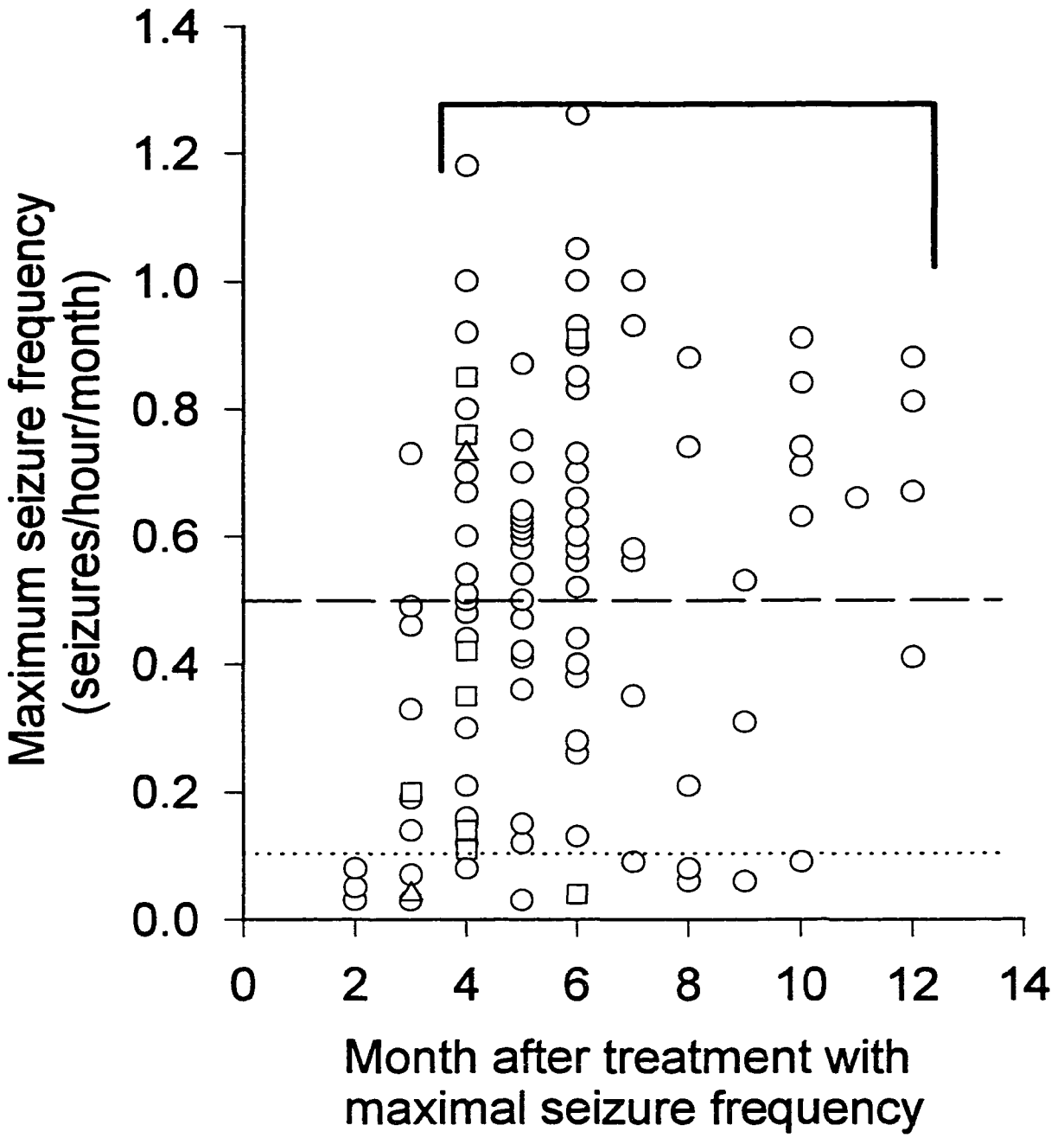

Figure 1.4: Maximal seizure frequency. Over 50% of the kainate-treated rats (62 of 117) had a maximal seizure frequency of >0.5 seizure/h (i.e., >1 seizure in 2 h of observation, open symbols above dashed line). A maximal seizure frequency of <0.1 seizure/h (i.e., <1 seizure in 10 h) was observed in only 17 kainate-treated rats (15%, open symbols below dotted line). In 86% of the treated rats (101 of 117), their maximal seizure frequency occurred >4 mo after kainate-induced status epilepticus (symbols under the bracket). Circles represent one rat, squares represent two rats, and triangles represent three rats for each point.

seizure frequency increased for several months after the onset of the first spontaneous seizure. Furthermore, this epileptic condition appeared to be chronic, and seizures were observed in nearly all animals until euthanasia or death, 5-22 months after the initial treatment.

Similarities in seizure patterns in kainate-treated rats and in human temporal lobe epilepsy

The behavioral data collected in this study suggest that, like most patients with human temporal lobe epilepsy (French et al., 1993; Glaser, 1993; Spencer and Spencer, 1994; Mathern et al., 1995), rats treated with multiple low doses of kainate display a latent period of weeks (or even months) to the onset of spontaneous recurrent seizures following the precipitating injury (i.e., kainate treatment). Latent periods in seizure onset of 1-3 weeks have also been reported following a single dose of kainate (Cavalheiro et al., 1982; Tanaka et al., 1992; Mathern et al., 1993). Since the animals in the present study were observed for only 6-8 h per week, we presumably overestimated the latent period (i.e., many kainate-treated rats may have had seizures before the first observed seizure). However, it is possible but unlikely that we did not observe infrequent motor seizures during the first few weeks following kainate injections. Given the large number of kainate-treated rats monitored from 1 day after treatment until euthanasia (n=98), only one rat was observed to have had a spontaneous motor seizure 2 days after treatment, which was probably a residual effect of kainate. In the remaining 97 rats, the median latent period was observed to be 10 weeks

after kainate treatment. Therefore, our data support the hypothesis of a latent period between kainate-induced status epilepticus and the occurrence of spontaneous motor seizures, similar to what is known to occur in humans with temporal lobe epilepsy (French et al., 1993; Glaser, 1993; Spencer and Spencer, 1994; Mathern et al., 1995).

Several other characteristics of the seizures and behavior of the kainate-treated rats also resembled temporal lobe epilepsy in humans. After the latent period, some of the animals only had a few observed motor seizures (even with several months of observation), but other animals had many seizures at a frequency as high as 1-2/h; this range in individual seizure frequency is reminiscent of epilepsy in the human population (French et al., 1993). The kainate-induced epileptic rats often appeared 'confused' (e.g., hyperactive exploration of their cage) after a seizure, which also resembles the post-ictal confusion in most humans with temporal lobe epilepsy (Engel, 1989; Fenwick, 1995). Although spontaneous remission can occur in the adult human population, temporal lobe epilepsy is generally a chronic condition (Hopkins and Shorvon, 1995); we found that spontaneous seizures continued until the kainate-treated rats died or were euthanized. Therefore, the latent period, the wide range across individuals in severity of the epileptic state, the behaviors associated with the epileptic seizures, and the long-term or chronic nature of the epilepsy appear to be similar in both human temporal lobe epilepsy and rats given this modified kainate treatment.

The progressive increase in seizure frequency

The observation that the frequency of seizures initially increased with time following kainate treatment supports the hypothesis that temporal lobe epilepsy is a progressive syndrome (Engel, 1989; French et al., 1993; Glaser, 1993), and possibly that 'seizures beget seizures' (Gowers, 1885). Using the electrically-induced limbic-status-epilepticus model, Bertram and Cornett (1994) also found that seizure frequency increased during the 6 months after the first spontaneous motor seizure, although seizure frequency could also plateau. In the kindling model, where the susceptibility to electrically evoked seizures increases with the number of evoked seizures (Cavazos et al., 1994), *spontaneous* motor seizures only occur after many stimulated seizures (i.e., 'overkindling'; McNamara, 1986; Shouse et al., 1990). The gradual increase in seizure frequency in kainate-treated rats may be due to progressive anatomical changes in the temporal lobe (i.e., neuronal loss, gliosis, axonal sprouting and/or synaptic reorganization), as seen in both human temporal lobe epilepsy and kainate-treated rats (e.g., Cronin and Dudek, 1988; DeLanerolle et al., 1989; Babb et al., 1991; Mathern et al., 1996; Buckmaster and Dudek, 1997a,b). Axonal sprouting and synaptic reorganization, for example, increase over several weeks and may take several months to reach maximal levels (Mathern et al., 1992, 1993). It is unclear whether the time-dependent increase in seizure frequency is because 'seizures beget seizures', or because of slowly developing kainate-induced changes in the anatomy and/or physiology of temporal lobe structures. Regardless of the cellular

mechanism(s), these behavioral observations support the hypothesis that chronic epileptogenesis involves a latent period and is initially progressive.

Other models of temporal lobe epilepsy

Several chronic models of temporal lobe epilepsy now appear to duplicate aspects of this syndrome (Engel, 1989). Generally, there are two types of treatments used to generate animal models of human temporal lobe epilepsy: (i) electrical stimulation of limbic structures, and (ii) injection of neurotoxic chemicals. Both methods are artificial and can have side effects. Electrical stimulation causes an intense and synchronous activation of neurons that is unnatural, and can also have secondary effects (e.g., lesions). Similarly, it is difficult to separate the secondary effects of kainate and other chemical treatments from their initial neurotoxic effects. This study, however, reports observational data on chronic, spontaneous, motor seizures that develop weeks and months after kainate treatment, when the injected kainate has dissipated. Segregating the initial neurotoxic effect from the process of epileptogenesis in chemical-based models is more of an issue for short-term studies, particularly those analyzing status epilepticus after kainate or pilocarpine treatment.

In addition to the kainate-treated rat model described here, the pilocarpine-treated rat (Turski et al., 1989; Cavalheiro et al., 1991; Mello et al., 1993) and the self-sustained limbic-status-epilepticus model (Lothman et al., 1990; Bertram and Cornett, 1993, 1994) are based upon status epilepticus as the precipitating injury. These two models, which involve injection of an excitotoxin or

intense electrical stimulation respectively, are associated with prolonged and repetitive seizure activity within the limbic circuit. In both models, anatomical changes resembling those seen in human mesial temporal sclerosis have been observed in the hippocampus following the initial treatment, and spontaneous recurrent generalized seizures arise following a latent period (e.g., Lothman et al., 1990; Mello et al., 1993; Bertram and Cornett, 1994). In comparison to the multiple-kainate-injection protocol, pilocarpine treatment has a higher mortality rate (i.e., 30% was observed by Mello et al. (1993), and 41% by Cavalheiro et al. (1991)), although virtually all of the surviving animals became epileptic (Cavalheiro et al., 1991). Therefore, the kainate model described in this report was about as effective as the pilocarpine model, but with less mortality. In the self-sustained limbic-status-epilepticus model, approximately 50-73% of the rats developed spontaneous recurrent seizures (e.g., Lothman et al., 1990; Bertram and Cornett, 1993) compared to 97% of rats injected with multiple low doses of kainate.

Two other commonly used models of temporal lobe epilepsy are the kindling model (McNamara, 1986; Cavazos et al., 1994) and the repetitive-perforant-path-stimulation model (Sloviter, 1994; Wasterlain et al., 1996). Kindled animals generally do not have spontaneous seizures, although there are some anecdotal reports of an occasional spontaneous seizure after many stimulated seizures (i.e., 'overkindling'; McNamara, 1986). Similarly, some anecdotal reports are available to suggest that animals treated with the repetitive-perforant-path stimulation paradigm develop spontaneous generalized seizures. Unlike kindling

and the repetitive-perforant-path-stimulation model, nearly every animal treated with multiple low-dose injections of kainate allowed to survive >4 months had many spontaneous seizures, and the seizures appeared to initially increase in frequency and continued until death. Therefore, the modified kainate model appears to have minimal mortality, and yet reliably and efficiently induces a chronic epileptic state.

The rationale behind this modified kainate-treatment protocol is that repetitive low-dose injections allow an investigator to modify the amount of administered kainate, dependent on the behavior of the animal, thereby decreasing the probability of an overdose but still insuring a prolonged period of status epilepticus. Preparation of kainate-treated rats in prior investigations entailed either the more labor-intensive procedure of injecting rats intrahippocampally or intraventricularly with kainate (e.g., Nadler et al., 1980; Cavalheiro et al., 1982; Mathern et al., 1993) or systemically injecting rats with a single large- dose of kainate (e.g., Cronin and Dudek, 1988; Cronin et al., 1992; Sloviter, 1992). These treatment protocols have often been associated with a relatively high mortality rate and a low percentage of rats becoming epileptic (Cavalheiro et al., 1982; Cronin and Dudek, 1988). Additionally, the epileptic condition produced in these animals may only be transient (Cavalheiro et al., 1982). Earlier experience from our laboratory (e.g., Cronin and Dudek, 1988; Cronin et al., 1992; Meier et al., 1992; Meier and Dudek, 1996), including many anecdotal and unpublished observations, indicated that single high-dose injections of kainate induced much higher mortality (sometimes >50%) and yet

did not consistently induce chronic epilepsy (i.e., often <50% of the treated rats were observed to have seizures). This modified kainate-treatment protocol (i.e., multiple low-dose injections of kainate) appears to have a greater efficacy compared to other kainate protocols (e.g., single injections), because the injections are performed intraperitoneally and the exposure to kainate is most likely extended. This multiple low-dose injection method allows slower diffusion to the brain compared to venous, intrahippocampal, and intraventricular injections. Although the total dose is higher with the multiple-injection protocol (i.e., 20-50 mg/kg) than with the typical single-injection protocol (15-18 mg/kg), some of the kainate is seemingly broken down during the procedure. Therefore, the maximum levels of kainate in the blood may be similar, but the exposure to basal levels of kainate needed to induce seizure activity is presumably longer.

Kainate-treated rats, like pilocarpine-treated rats, are representative of severe temporal lobe epilepsy or possibly intractable epilepsy, because the seizure frequency observed in the treated rats can be extremely high. The kindling and repetitive-perforant-path-stimulation models probably portray humans with a less severe form of temporal lobe epilepsy, because spontaneous motor seizures are rare. We cannot be certain, however, that many more cases of severe human temporal lobe epilepsy exist because patients are routinely treated with antiepileptic drugs.

Conclusions

In conclusion, multiple low-dose injections of kainate lead to an epileptic state where seizures (i) occur after a latent period of weeks, (ii) increase in frequency for many months, (iii) can occur as often as 1-2/h of observation, and (iv) appear to be chronic. The presence of a latent period of weeks supports the hypothesis that the initial damage from the precipitating injury (i.e., status epilepticus) is by itself insufficient to be the basis for chronic epileptogenesis. Along with the latent period, the increase in seizure frequency during the months following the first chronic seizure emphasizes the progressive nature of temporal lobe epilepsy. These data support but do not prove the idea that 'seizures beget seizures'. The latent period, the initial progressive increase in seizure frequency, and the chronic or long-term nature of the epileptic state suggest that the mechanism(s) of the epileptogenesis is a structural change that requires prolonged periods to develop and does not spontaneously reverse itself.

CHAPTER TWO

Spontaneous motor seizures of rats with kainate-induced epilepsy: effect of time of day and activity state

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ABSTRACT

Kainate treatment in rats can result in a chronic behavioral state that is similar to human temporal lobe epilepsy. We tested the hypothesis that, like some humans with epilepsy, rats with kainate-induced epilepsy have more spontaneous motor seizures during inactivity (i.e., little to no volitional movement, including apparent sleep) than during activity (i.e., apparent volitional movement, as in walking, grooming, eating, etc.). Rats were given intraperitoneal (IP) injections of kainate (5 mg/kg) every hour so that class III/IV/V seizures were elicited for ≥3 h. Seizure behavior was video-monitored (24 h for 5-6 days, n=32 rats at 3 months and n=23 rats at 4 months after treatment) to examine the occurrence of seizures as a function of light versus dark (12 h-12 h light-dark cycle) and inactivity versus activity. Significantly more spontaneous motor seizures occurred during inactive versus active states (82% vs. 18%, P=0.0001). Although more seizures occurred during the light period than the dark, the difference was not significant (62% vs. 38%, P>0.1). These data suggest that the frequency of spontaneous motor seizures in the rat with kainate-induced epilepsy depends primarily on activity state rather than time of day (i.e., time during the light-dark cycle). The effect of inactivity on the occurrence of seizures in the rat with kainate-induced epilepsy appears similar to some forms of human epilepsy.

INTRODUCTION

In many forms of human epilepsy (e.g., generalized epilepsies such as juvenile myoclonic epilepsy and absence epilepsy), clinical studies have shown

that spontaneous seizures commonly occur during sleep or upon arousal (Janz, 1962; for reviews, see: Janz, 1974; Kellaway, 1985; Meierkord, 1994; Hopkins, 1995; Niedermeyer, 1996; Shouse et al., 1996). Sleep and generalized epileptic seizures have been hypothesized to be associated because both phenomena use the same thalamocortical pathways for initiation (Avoli and Gloor, 1981). However, the relationship of sleep and spontaneous seizures in patients with partial epilepsies has not been well established. Other clinical studies (i.e., both partial and generalized epilepsies) have shown that motor seizures are more likely to take place during quiescent states (e.g., relaxed) compared to physical exercise (e.g., aerobic activity; Gotze et al., 1967; Livingston and Berman, 1973; Nakken et al., 1990). The mechanism responsible for an increase in the frequency of spontaneous seizures during periods of inactivity is unknown. It is also unknown whether these relationships (i.e., sleep and seizures; inactivity and seizures) exist in animal models of partial epilepsy.

The relationship between sleep and epilepsy has been studied in some animal models. The amygdala-kindled cat has provided data linking sleep and chronic sleep disorders with increased seizure susceptibility (Shouse et al., 1992, 1994), but this model does not consistently display chronic epilepsy with a prolonged period of recurrent spontaneous seizures. In both the pilocarpine and self-sustained limbic-status-epilepticus rat model, more spontaneous seizures have been observed during the day than the night (Cavalheiro et al., 1991; Bertram and Cornett, 1994; Quigg et al., 1998), although details about the

behavioral state (e.g., inactivity or activity) during these seizures were not reported.

In this study, we have used the rat with kainate-induced epilepsy as an animal model of temporal lobe epilepsy (Nadler, 1981; Ben-Ari, 1985). Following repeated hourly injections of kainate (i.e., 5 mg/kg/h, IP), rats consistently develop a chronic epileptic state (Hellier et al., 1998). In this investigation, we tested the hypothesis that more spontaneous motor seizures occur during inactivity (i.e., little to no volitional movement, including apparent sleep) in rats with kainate-induced epilepsy, as has been reported in many patients with epilepsy (see above). We found that the occurrence of spontaneous motor seizures in rats with kainate-induced epilepsy was increased during inactivity.

MATERIALS AND METHODS

Kainate treatment

Our protocol for kainate treatment, which consistently results in the development of spontaneous motor seizures, has been described in detail elsewhere (e.g., Hellier et al., 1998). Following arrival, animals were maintained in a room with a controlled light-dark cycle (lights on from 0700-1900 h) and a constant temperature (72-74 °F). Thus, the animals were entrained with a 12 h-12 h light-dark cycle one week prior to kainate treatment, and continued in the same 12 h-12 h light-dark cycle until euthanasia. Male (n=20) and female (n=18) Sprague-Dawley rats (150-200 g, Harlan) were given hourly injections of kainate (5 mg/kg, IP). After 3-4 injections, most of the treated rats began to have

overt motor seizures (i.e., class III/IV/V), and seizure severity was scored according to a modified Racine's scale (Racine, 1972; Ben-Ari, 1985). Kainate treatment was continued until class III/IV/V seizures were elicited for ≥3 h, yielding a total dose of 20-50 mg/kg of kainate. If a rat began showing excessive inactivity (i.e., barely moving), excessive activity (i.e., exaggerated running and/or jumping), or ≥10 class IV/ V seizures/h, subsequent injections were either reduced to 2.5 mg/kg or eliminated because we have observed that these behaviors frequently precede death if treatment is not decreased (Hellier et al., 1998). Motor seizure severity was defined as follows: class III, rats displayed forelimb clonus with a lordotic posture; class IV, rats reared with concomitant forelimb clonus; and class V, rats had a class IV seizure and fell over. Class I and II seizures were not scored. All surviving rats (16% mortality during treatment) received lactated Ringer's subcutaneously and moistened rat chow as needed for the first week after treatment.

Video monitoring and seizure analysis

Following kainate treatment, animals were placed in labeled cages and video-monitored for 5-6 consecutive 24-h periods (12 h-12 h light-dark cycle). Animal behavior and seizures were recorded on 8-h videotapes from a MTI 65 Silicon Intensified Target camera with automatic gain control. Night recordings were performed with a Kodak 1A filter over a safelight, and day recordings were accomplished with diffuse fluorescent light.

Video monitoring was performed 3 months (n=32; 16 male and 16 female) and 4 months (n=23; 8 male and 15 female) after kainate treatment (i.e., 90 and 120 days after treatment, respectively), when most treated rats are observed to have spontaneous motor seizures (Hellier et al., 1998). Three months after treatment, 32 rats were video-monitored for 144 h; while at 4 months after treatment, 15 of these rats were video-monitored for 144 h and 8 rats for 120 h. Nine of the rats from the 3-month group (n=32) were euthanized for electrophysiological experiments (see Wuarin and Dudek, 1996; Buckmaster and Dudek, 1997a,b; Patrylo and Dudek, 1998) before the second period of videotaping at 4 months. Therefore, the 9 rats with several seizures were used, and hence were not available for the 4-month group (i.e., n=23). The rats used for electrophysiological experiments were therefore not necessarily the same as those animals that continued in the present study, which is why some of the comparisons for seizure frequency were made using only the 23 rats available at *both* 3 and 4 months after treatment.

Three trained observers independently viewed all videotapes and recorded motor seizure activity and behavior. Seizure activity during video monitoring was rated by the same method used during kainate treatment (i.e., class III/IV/V seizures were scored). Motor seizures were assessed from analysis of behavioral postures (e.g., lordosis, straight tail, forelimb clonus, and/or rearing) during the fast-forward speed of the video-recorder. Once a behavioral posture was seen, the videotape was rewound to the beginning of the behavior and examined at *real-time speed* (i.e., 32 frames per second).

Therefore, all motor seizures were scored at real-time speed. The first observer analyzed the 3-month data, the second observer analyzed both the 3- and 4-month data, and the third observer reviewed all videotapes after the first and second observers to ensure motor seizures were not missed and were scored correctly. All observers were unaware of the results of any other videotape analysis. If a discrepancy occurred in the number of seizures, the first or second observer re-watched the videotape to check the third observer. The same individual trained all three observers, and there was complete agreement (100%) in absolute number of seizures and a strong agreement (98%) in the timing of seizures (i.e., light versus dark periods) between the two independent observers.

The animal was scored as "inactive" if there was 30 s of little to no volitional movement (i.e., animals were considered inactive even if they displayed slight head movement), or following 30 s of apparent sleep (i.e., lying still with head down and eyes apparently closed). Spontaneous motor seizures that occurred within 30 s after arousal were also considered to be seizures during inactivity. These behaviors were combined into one group, because in many forms of epilepsy, enhanced seizure frequency occurs not only during sleep, but also upon arousal (Janz, 1962; Bazil and Walczak, 1997; Crespel et al., 1998; for reviews see: Janz, 1974; Meierkord, 1994; Hopkins, 1995; Niedermeyer, 1996; Shouse et al., 1996). Motor seizures were considered to be during activity when the animal had spent more than 30 s in apparent volitional movement (e.g., grooming, walking, and eating). Although our definition required that the animal show a specific behavior for more than 30 s preceding a seizure, post hoc

analysis revealed that most rats were in a particular behavioral state (i.e., inactive or active) for several minutes prior to the onset of a seizure.

Two male and one female rats with kainate-induced epilepsy were observed to experience status epilepticus (i.e., a motor seizure every 1-2 min for 6-8 h), and these seizures were not included in the analysis. These animals did not die during the period of status epilepticus.

Normalization of seizure frequency for proportion of time in behavioral state

As nocturnal animals, rats exhibit extended bouts of inactivity during the light period, but these inactive states occur less frequently during the dark period. In order to take into account the proportion of time spent in the two possible behavioral states as a function of the light-dark period, we considered the seizure frequency relative to the percent time spent in either inactivity or activity. The average length of time that rats spent either inactive versus active was assessed in 9 rats with kainate-induced epilepsy at every 10-min interval for each hour of a continuous 24-h light-dark cycle. The behavior exhibited at the beginning of each 10-min epoch was used as a sample to assess the relative amount of time in either behavior. Therefore, seizure frequencies determined for the 3- and 4-month groups were normalized to the average amount of time spent in either behavioral state (i.e., inactivity versus activity).

Statistical analysis

A mixed model analysis of variance (ANOVA) was used to determine statistical differences between normalized seizures during the light-dark cycle and inactivity versus activity at 3 and 4 months after treatment. Log transformations were performed on the behavioral data because variances were not homoscedastic (i.e., evenly distributed), as required for the statistical procedure (ANOVA). We designed a saturated model using the log of normalized seizures as the response, and group (rats with either a high [$n \geq 50$] or low [$n \leq 25$] number of seizures), activity level, and light-dark as categorical explanatory variables. After running the first iteration of the model, we eliminated non-significant interactions and reduced the model to include only significant interactions. Statistical significance was accepted at $P \leq 0.05$, and all categories were considered *a priori*.

RESULTS

Seizure activity during 24-h video monitoring of rats with kainate-induced epilepsy

The 32 rats with kainate-induced epilepsy in this study were observed at 3 months after treatment, and 23 of them were also observed at 4 months. Comparing only those rats studied at *both* 3 and 4 months after treatment, the average number of seizures was similar in males (i.e., 3.8 seizures/rat at 3 months and 10.5 seizures/rat at 4 months) and females (i.e., 2.2 seizures/rat at

3 months and 13.0 seizures/rat at 4 months). Accordingly, all data were analyzed across time independent of sex.

In the group studied 3 months after treatment, 75% (24 of 32) of the rats had one or more spontaneous motor seizures. The number of seizures per rat averaged 18.7±5.7 (± s.e.m.) and ranged from 1 to 81 seizures (total: 449). In the group studied 4 months after kainate treatment, 91% (21 of 23) of the rats had ≥1 spontaneous motor seizures, with an average of 13.3±6.2 seizures/animal (range: 1-128; total: 280). Comparing only those rats studied at *both* 3 and 4 months after treatment, the percent of rats having one or more spontaneous motor seizures increased from 65% at 3 months to 91% at 4 months. Likewise, when seizure frequency was compared for only the rats studied at *both* 3 and 4 months, the mean number of spontaneous seizures increased from 2.5±0.6 (±s.e.m.) at 3 months to 12.2±5.7 seizures/rat at 4 months. These results with 24-h video monitoring corroborate earlier data suggesting that seizure frequency increases as a function of time following kainate treatment (for further details see Hellier et al., 1998). The seizure history of all 32 of the rats with kainate-induced epilepsy in the present study was included in the previous report (Hellier et al., 1998).

Spontaneous seizures during the light-dark cycle

The distribution of spontaneous motor seizures during the 24-h light-dark cycle is shown in Figure 2.1. At 3 months after treatment, 288 of 449 (64%) of the seizures were observed during the light period compared to 36% during the

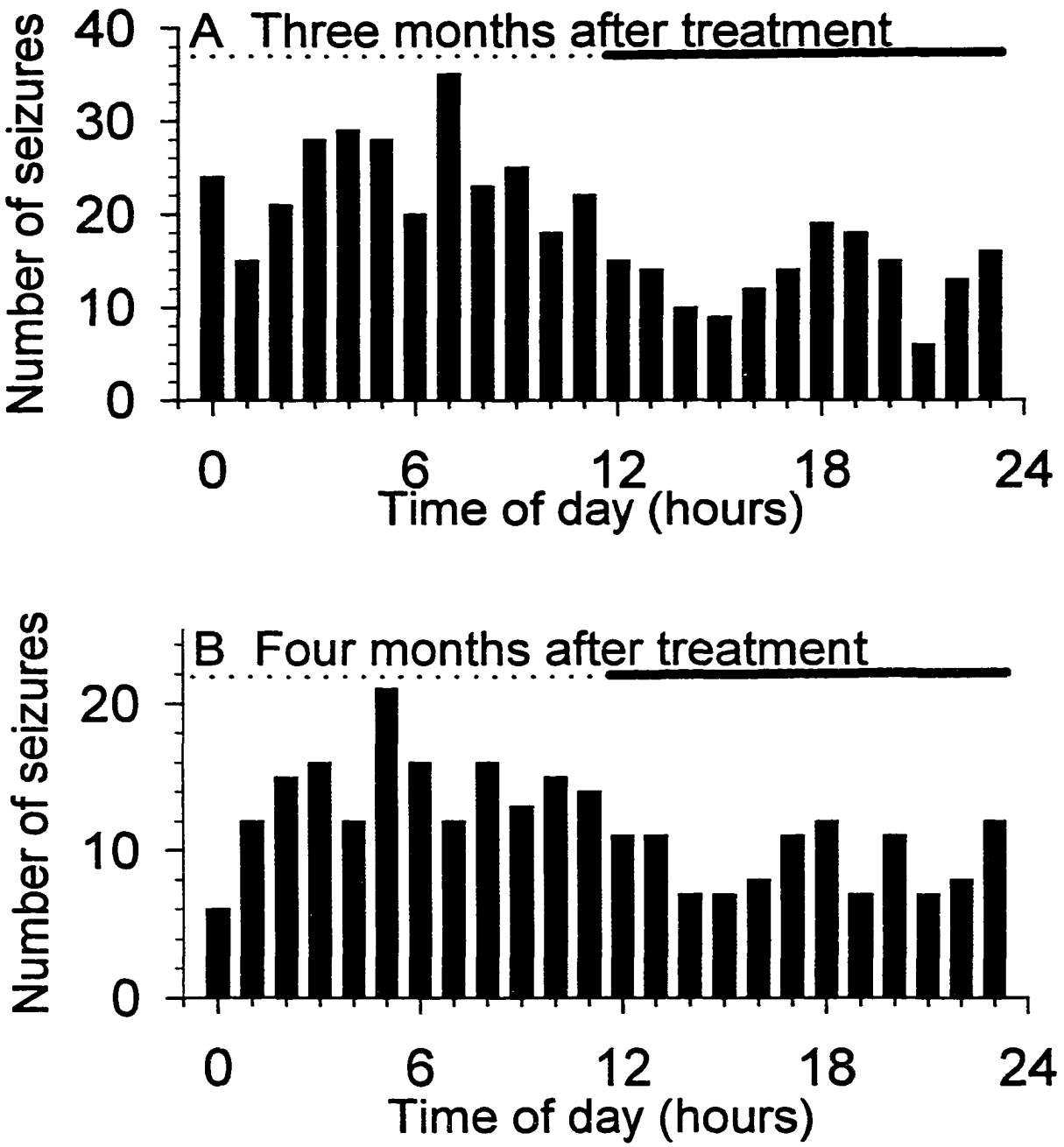

Figure 2.1: Number of spontaneous motor seizures observed in kainate-induced epileptic rats during 5-6 days of 24-h video monitoring. For this and subsequent figures, the dotted horizontal bar marks the light period; the dark horizontal bar indicates the period of darkness. All rats with spontaneous seizures were used for the analyses (n=24 at 3 months and n=21 at 4 months after treatment). (A) In the 3-month group, 64% of the motor seizures occurred during the light phase (0-11 h). (B) In the 4-month group, 60% of the spontaneous motor seizures occurred during the light period.

dark period (Fig. 2.1A). A similar effect of the light-dark cycle on seizure frequency was observed in the 4-month group where 168 of 280 (60%) of the seizures were observed during the light period (Fig. 2.1B). Although more seizures occurred during the light phase than the dark, the difference was not significant ($P>0.1$ at 3 months and $P>0.9$ at 4 months after treatment).

Spontaneous motor seizures as a function of inactivity and activity

Figure 2.2 shows the relationship between the occurrence of spontaneous motor seizures and activity states as a function of time. In the 3-month group, rats experienced more spontaneous seizures during inactivity (82%) than during the active state (18%; Fig. 2.2A). Likewise, the 4-month group had more spontaneous motor seizures during inactivity (83%) than during the active state (17%; Fig. 2.2B). These data suggest that spontaneous motor seizures are much more likely to occur during periods of inactivity in rats with kainate-induced epilepsy.

Synopsis of the raw data analysis

A summary of the relationships between spontaneous motor seizures and the light-dark cycle and inactivity versus activity is shown in Figure 2.3. Overall, a greater proportion of motor seizures occurred during the light phase and during periods of inactivity. A similar relationship was also observed when rats were grouped by a low (e.g., 1-23) or high (e.g., 50-128) number of seizures (data not shown). Although more spontaneous motor seizures occurred during the light

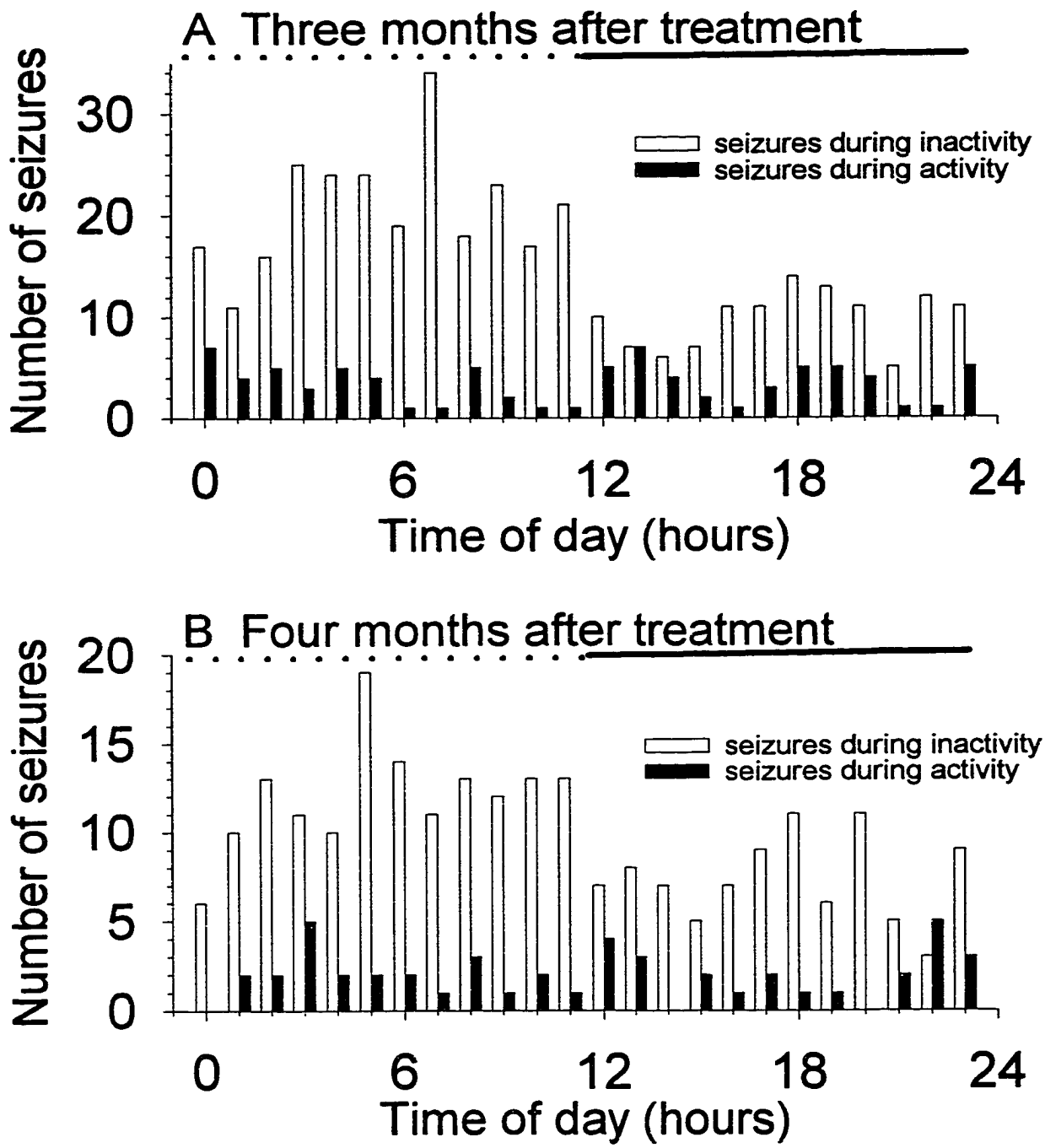

Figure 2.2: Spontaneous motor seizures during inactivity or activity. (A) Three months after treatment, more spontaneous seizures occurred during inactivity (82%) than during the active state (18%). (B) Four months after treatment, rats had more spontaneous motor seizures during inactivity (83%) than during the active state (17%). The open bars indicate seizures during inactivity; the closed bars represent seizures during activity.

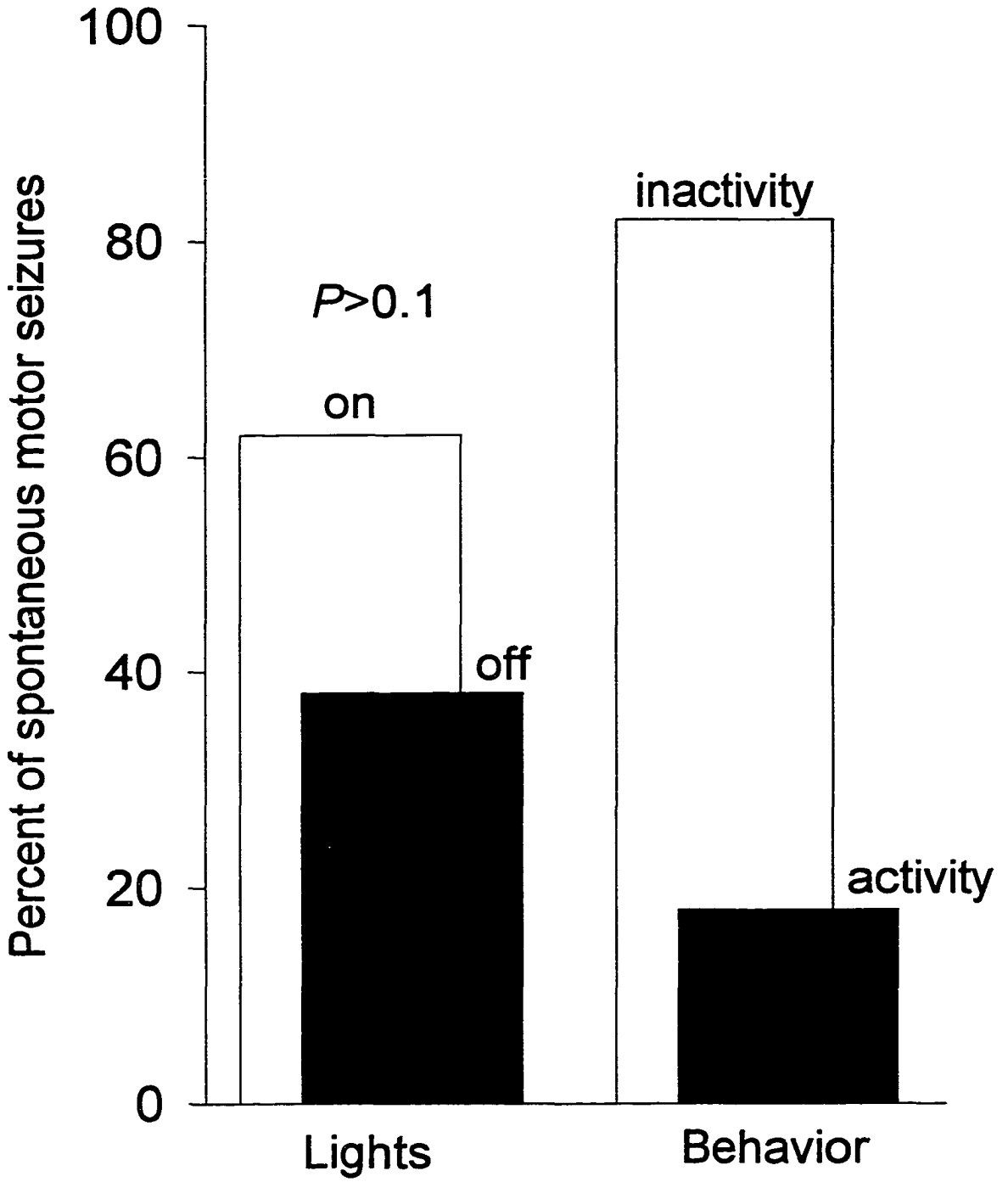

Figure 2.3: Summary of spontaneous seizures as a function of light versus dark and inactivity versus activity at 3 months after treatment. The left set of bars shows no significant difference between spontaneous motor seizures occurring in the light or dark phases. As shown in the right set of bars, more seizures occurred during inactivity (82%).

phase compared to the dark, the difference was not found to be significant (P>0.1). These data suggest that the occurrence of spontaneous motor seizures depends primarily upon the activity state of the animal and not upon the light-dark cycle.

Normalization of behavioral state

Although the data described above suggest that seizures were more likely to occur during inactivity, rats tend to spend more of their time inactive than active during the day (i.e., light period). It could be postulated that more seizures occurred during inactivity simply because the animals spent more time in this behavior. Therefore, we calculated the relative amount of time rats spent in each behavioral state (Fig. 2.4, see Materials and Methods). Rats were inactive during 82% of the light period and 40% of the dark period (Fig. 2.4A, left versus right panels). Overall, rats were inactive 61% of the 24-hr light-dark cycle (Fig. 2.4B).

Significantly more seizures occurred during inactivity

Figure 2.5 shows the percentage of spontaneous motor seizures during the light and dark periods, normalized for the duration of time spent in each behavior. When a log transformation of the normalized seizure frequency was performed (see Materials and Methods), the frequency of seizures was significantly greater during inactivity than during activity at both 3 months (P=0.0001) and 4 months after treatment (P=0.0062). In the dark period, significantly more seizures occurred during inactivity compared to activity

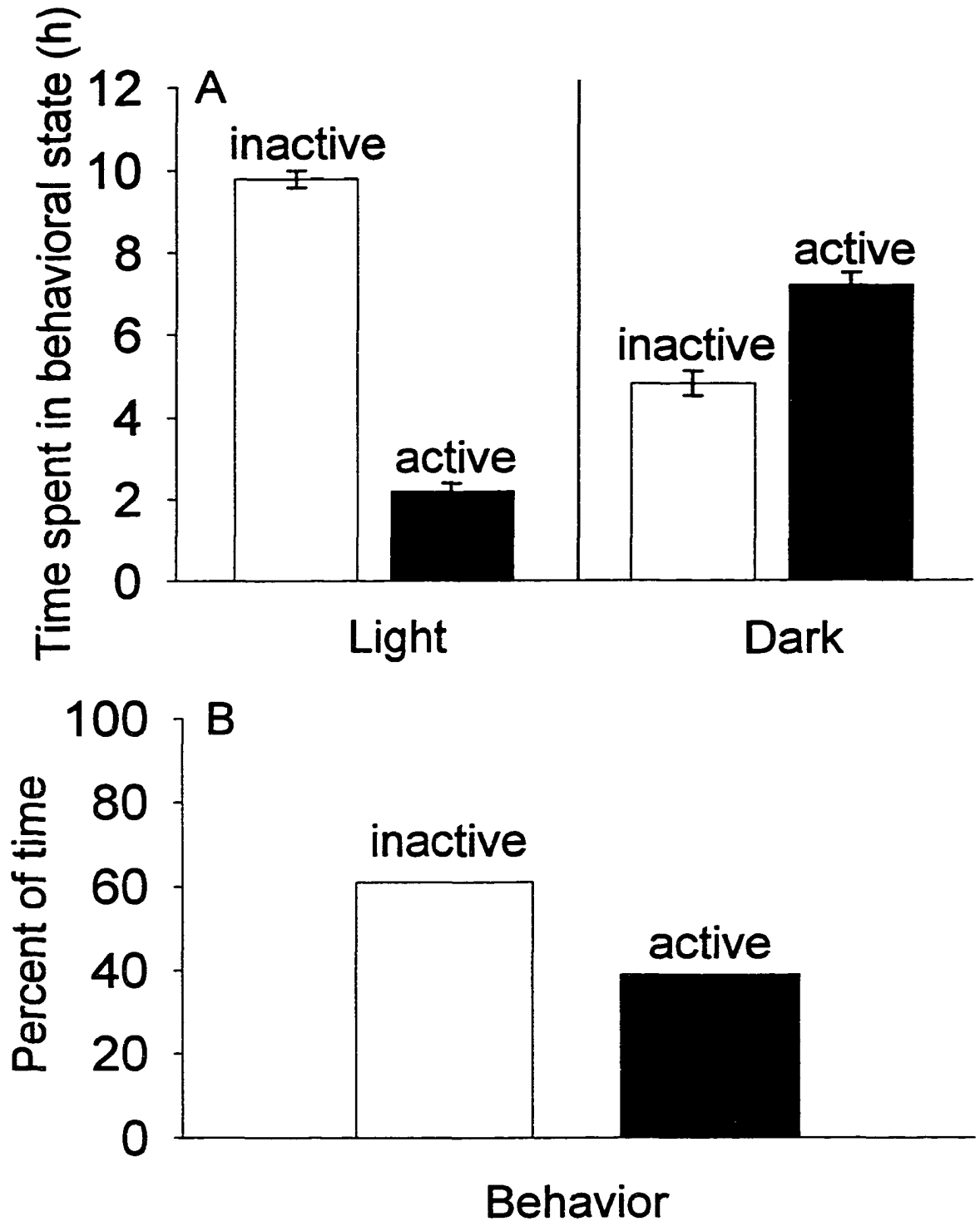

Figure 2.4: Proportion of time in different behavioral states (i.e., inactivity versus activity). (A) Rats spent more time inactive (82%) in the light phase compared to the dark phase (40%). (B) The relative amount of time rats spent in each behavioral state across the 24-h cycle.

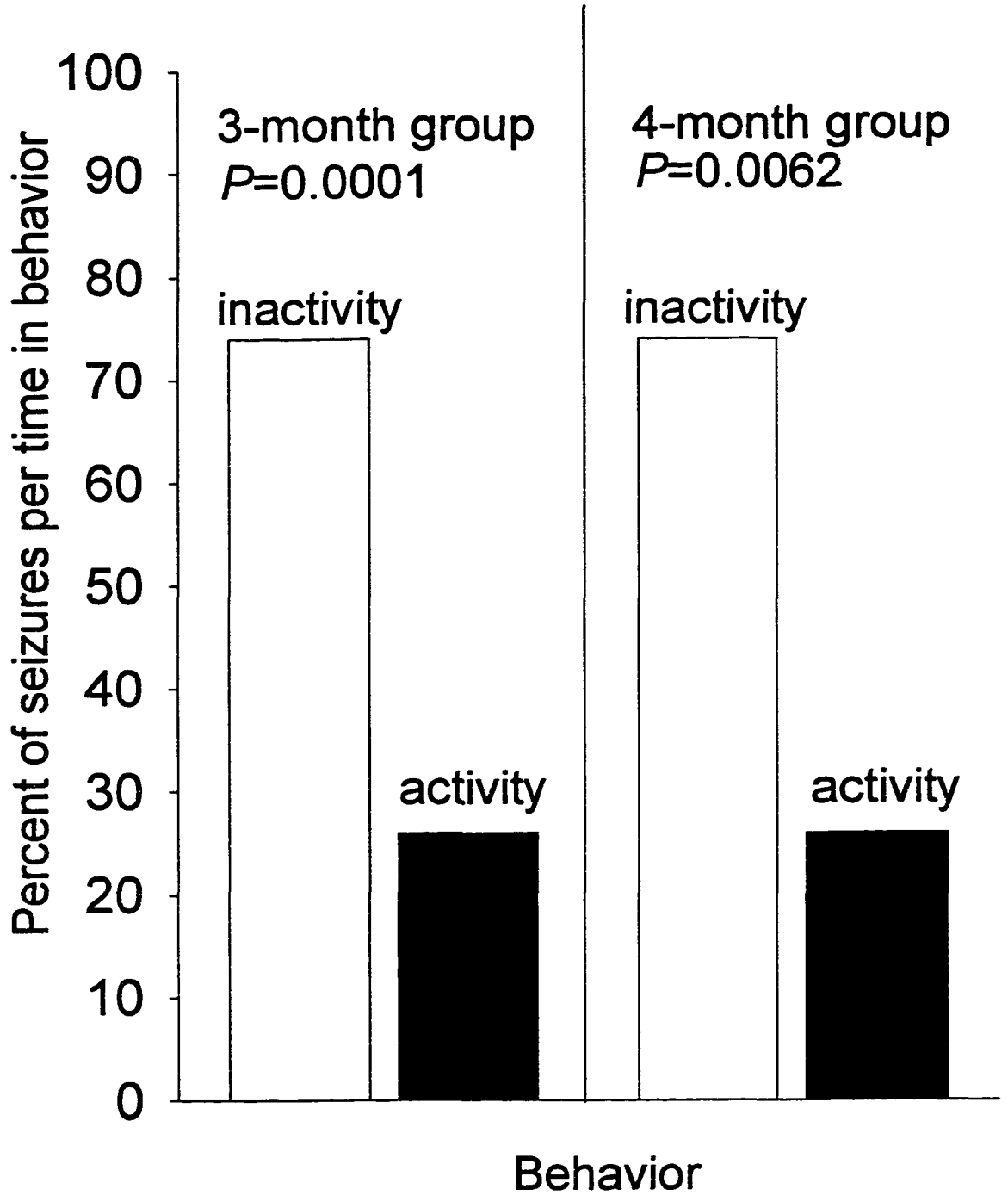

Figure 2.5: Percentage of seizures normalized for the amount of time in each behavioral state. Significantly more spontaneous motor seizures occurred during inactivity compared to activity during the 24-h light-dark cycle (see Results for analysis).

(P=0.009, see right side of Fig. 2.2), even though less time was spent in these behaviors (Fig. 2.4A, right panel). Thus, these data support the hypothesis that the occurrence of spontaneous motor seizures depends on behavioral state.

DISCUSSION

The principal finding of this study is that rats with kainate-induced epilepsy had significantly more spontaneous motor seizures during periods of inactivity. The increased frequency of spontaneous seizures was not a *direct* consequence of the amount of time spent in a behavioral state nor did it appear to depend on the light-dark cycle. Therefore, these data support the hypothesis that the occurrence of spontaneous motor seizures in the rat with kainate-induced epilepsy depends on the state of behavioral activity (i.e., active with volitional movements versus inactive, including asleep).

Differentiation between motor seizures and other behaviors

Spontaneous motor seizures were identified from other activities (e.g., grooming, eating, or arousal from apparent sleep) by employing the same scoring method used during kainate treatment (i.e., class III/IV/V seizures were rated according to the Racine scale [1972]). Several laboratories have used this classification scheme to score seizures in a wide range of studies, particularly those using the kindling model (e.g., McNamara, 1986). *Spontaneous* seizures might seem more difficult to differentiate from ongoing behaviors than seizures that would be time-locked with electrical stimulation during a kindling protocol. In

either case, however, the essential issue is distinguishing a motor seizure from "background behavior". We did not consider class I or II seizures (i.e., facial clonus or head nodding), because these are more difficult to distinguish from ongoing behaviors. This may be an under appreciated problem with other studies, particularly those that involve the kindling model. Finally, the combination of real-time video analysis, with the ability of the observer to review the putative seizures repeatedly, and multiple blind observers provided safeguards against false-positive identification of motor seizures.

Detection of motor seizures during the dark period

We have considered that we may have underestimated the number of spontaneous motor seizures during the dark period (i.e., recordings were performed with a red filter over a safelight). Although we used a Silicon-Intensified Target camera with automatic gain control, recordings in the dark still had slightly less contrast compared to the recordings from the light phase. For the same reasons that were stated above, we believe that we did not miss any motor seizures in the dark period. Each observer replayed the relevant part of the tape at real-time speed when there was any questionable behaviors that may or may not have been a seizure (e.g., straight tail, rearing, or forelimb clonus), and this was examined even more carefully during the dark period when rats tend to be more active. Because all videotapes were viewed at least twice (see Materials and Methods), we were able to compare the precise time of every seizure, as assessed by each observer. The near-perfect agreement of the seizures by

independent observers provides evidence that all motor seizures were detected during the dark period.

Normalization for behavioral state during the light-dark cycle

Human clinical studies on seizure susceptibility have focused on sleep state (e.g., Janz, 1974; Bazil and Walczak, 1997; Crespel et al., 1998) rather than the light-dark cycle. In contrast, previous reports on animal models of temporal lobe epilepsy have primarily focused on diurnal fluctuations in the frequency of spontaneous seizures (Cavalheiro et al., 1991; Bertram and Cornett, 1994; Quigg et al., 1998). One of our goals was to assess the relative importance of behavioral versus diurnal influences on seizure susceptibility. The increased occurrence of seizures during the light versus dark period was not found to be significant in our data, yet seizure frequency was significantly greater during periods of inactivity. A fundamental problem that had to be addressed, however, was the relative amount of time the rats spent active versus inactive in the light and dark periods. Was the increased occurrence of seizures during periods of inactivity merely because the animals were inactive most of the time? We evaluated the amount of time that the rats were active versus inactive by the hour throughout the 24-h day in order to control for this potential problem. Seizures were substantially more frequent during inactive periods versus active periods (i.e., 82% versus 18%, see Fig. 2.3) even when normalized for the amount of time spent in these behaviors (i.e., 74% versus 26%, see Fig. 2.5). Particularly important in this regard was the observation that more seizures

occurred during inactivity in the dark period (see Fig. 2.2, right side of histogram). This result was present even though the rats spent a greater proportion of their time in the active state while in the dark (see Fig. 2.4A, right panel). These observations suggest that behavioral state modulates seizure susceptibility in the rat with kainate-induced epilepsy, which is similar to generalized and partial epilepsy in humans (Gotze et al., 1967; Livingston and Berman, 1973; Nakken et al., 1990).

Behavioral activity and seizures

Understanding the behavioral situations associated with the increased likelihood of seizures is obviously of practical importance. For example, it appears that many patients with epilepsy are less likely to have seizures during strenuous exercise compared to low-level activity (Livingston and Berman, 1973; Engel, 1989; Nakken et al., 1990), and increased stress is hypothesized to increase the likelihood of seizures (Roth et al., 1994). Anecdotal observations of rats with kainate-induced epilepsy in the present study revealed spontaneous seizures during a wide range of behaviors (e.g., walking, exploring, whisking, eating, grooming, and fighting with cage-mate). None of these particular behaviors was clearly associated with an increased or decreased likelihood of seizures. A rigorous analysis of volitional behaviors, however, could reveal differences in their propensity to be associated with seizures.

Electroencephalographs, sleep, and seizures

In most clinical studies on humans with epilepsy, video-electroencephalographic recordings have been used to assess when seizures occur as a function of behavioral state (e.g., sleep versus wakefulness). Electroencephalographic recordings were not available for this study, so rats were considered to be inactive versus active. Our definition of inactivity clearly included sleep; our behavioral observations suggest that most but not all periods of inactivity were sleep. Thus the inactive period must be considered heterogeneous, because neuronal mechanisms involved in inactive-awake behaviors are presumably quite different from those responsible for sleep and its several stages. For years, many connections have been proposed between sleep and interictal spikes and epileptiform bursts in several types of human epilepsy and animal models of human epilepsy (e.g., Kellaway, 1985). Other animal studies have also found associations between sleep spindles and acute seizures due to pharmacological manipulations (e.g., penicillin-induced seizures in cats; Avoli and Gloor, 1981). Therefore, quantitative electroencephalographic analyses during sleep and its various stages should reveal possible conditions that promote spontaneous motor seizures in the rat with kainate-induced epilepsy.

Other studies on animal models of temporal lobe epilepsy

Relatively little work has linked spontaneous seizures in animal models of temporal lobe epilepsy (e.g., kainate- and pilocarpine-treated rats and rats subjected to electrical stimulation of limbic structures) to documented sleep or

even other behaviors or environmental conditions. The most comparable studies have shown a diurnal effect on seizures, with more motor seizures during the light phase (Cavalheiro et al., 1991; Bertram and Cornett, 1994; Quigg et al., 1998). In a recent study directed specifically at this particular issue, rats that were subjected to electrically induced limbic-status-epilepticus had more seizures during the light period (i.e., 63%, Quigg et al., 1998). The data presented here show that rats with kainate-induced epilepsy also had 60-64% of spontaneous motor seizures during the light period (see Fig. 2.1). Our analysis did not show a significant diurnal effect, but different ambient conditions (e.g., changing levels and patterns of noise in the vivarium) could have masked an otherwise significant difference. We found, however, a particularly prominent effect of behavioral state (i.e., seizures occurred mainly during periods of inactivity).

Conclusions

Data from 24-h video monitoring showed that rats with kainate-induced epilepsy had significantly more spontaneous motor seizures during periods of inactivity (82%).

CHAPTER THREE

Assessment of Hippocampal Inhibition and Epileptiform Activity in Freely-Behaving Rats during the First Week after Kainate Treatment

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ABSTRACT

Mossy fiber sprouting has been hypothesized to restore or enhance inhibition after kainate treatment (Sloviter, 1992); however, the time course in which inhibition recovers shortly after kainate-induced status epilepticus has not been well examined. Therefore, we tested the hypothesis that inhibition is restored within the first week after kainate treatment, when little if any mossy fiber sprouting has occurred in the inner molecular layer of the dentate gyrus.

Chronic in vivo recordings were performed in male Sprague-Dawley rats 1, 4, and 7-8 days after kainate (5 mg/kg; IP; n=17) or saline (n=11) treatment. In the perforant-path, a single stimulus-response series (0.05 Hz) was performed in each animal, as well as a paired-pulse stimulation protocol (0.05 Hz, 20 ms interstimulus interval) to assess synaptic inhibition. At 7-8 days after treatment, animals were either euthanized for histological studies or allowed to survive to confirm that they became epileptic.

Histological analysis revealed that kainate-treated rats had a significant decrease in the number of hilar neurons along the septotemporal axis of the hippocampus, compared to controls (P=0.013). Timm staining, however, revealed little to no mossy fiber sprouting within the hippocampus at this time. Spontaneous interictal spikes were observed as early as 1 day after kainate treatment, and these events were seen throughout the testing period in 94% of the treated rats. The first day after kainate treatment, some rats showed multiple population spikes (35% of rats), prolonged field postsynaptic potentials (PSPs,

76% of rats), and loss of paired-pulse inhibition (29% of rats) to perforant path stimulation. By 7-8 days after treatment, however, nearly all kainate-treated rats showed partial or full recovery in number of population spikes, field PSP duration, and paired-pulse inhibition. Thus, inhibition was reduced in only some of the kainate-treated rats; when inhibition was reduced, partial to full recovery occurred within 1 week, when little or no mossy fiber sprouting had developed. These results suggest that a decrease in synaptic inhibition is not a prerequisite for chronic epileptogenesis in the dentate gyrus, and that the recovery of inhibition is not correlated temporally with mossy fiber sprouting in the inner molecular layer.

INTRODUCTION

Human temporal lobe epilepsy is behaviorally characterized by a latent period between the initial injury and the onset of recurrent seizures, variability of seizure frequency within a population, and a permanent epileptic state (Engel, 1989; French et al., 1993; Spencer and Spencer, 1994). These same characteristics have been shown in kainate-treated rats, an animal model of temporal lobe epilepsy (Hellier et al., 1998). In addition to these behavioral characteristics, hippocampal volume decreases due to neuronal loss and new axon collaterals sprout into the inner molecular layer of the dentate gyrus in many humans with temporal lobe epilepsy (e.g., de Lanerolle et al., 1989; Sutula et al., 1989; Houser et al., 1990; Babb et al., 1991; Mathern et al., 1995) and in

the kainate-treated rat (e.g., Nadler et al., 1980; Cronin and Dudek, 1988; Mathern et al., 1992; Wuarin and Dudek, 1996; Buckmaster, 1997a,b). It has been hypothesized that the development of this new circuitry is responsible for the latent period to seizure onset and causes the permanent epileptic state. However, it is not clear if these new circuits are responsible for increasing or decreasing seizure susceptibility in the dentate gyrus of humans with temporal lobe epilepsy. Previous experimental data suggest that mossy fiber sprouting can cause either increased inhibition or excitation in the kainate-treated rat (Sloviter, 1991; Wuarin and Dudek, 1996; Patrylo and Dudek, 1998).

From in vivo experiments, Sloviter (1992) observed that kainate-treated rats have an initial reduction of paired-pulse inhibition 2-4 days after treatment. However, this loss of inhibition was later restored months after treatment, when robust mossy fiber sprouting was present. This result led to the hypothesis that inhibition is lost initially, and that significant mossy fiber sprouting leads to restored or increased inhibition in the dentate gyrus. This hypothesis, however, does not address why spontaneous seizures occur several months or years following a cerebral injury in humans with temporal lobe epilepsy (Engel, 1989; French et al., 1993; Spencer and Spencer, 1994).

Other models of temporal lobe epilepsy (e.g., kindling and pilocarpine treatment) also show significant mossy fiber sprouting months after treatment. However, in vivo kindling experiments revealed that inhibition was increased within 24 h following the first afterdischarge (de Jonge and Racine, 1987; Maru

and Goddard, 1987). Milgram et al. (1991) tested inhibition in vivo in kainate-treated rats (i.e., paired-pulse stimulation) and found a progressive loss of inhibition that recovered within 24 h after kainate treatment. A potential problem with this experiment was that following single injections of kainic acid, a dose of sodium pentobarbital was delivered to stop the seizures 1 h after they began. This would potentially alter the inhibition if there were residual effects of the drug treatment during the 24-h period. The mechanism responsible for the rapid recovery of inhibition is still unknown. In addition, it is also not clear whether a loss or decrease of inhibition is a critical factor in developing temporal lobe epilepsy.

In this study, we have used the rat with kainate-induced epilepsy as an animal model of temporal lobe epilepsy (Nadler, 1981; Ben-Ari, 1985). Following repeated hourly injections of kainate (i.e., 5 mg/kg/h, IP), rats consistently develop a chronic epileptic state (Hellier et al., 1998). We implanted recording and stimulating electrodes to determine spontaneous and evoked responses of both epileptiform activity and inhibition in rats during the first week after kainate or saline treatment. In this investigation, we tested the hypothesis that if inhibition is decreased after kainate treatment, it recovers within 1 week, a period during which little if any mossy fiber sprouting has developed.

MATERIALS AND METHODS

Animals

Surgery

Male Sprague-Dawley rats (175-350 g) were used for all experiments. Rats were initially injected with penicillin (60,000 units/d, subcutaneously, SC) and atropine (1 mg/kg, SC) to prevent infection and cardiorespiratory complications associated with survival surgery and general anesthesia. Subsequently, rats were anesthetized with secobarbital (5 mg/ 100 g body wt, intraperitoneally, IP). The head was placed in a stereotaxic apparatus, a midsagittal incision was made on the scalp, and the skin reflected with hemostats. Using bregma as a reference, nine holes were burred in the skull for implantation of recording, stimulating, and grounding electrodes as well as three support screws. The wire sizes and placement of electrodes for the surgery are listed in Table 3.1. All electrodes were cemented in place with dental acrylic and then gathered into a miniature connector (McIntyre Miniature Connector, Science Technology Centre, Carleton University). Animals recovered from surgery under a heat lamp, and were injected with lactated Ringer's (3-5 ml, SC). A Tylenol-codeine elixir (3 ml / 250 ml H_2O) and penicillin (60,000 units/d, SC) were provided for 3 days after surgery.

Kainate treatment

Kainate treatment has been described in detail elsewhere (e.g., Wuarin and Dudek, 1996; Hellier et al., 1998; Patrylo and Dudek, 1998). Briefly, rats

Table 1: Type and location of implanted electrodes as referenced by bregma. Depth of electrode was measured below dura unless otherwise mentioned. Electrodes were made of stainless steel, Teflon-coated wire.

Electrode	Hemisphere	Lateral	Caudal	Depth	Wire size
Depth recording (dentate gyrus)	Right	2.5 mm	4.0 mm	2.7-2.9 mm	0.003"
EEG recording	Left	1.0 mm	4.0 mm	on dura	0.007"
Stimulating (angular bundle)	Right	4.1-4.3 mm	7.8-8.2 mm	2.5-3.5 mm	0.0045"
Stimulating indifferent	Left	4.4 mm	8.0 mm	on dura	0.007"
Electrode	**Hemisphere**	**Lateral**	**Rostral**	**Depth**	**Wire size**
Ground	both	2.0 mm	3.5 mm	on dura	0.013"

were given hourly injections of kainate (5 mg/kg, IP) or saline, and seizure activity was rated according to a modified Racine's scale (i.e., class III, IV, and V seizures; Ben-Ari, 1985; Racine, 1972). To minimize mortality, injections were reduced or eliminated if an animal showed excessive inactivity or activity. After the first injection, rats (n=17 kainate and n=11 saline) were placed in the recording chamber to record electrographic epileptiform activity induced by kainate treatment. Electrographic recordings were also made from saline-treated rats. These sessions were performed throughout the injection series, and data were collected for ≥30 minutes. Kainate treatment was continued until class IV and V seizures were elicited for ≥3 h. Rats were then injected with lactated Ringer's (3-5 ml, SC), and given apple slices for the first week after treatment.

Video monitoring and seizure analysis

Directly following kainate treatment, 24-h continuous videotaping was performed as described earlier (Hellier and Dudek, 1999). Briefly, 26 kainate- (n=12 implanted and n=14 non-implanted) and 12 saline-treated (n=4 implanted and n=8 non-implanted) rats were placed in labeled cages and video-monitored for seven consecutive 24-h periods (12 h-12 h light-dark cycle). Animal behavior and seizures were recorded on 8-h videotapes from a MTI 65 Silicon Intensified Target camera with automatic gain control. Night recordings were performed with a Kodak 1A filter over a safelight, and day recordings were accomplished with diffuse fluorescent light.

Two trained observers independently viewed all videotapes and recorded motor seizure activity and behavior. Seizure activity during video monitoring was

rated by the same method used during kainate treatment (i.e., class III/IV/V seizures were scored). Motor seizures were assessed from analysis of behavioral postures (e.g., lordosis, straight tail, forelimb clonus, and/or rearing) during the fast-forward speed of the video-recorder. Once a behavioral posture was seen, the videotape was rewound to the beginning of the behavior and examined at *real-time speed* (i.e., 32 frames per second). Therefore, all motor seizures were scored at real-time speed.

Electrophysiology

Chronic in vivo recordings

Rats were placed in a recording chamber and the electrodes connected to a commutator system that allowed the animal to move freely. Rats were monitored both behaviorally and electrographically for 1-3 h during each recording session. Baseline electrophysiological recordings were performed 5-7 days after surgery. Spontaneous activity and single- and paired-pulse responses to perforant path stimuli were studied in all rats. Chronic in vivo recordings (dentate gyrus field potentials and surface electroencephalograms) were performed before and 1, 4, and 7-8 days after treatment. The electroencephalogram (EEG) and dentate field responses were filtered (0.5 Hz -10 kHz), amplified (100x), displayed on an oscilloscope, digitized, and stored on videotape and computer for later analysis. A perforant-path stimulus-response series was performed using single (0.05 Hz) and paired-pulse stimuli (20 ms interstimulus interval at 0.05 Hz) to assess inhibition. At 7-8 days after

treatment, animals were either allowed to survive to confirm that they became epileptic or euthanized for histology.

Data Analysis

All data analyses were performed using pCLAMP software (pCLAMP6, Axon Instruments). Traces used in the analyses were an average of ten responses. The length of the field PSP was measured from the stimulus artifact to the time when the field PSP crossed the baseline. Population spike amplitudes were calculated by taking the mean of the fast descending and the fast ascending components. Only those population spikes with distinct fast components were used for data analysis. Paired-pulse index was calculated by taking the ratio of the amplitudes of the population spike from the second response by the first response. A paired-pulse index ≤ 1 was used to determine that inhibition was intact compared to a paired-pulse index >1 which was used to determine that the response was facilitated.

Anatomy

Staining

Timm staining was used to visualize mossy fiber sprouting in the inner molecular layer while cresyl violet staining was used to quantify hilar cell populations. Animals were transcardially fixed with 0.37% sodium sulfide, then with 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed, hemisected, and post-fixed overnight at 4 °C. Both hippocampi were extracted

from the neocortex and placed in 30% sucrose in 0.1 M phosphate buffer for cryo-protection. Each hippocampus was then sectioned transversely (30 μm) with a sliding microtome, and alternate sections (i.e., every 10th and 11th sections) were mounted on slides and allowed to air dry overnight. Alternate sections were processed with cresyl violet stain or a modified Timm staining protocol (Babb et al., 1991). The Timm developer consisted of 180 ml of 50% gum arabic, 30 ml of 2.25 M citrate buffer, 45 ml of 0.5 M hydroquinone, and 50 ml of 0.04% silver lactate. The sections were allowed to develop for 70-90 min, and then rinsed with water, counter-stained with cresyl violet, dehydrated, and coverslipped.

Quantitative analysis

Hilar cell counts and mossy fiber sprouting were separately analyzed without the observer's knowledge of the experimental treatment. Estimation of the hilar cell population was calculated using the optical fractionator method (West et al., 1991) with the computer software Stereo Investigator 3.12 (MicroBrightField Inc., Colchester, Vermont). Total section thickness was used to determine dissector height (i.e., at least a 2-μm border from the bottom of the section), and only "caps" were counted. Caps were defined as large nuclei with diffuse chromatin containing a nucleolus that came into focus while focusing through the dissector height; only those caps within the counting frame were counted. The counting frame was previously determined by a pilot study (i.e., 65 μm x 65 μm). Counting frames were randomly distributed throughout the

hilus, according to the method described by West et al. (1991). The hilus was determined by: 1) drawing a line directly next to the granule cell layer from the tip of the inner blade to the tip of the outer blade, and 2) extending the line from both tips to the proximal end of the CA3 pyramidal cell layer. For sections with severe loss of CA3 pyramidal cells, the proximal end of the CA3 was estimated by observing patterns of gliosis and remaining neurons that were different in CA3 compared to the hilus.

Mossy fiber sprouting was analyzed by determining the amount of dark reaction product in the inner molecular layer of the dentate gyrus. The 0-3 scoring method designed by Tauck and Nadler (1985) was used to rate the amount of sprouting. A Timm score was applied to the inner blade, apex, and outer blade of each section, and an average Timm score was calculated (saline: n=203 sections, kainate: n=274 sections). The mean Timm scores were then used for statistical analysis between hippocampi of control and kainate-treated rats.

Hilar cell populations and the amount of Timm staining in the inner molecular layer was compared between saline- and kainate-treated rats along the septotemporal axis of the hippocampus. Because there is a large variability between hippocampal lengths, the data were normalized by recording each section position as percent of the distance from the septal pole (e.g., 0-9.9%, 10-19.9%, etc.).

Statistics

A power analysis was performed to determine the number of animals needed for a significant difference between means, with a 90% confidence that the p-value would be 0.05 for all statistical tests. Using means and standard deviations from a modest-sized group of implanted rats (e.g., n=10) to run the power analysis, it was calculated that we needed: i) 12 rats to determine if the number of population spikes and the length of the field PSP before kainate treatment was significantly different from 1 day after treatment (Power=0.926 and 0.939, respectively); ii) 13 rats to determine if the number of population spikes before kainate treatment was significantly different from 7 days after treatment (Power=0.911); and, iii) 16 rats to determine if the length of the field PSP before kainate treatment was significantly different from 7 days after treatment (Power=0.906). Analysis of variance (ANOVA) with multiple comparisons was performed to determine if significant differences in field responses (i.e., length of field PSP, number of population spikes, and paired-pulse index) existed between pretreatment and 1, 4, and 7 days after treatment. Student's t-test or analysis of variance with multiple comparisons was used to determine significant differences between estimations in the number of hilar cells as well as the amount of Timm stain in the inner molecular layer of the dentate gyrus. A linear regression was used to determine the relationship between the percent of recovery versus mean Timm score. Significance for all tests was accepted when $P<0.05$, and all interactions were performed *a priori*.

RESULTS

Epileptiform activity and status epilepticus during kainate treatment

After the first injection, rats (n=17 kainate and n=11 saline) were placed in the recording chamber to record electrographic activity. Each rat was recorded for ≥30 min during three or four sessions throughout the injection series. Therefore, electrographic seizure activity was recorded for 1.5-2 h in each rat. The 1.5-2 h recording sessions were analyzed in 12 kainate-treated rats to estimate the frequency of non-convulsive and convulsive seizures. The mean seizure frequency of kainate-treated rats was 18.9±2.4 (±S.E.M.) seizures/h during treatment (range: 11.3–41.1). No saline-treated rats were observed to have seizures during treatment.

Kainate-induced electrographic non-convulsive and convulsive seizures were recorded in the granule cell layer of the dentate gyrus (Fig. 3.1). This electrographic epileptiform activity, however, was not associated with a specific class of behavioral seizure (i.e., similar electrographic seizure events occurred during both non-convulsive and class III/IV/V seizures). The EEG electrode (located on the surface of the dura mater) did not detect non-convulsive seizures. However, the EEG electrode recorded repetitive sharp waves during convulsive seizures (Fig. 3.1B). These 1-Hz waveforms with amplitudes of 1-2 mV coincided with the ictal discharge in the dentate electrode, suggesting that seizures were generated and spread from the limbic structures to the neocortex.

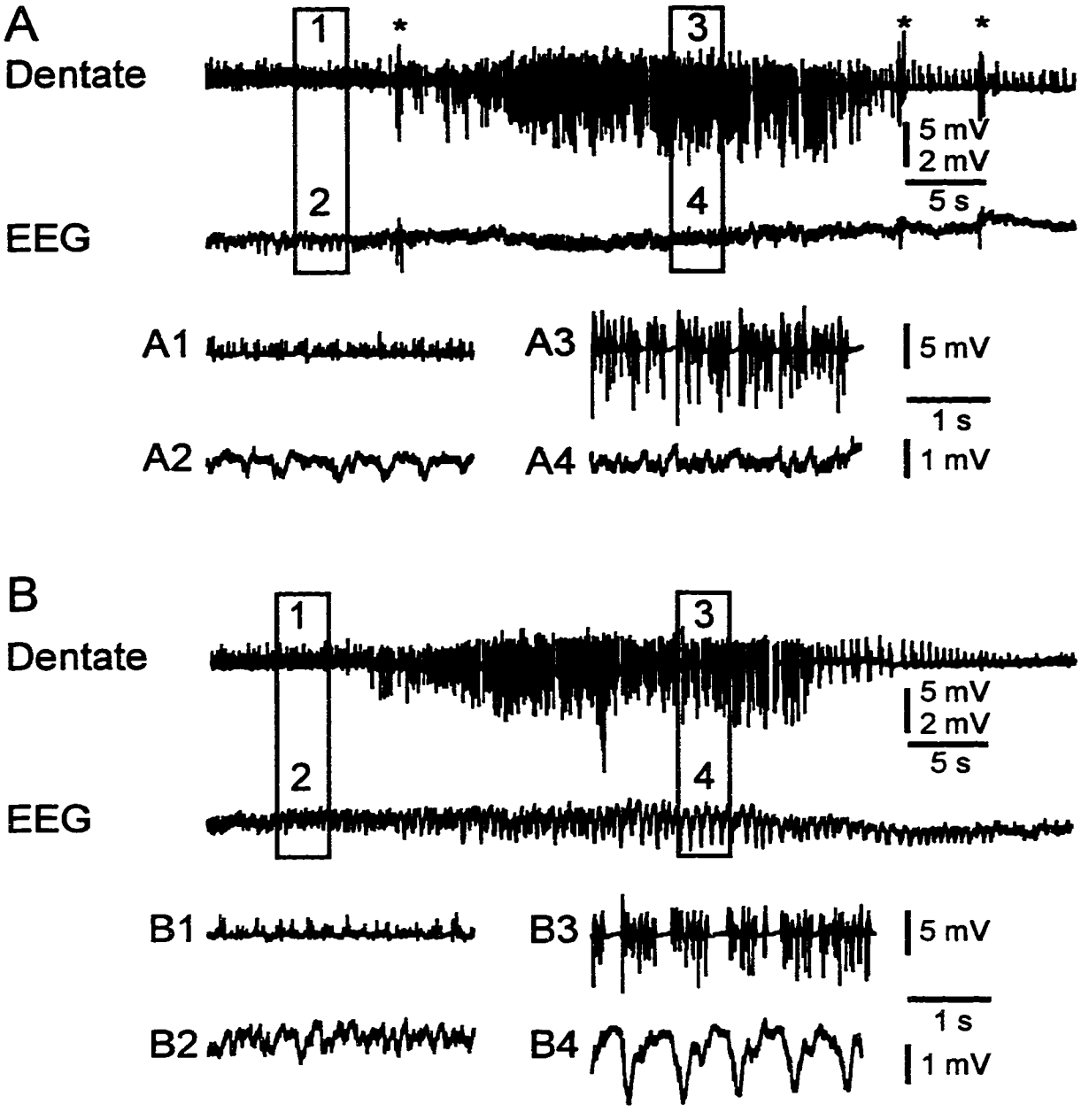

Figure 3.1: Granule cell layer (dentate) and surface (EEG) recordings of non-convulsive and convulsive seizures during kainate treatment. (A) Non-convulsive seizure during kainate treatment. In the expanded traces, A3 and A4, seizure activity is shown in the dentate electrode, but it is absent in the EEG trace. (B) Stage IV motor seizure during kainate treatment. Note the epileptiform activity in the expanded dentate and EEG recordings (B3 and B4, respectively). All dentate traces are of the same gain (calibration 5 mV). The EEG traces are calibrated at 1 mV (expanded traces) or 2 mV. The top two traces in each panel are of similar time scale (5 s) while the numbered traces are at a faster time scale (1 s).

During kainate treatment, rats experienced electrographic status epilepticus 4-5 h after the first injection (Fig. 3.2). This activity was recorded in both electrodes. In the dentate, the status epilepticus consisted of non-convulsive ictal activity (2.5-10 Hz) with episodic convulsive ictal events (10-30 Hz). Similarly, the EEG electrode recorded repetitive sharp-waves (2.5-5 Hz) that coincided with the non-convulsive and convulsive ictal discharges in the dentate. Following a motor seizure, non-convulsive ictal events persisted until the onset of the next motor seizure. Therefore, this activity was classified as electrographic status epilepticus. Months after treatment, all of the kainate-treated rats (n=8) allowed to survive beyond the first week were observed to have spontaneous motor seizures. Thus, by definition, these rats became epileptic.

Hilar neuron loss and Timm staining in the inner molecular layer

Hilar neuron populations and mossy fiber sprouting were quantified throughout the septotemporal axis of the hippocampus of kainate-treated (n=9) and control rats (n=6). The observer was unaware of the experimental treatment during the analyses of the hippocampi. The region used to quantify hilar neurons was defined by a line drawn along the inner margin of the granule cell layer from the tips of the inner and outer blades to the proximal end of CA3 (Fig. 3.3). Timm staining in the inner molecular layer was analyzed by using the scale of Tauck and Nadler (1985, see Materials and Methods). For estimating hilar neuron populations, 275 sections from kainate-treated rats (n=8) and 206 sections from

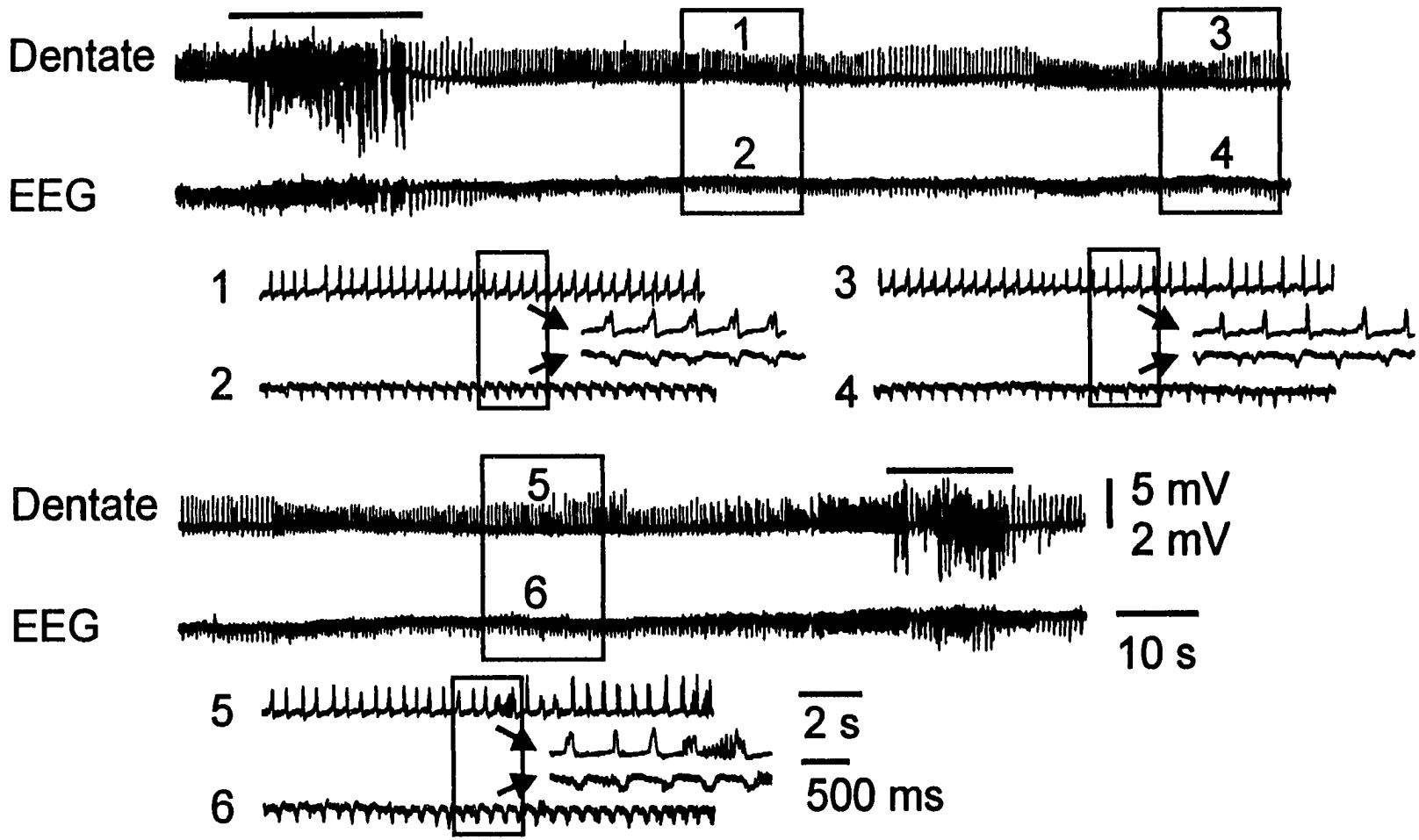

Figure 3.2: Electrographic status epilepticus during kainate treatment. The dentate and EEG traces are continuous. Stage IV motor seizures that were recorded at both the beginning and end of the traces are marked with a bar. Between the motor seizures, non-convulsive ictal activity with a frequency of 2.5-10 Hz was observed in both the dentate and EEG recordings. All dentate traces are of the same amplitude (calibration 5 mV), as are the EEG traces (calibration 2 mV). The dentate and EEG traces are of similar time scale (10 s). The numbered traces are at a faster time scale (2 s), and the arrows point to traces with the fastest time scale (500 ms).

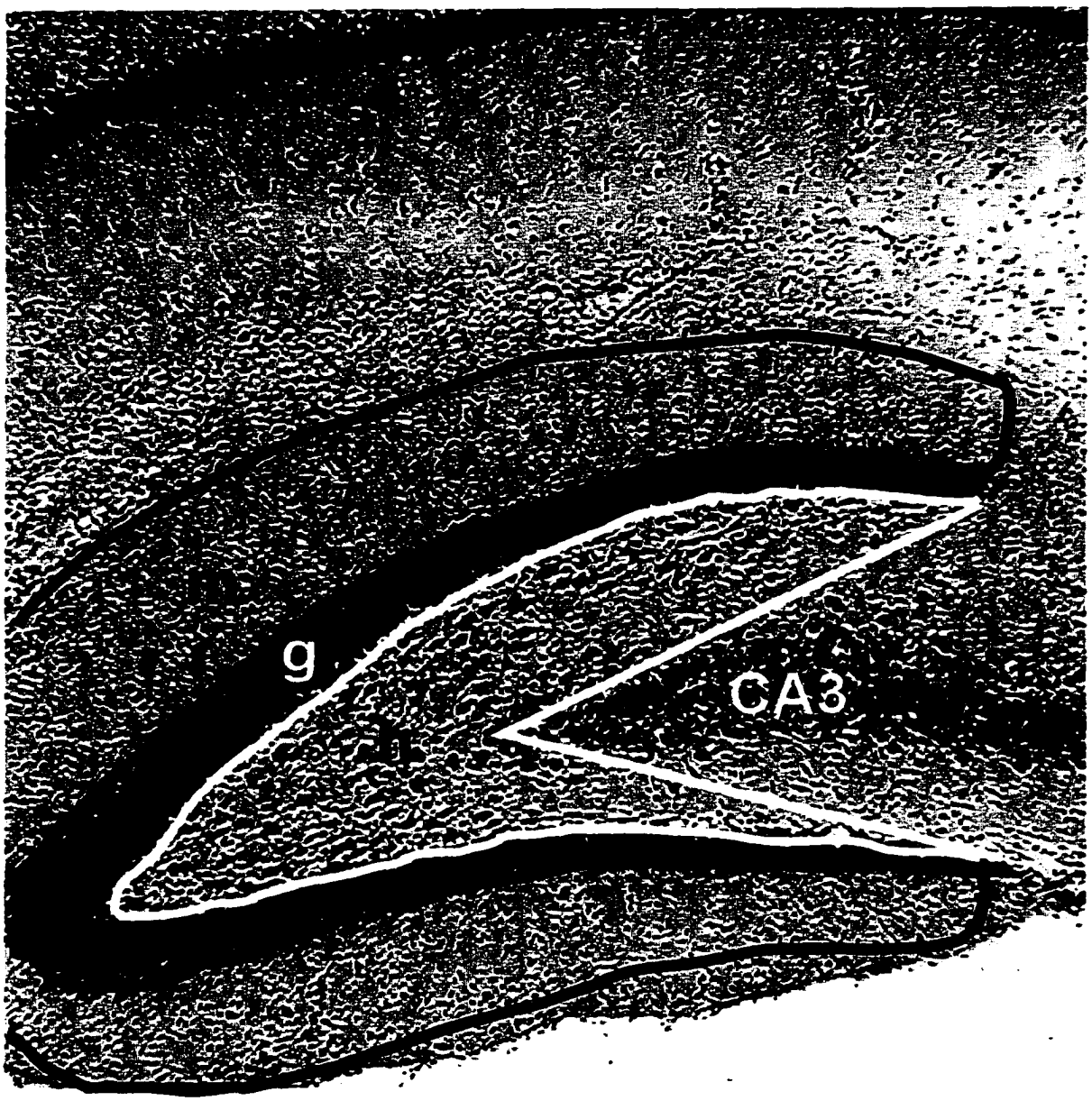

Figure 3.3: Regions used for counting neurons and analyzing Timm stain. The hilus was determined by: 1) drawing a line along the inner margin of the granule cell layer from the tip of the inner blade to the tip of the outer blade, and 2) extending the line from both tips to the proximal end of the CA3 pyramidal cell layer (white line). Abnormal Timm staining was scored as the amount of dark reaction product in the inner molecular layer and through the granule cell layer (black line).

controls (n=6) were used. Similarly, 274 sections from kainate-treated rats (n=9) and 203 sections from saline-treated rats (n=6) were analyzed for abnormal Timm staining in the inner molecular layer.

Cresyl violet-stained hippocampi from saline- and kainate-treated rats 7 days after treatment are illustrated in Figure 3.4. Hippocampi from control animals revealed a mixed population of neurons in the hilus, and the CA3 pyramidal cell layers were tightly packed (Figs. 3.4A-D). In contrast, hippocampi from kainate-treated rats had fewer neurons in the hilus and showed extensive gliosis. The greatest difference in the number of hilar neurons was observed in sections from the temporal end of the hippocampus (Fig. 3.4D vs. 3.4H). Cresyl violet staining also revealed neuron loss in the CA3 pyramidal layers of most kainate-treated rats (Figs. 3.4E-H); however, these neurons were not quantified.

Through the septotemporal axis of the hippocampus, saline-treated rats revealed intense Timm staining in the hilus that extended to the proximal dendrites of the CA3 at 7 days after treatment (Fig. 3.5A). There was little to no dark reaction product in the inner molecular layer of the dentate gyrus. Control rats had 201 of 203 (99%) hippocampal sections with a Timm score of 0 or 1 (Fig. 3.5B). The mean Timm score was 0.06 ± 0.04 ($\pm$ S.E.M.) in saline-treated rats. Timm staining in kainate-treated rats was primarily concentrated in the hilus; however, an occasional strand of reaction product projected through the granule cell layer and into the molecular layer. Kainate-treated rats had 96% (262 of 274) of sections with a Timm score of 0-1 in the inner molecular layer,

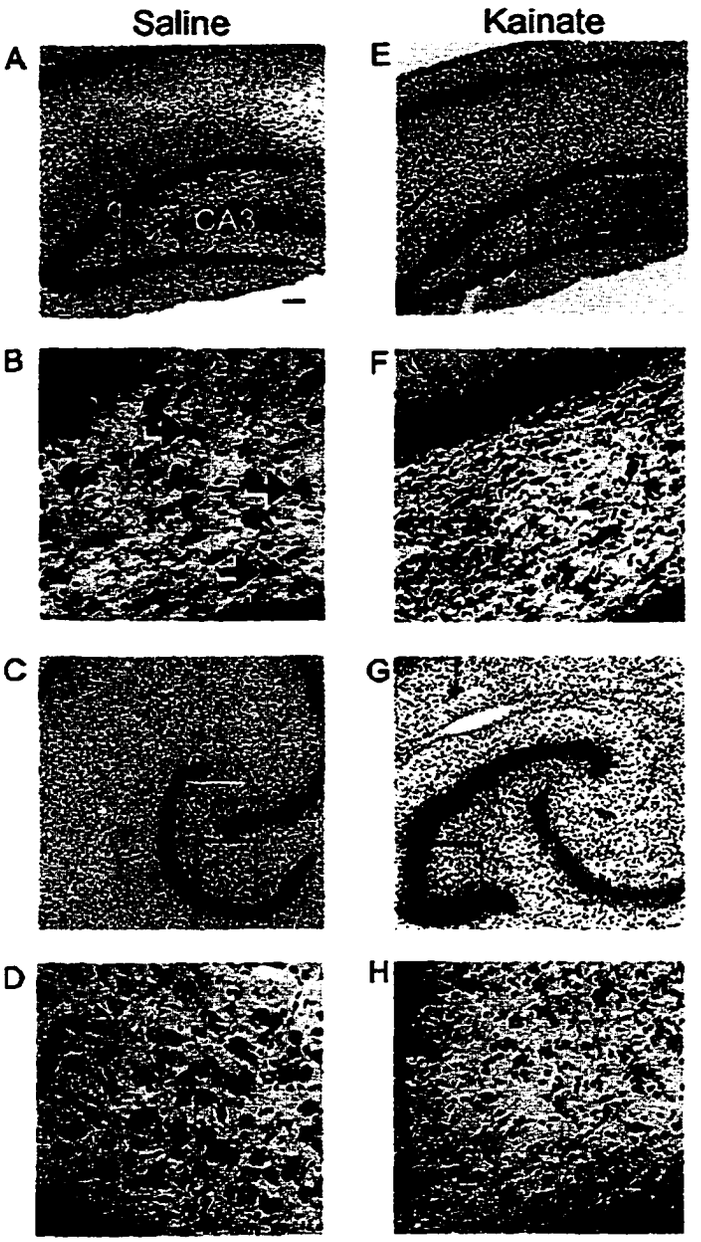

Figure 3.4: Cresyl violet-stained hippocampal sections of saline- (A-D) and kainate-injected rats (E-H) 7 days after treatment. Sections from the septal pole of the hippocampus (A,B,E,F) show partial loss of hilar neurons in a kainate-treated rat (E,F). Severe loss of hilar neurons occurred at the temporal pole in the kainate-treated rat (G,H). Note the neurons in the hilus and the tightly packed CA3 pyramidal cell layer in the control rat compared to the prominent gliosis in the hilar region and the loss of CA3 pyramidal cells in the kainate-treated rat. The arrows point to neurons located in the hilus, and the boxed regions are magnified. Molecular layer, m; granule cell layer, g; hilus, h; CA3 pyramidal cell layer, CA3. Scale bar: 100 μm in A, C, E, and G; 10 μm in B, D, F, and H.

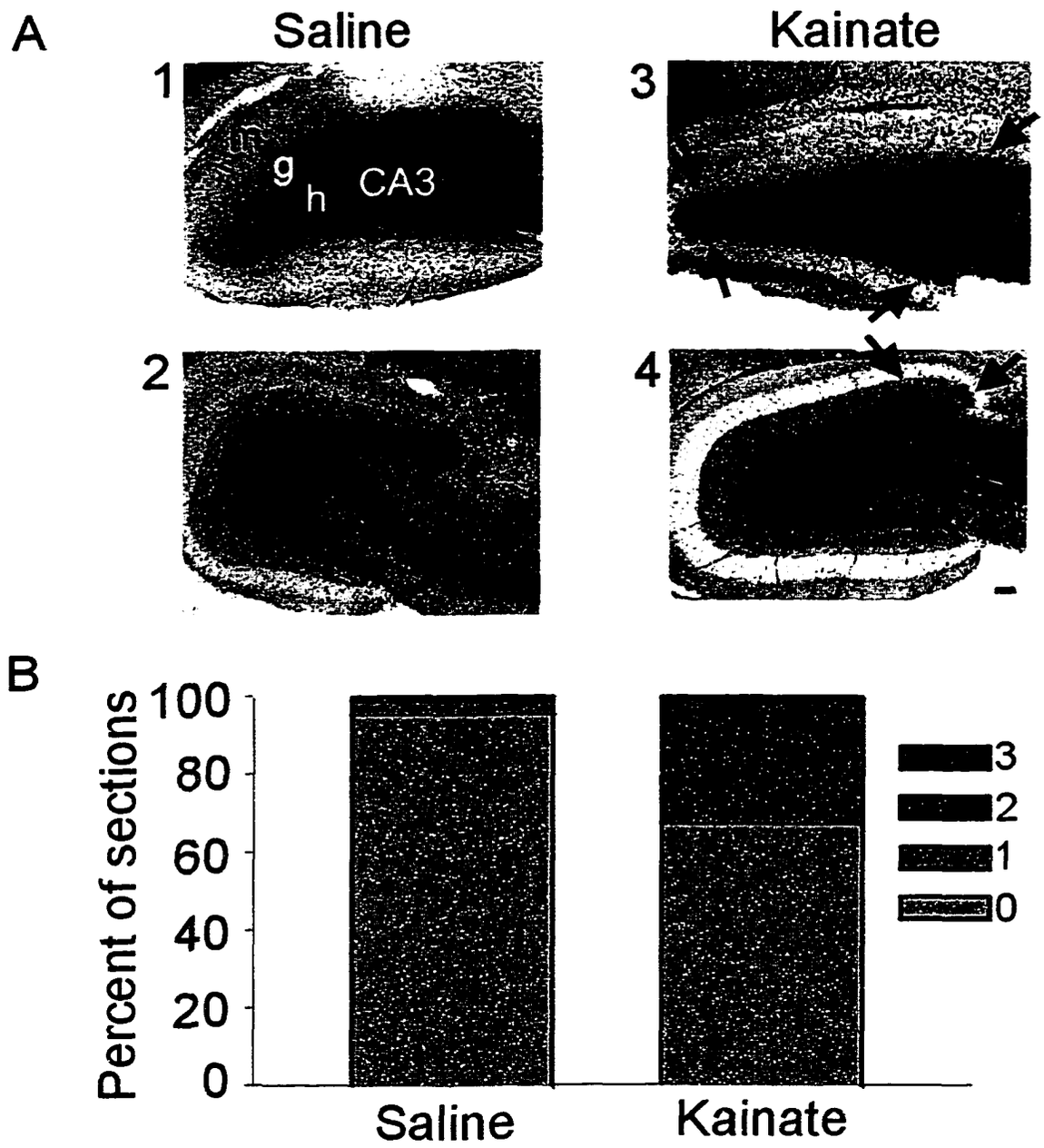

Figure 3.5: Timm- and cresyl violet-stained hippocampal sections of saline- and kainate-injected rats 7 days after treatment. Sections from a control rat at the septal (A1) and temporal poles (A2) of the hippocampus lack dark reaction product in the inner molecular layer of the dentate gyrus. In a kainate-treated rat, the septal (A3) and temporal sections (A4) have little Timm staining in the granule cell layer and molecular layer. The arrows indicate dark reaction product in the inner molecular layer. Scale bar: 100 μm. (B) In both controls and kainate-treated rats, most hippocampal sections (99% and 96%, respectively) had a Timm score of 0 or 1 (Tauck and Nadler, 1985).

and the mean Timm score was 0.4 ± 0.15 (± S.E.M.). Therefore, very little Timm reaction product was observed in the inner molecular layer in both control and kainate-treated rats.

To compare neuronal loss observed in rats months after kainate-treatment (Mathern et al., 1992; Buckmaster and Dudek, 1997a,b) with our rats at 7 days after kainate treatment, we counted hilar neurons throughout the hippocampus using the optical fractionator method (West et al., 1991). The sections were binned by percent distance from the septal end to determine hilar population and mean Timm score (see Materials and Methods). Hippocampal sections from controls had few hilar neurons at the most septal end with progressively more neurons at the temporal pole. In contrast, kainate-treated rats had fewer neurons per section, and the difference was significant in sections at the temporal pole ($P<0.01$, ANOVA, Fig. 3.6A). Kainate-treated rats displayed more sections with Timm reaction product in the molecular layer compared to controls (Fig. 3.6B). The extent of abnormal Timm staining, however, was significant only at the extreme temporal end ($P=0.0001$, ANOVA). We further normalized the data by grouping the sections into thirds (i.e., 0-33%, 34-66%, and 67-100%) and recalculating the averages. With the new bins, hilar neuron counts in kainate-treated rats were significantly smaller compared to controls throughout the septotemporal distance ($P<0.005$, Student t-test, Fig. 3.6C). There was also a significant increase in abnormal Timm staining in sections from kainate-treated rats compared to controls ($P<0.0005$,

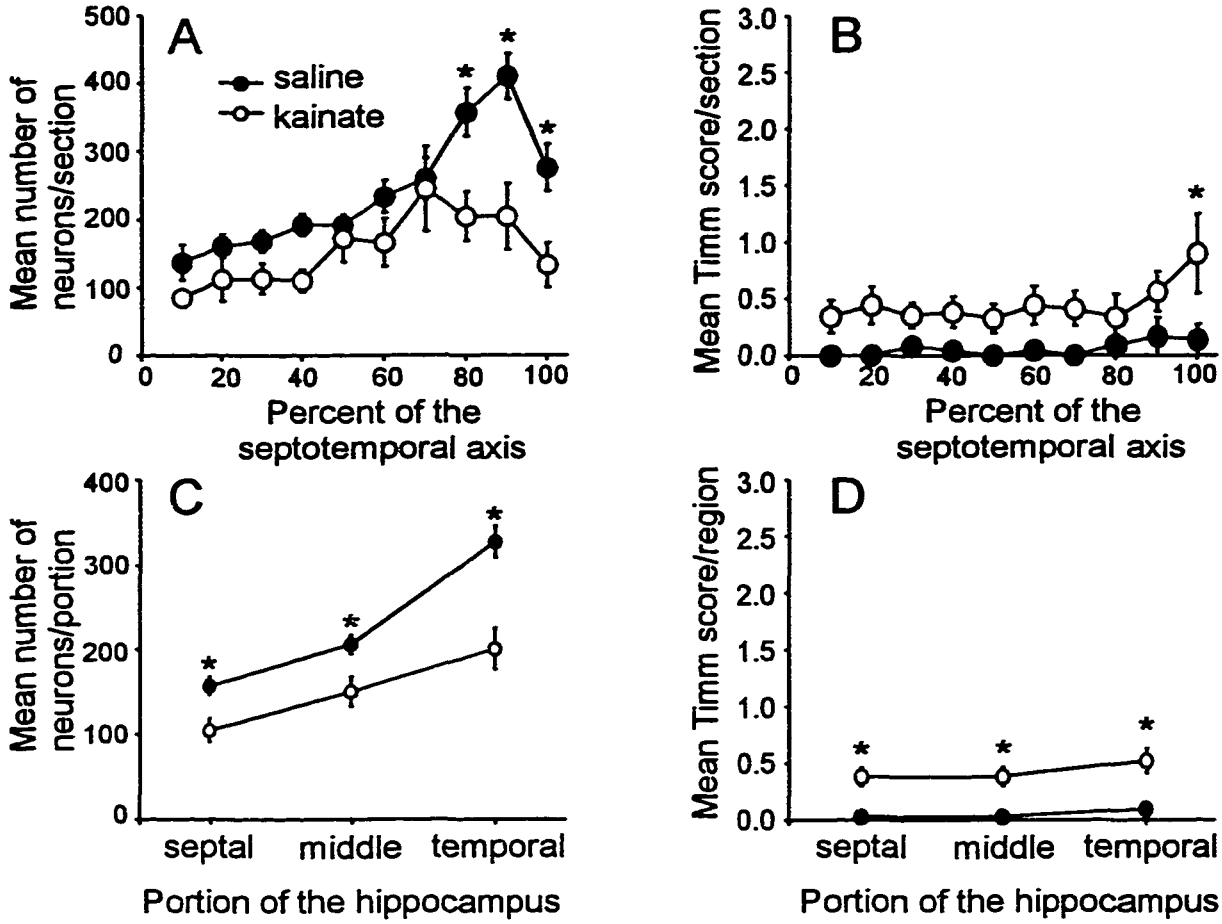

Figure 3.6: Septotemporal distribution of hilar neurons and Timm staining in control (n=6, closed circles) and kainate-treated rats (n=8, open circles). (A) Saline-treated rats had more cresyl violet-stained neurons in the temporal pole (100% septotemporal distance) compared to the septal pole (0% septotemporal distance). Kainate-treated rats had similar number of neurons near the septal pole; however, the number of neurons significantly decreased in sections approaching the temporal pole ($P<0.01$, ANOVA). (B) Kainate-treated rats had more dark reaction product in the granule cell layer and inner molecular layer compared to control rats. Abnormal Timm staining was significantly increased only at the most temporal end of the hippocampus ($P=0.0001$, ANOVA). (C) When the data were further normalized (i.e., 0-33%, 34-66%, and 67-100% of septotemporal distance), there were significantly fewer neurons in all three regions of the hippocampi in kainate-treated rats ($P<0.005$, Student t-test). (D) Similarly, the difference in the mean Timm score between controls and kainate-treated rats was small but significantly different ($P<0.0005$, Student t-test). Error bars represent standard errors of the mean, and at some cases are smaller than the data point symbol. The asterisks represent significant differences between data point pairs.

Student t-test, Fig. 3.6D). Although our kainate-treated rats had neuronal loss at 7 days after treatment, they also had modest Timm staining in the inner molecular layer compared to rats with kainate-induced epilepsy (e.g., Buckmaster and Dudek, 1997a,b).

Spontaneous motor seizures observed during 24-h video monitoring

To assess the latent period between kainate treatment and the first spontaneous motor seizure, rats were video-monitored (n=26 kainate and n=12 saline) for seven consecutive 24-h periods, beginning immediately after treatment. Twenty-one of 26 (81%) kainate-treated rats had no behavioral seizures (seizure frequency = 0.0 seizure/h) for the first week after treatment (Fig. 3.7A). The remaining five rats had at least one behavioral motor seizure during the first post-treatment week (seizure frequency = 0.006-0.095 seizure/h). Of these five animals, two rats experienced motor seizures only within the first 27 h after treatment. The remaining three rats exhibited their first motor seizure at 5-7 days after treatment (Fig. 3.7B). For most kainate-treated rats, the latent period between the initial injury and first spontaneous motor seizure is > 7 days after treatment. These results with 24-h video monitoring corroborate previous data that suggest the latent period is longer in kainate-treated rats than in other animal models of temporal lobe epilepsy (for further details see Hellier et al., 1998).

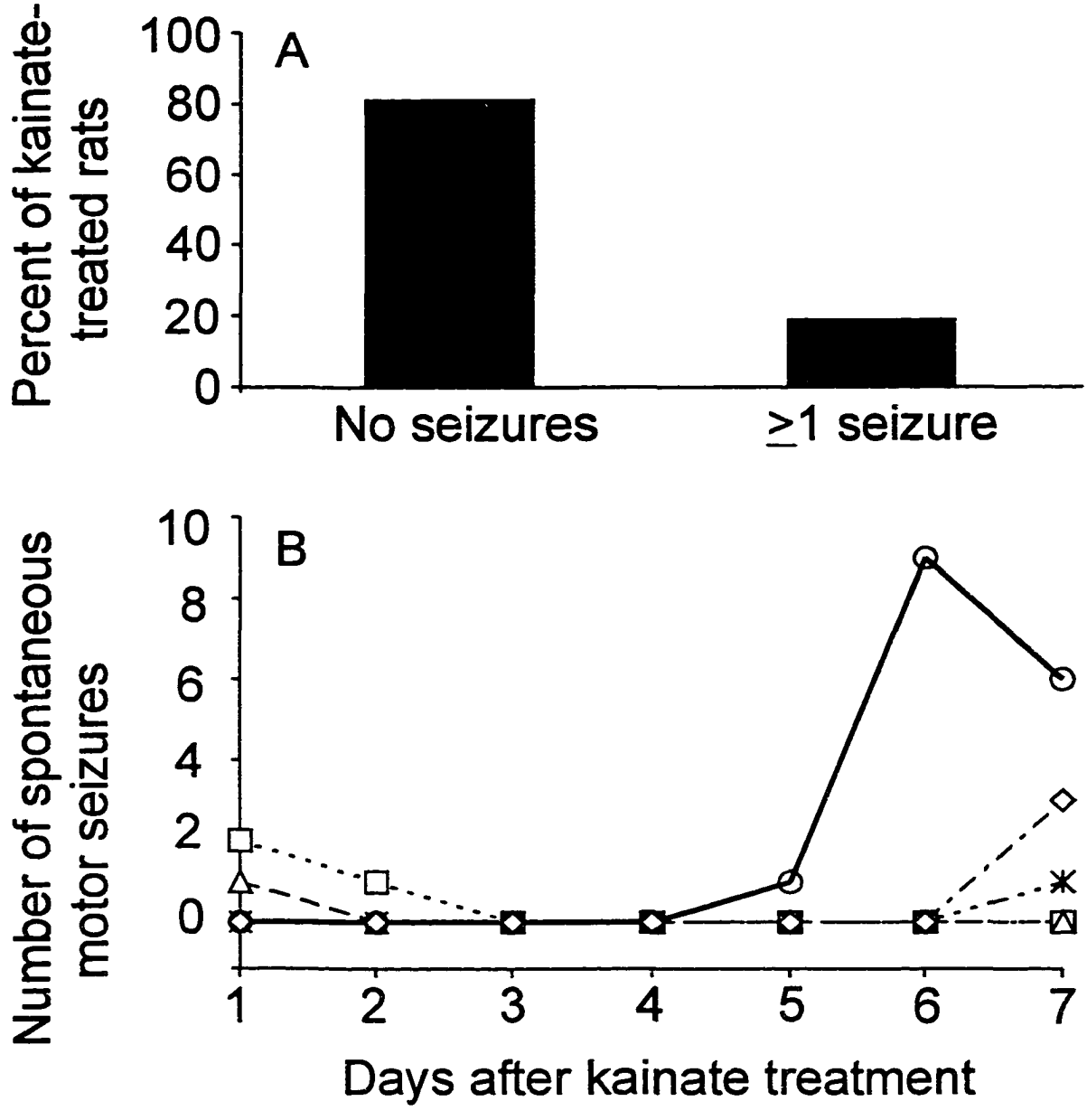

Figure 3.7: During the first week after kainate treatment, few spontaneous motor seizures were observed in kainate-treated rats. (A) Continuous video monitoring (24-h) showed that 81% of kainate-treated rats did not have any spontaneous motor seizures during the first week after treatment. The remaining 19% of rats, however, were observed to have >1 behavioral seizure. (B) The seizure frequencies of five rats are shown as a function of time after kainate treatment. Of these five animals, two rats experienced motor seizures within the first 27 h after treatment; and, three rats had their first motor seizure at 5-7 days after treatment.

Spontaneous electrographic activity after treatment

All rats (n=17 kainate and n=11 saline) were placed in a recording chamber to record spontaneous electrographic activity 1, 4, and 7 days after treatment. Spontaneous activity was recorded for ≥10 min prior to the stimulation-response series. Therefore, for each rat, spontaneous electrographic activity was recorded for at least 30 min. To estimate the frequency of spontaneous interictal spikes in the dentate gyrus of kainate-treated rats, a 2-min sample of the recording sessions at 1 and 7 days after treatment were analyzed in 12 rats. Eight of the 12 kainate-treated rats were observed to have interictal spikes during the 2-min sample recording. Of the four remaining animals, only one rat failed to exhibit spontaneous interictal spikes during any of the recording sessions.

Spontaneous interictal events were observed in the dentate gyrus in 94% of rats as early as 1 day after kainate treatment and were recorded throughout the 1-week testing period (Fig. 3.8). Interictal spikes were often synchronous in both the granule cell layer electrode and the surface EEG. The mean frequency of interictal spikes in kainate-treated rats was 0.21±0.02 spikes/min (±S.E.M.), and the mean length was 252.7±61.7 ms 1 day after treatment. These events, however, increased in frequency to 0.30±0.04 Hz, while the mean duration decreased to 222.5±35.5 ms 7 days after treatment. These results suggest that kainate-treated rats had abnormal electrographic events in the dentate gyrus shortly after treatment that persisted throughout the testing period.

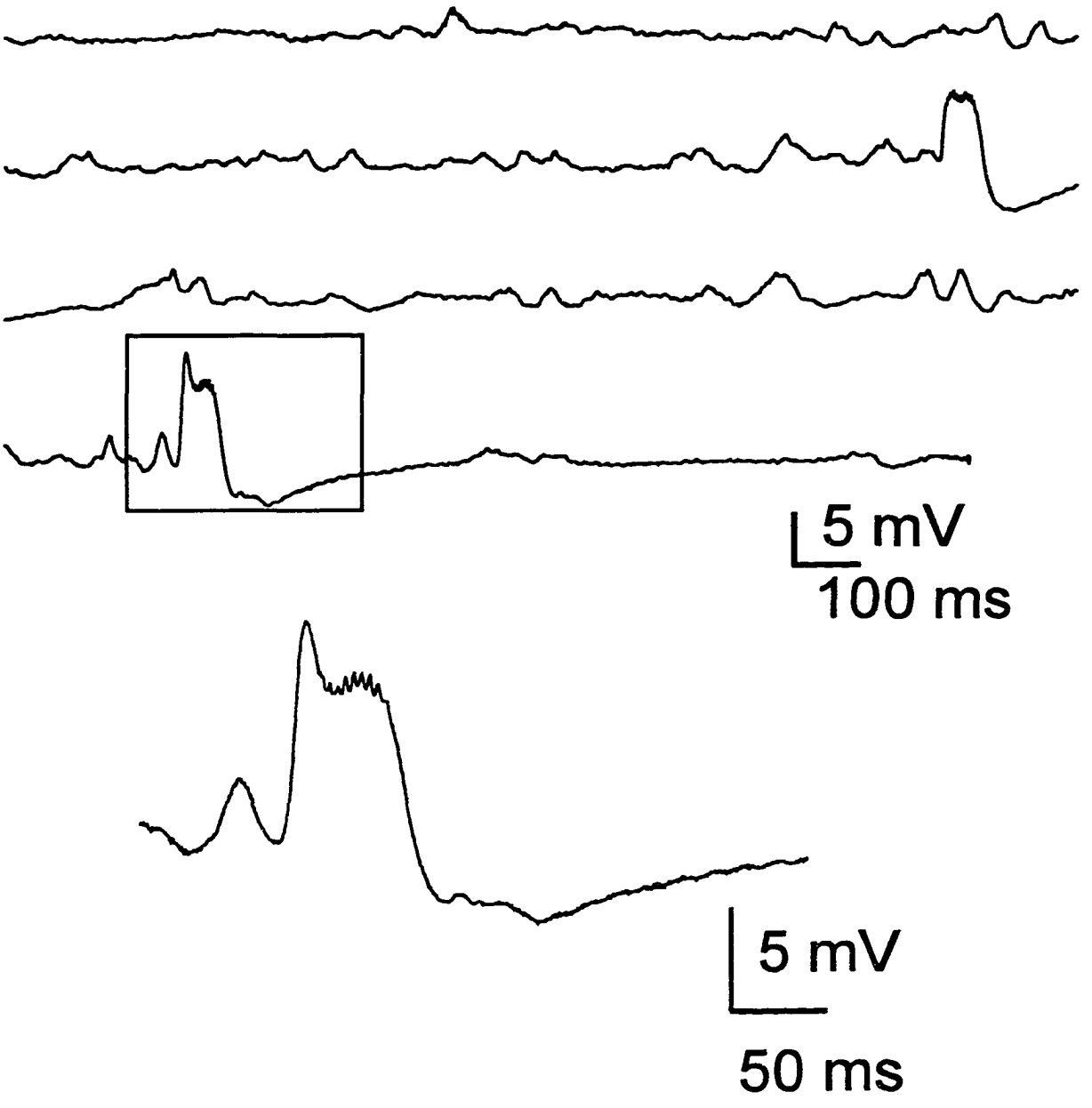

Figure 3.8: Spontaneous interictal events. In the granule cell layer of the dentate gyrus in kainate-treated rats, spontaneous interictal events were observed from the first day after treatment until euthanasia. These events varied in frequency and duration among kainate-treated rats.

Chronic in vivo recordings were performed before and 1, 4, and 7-8 days after treatment to record spontaneous activity and responses to perforant path stimuli. All rats (n=17 kainate and n=11 saline) were monitored both behaviorally and electrographically for 1-3 h during each recording session so that, each rat was monitored for at least 4 h during the first post-treatment week. During these sessions, neither electrographic nor behavioral seizure activity was observed in either saline- or kainate-treated rats.

Response to perforant path stimuli during the first week after treatment

To assess inhibition and epileptiform activity, rats were given single or paired stimuli to the perforant path during the first week after treatment. Three components of the responses were measured: 1) number of population spikes, 2) length of the field PSP, and 3) paired-pulse index (see Materials and Methods). In all controls, single stimuli to the perforant path evoked 1-2 population spikes, and the mean field PSP duration ranged from 12.6±0.8 to 13.1±0.9 ms (±S.E.M., Fig. 3.9A). Similarly, responses from paired-pulse stimulation revealed strong inhibition in all control animals. The mean paired-pulse index ranged from 0.37±0.2 to 0.48±0.2 (Fig. 3.9B). These data suggest that saline treatment does not induce epileptiform activity, and it does not affect inhibition.

Following a single stimulus of the perforant path 1 day after treatment, 8 of 17 (47%) kainate-treated rats showed no increase in the number of

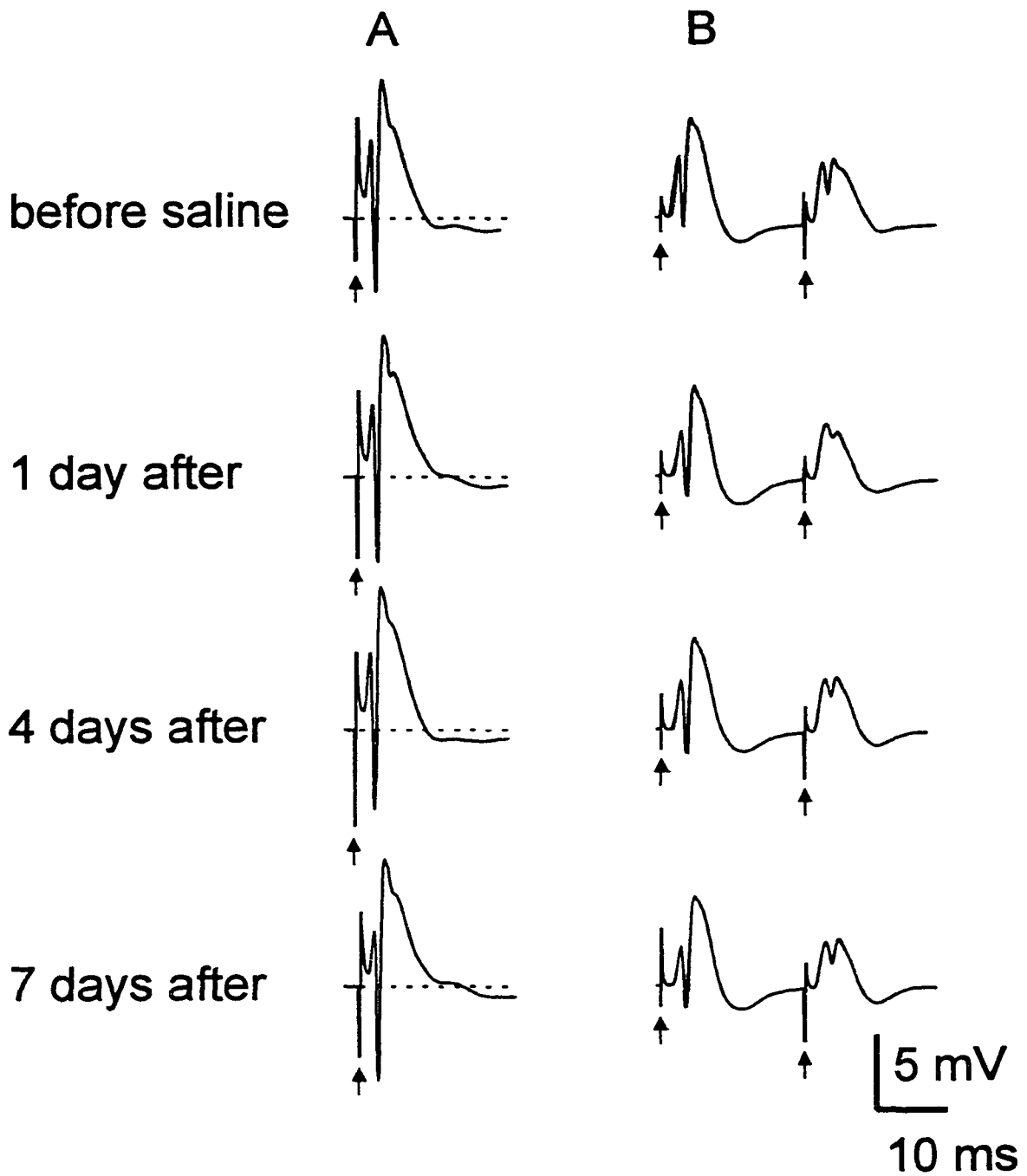

Figure 3.9: Response to perforant path stimuli during the first week after saline treatment. For this and all following figures, the traces are averages of 10 evoked events, and arrows point to truncated stimulus artifacts. (A) Single stimuli produced responses with 1-2 population spikes and uniform lengths of field PSP throughout the testing paradigm. (B) Paired-pulse stimulation produced inhibition in the response to the test stimulus compared to the conditioning stimulus.

population spikes (Fig. 3.10). However in the remaining 53% (9 of 17) of kainate-treated rats, a modest increase in population spikes was observed 1-8 days after treatment (i.e., 3-5 population spikes). At 7-8 days after treatment, 65% of kainate-treated rats had the same number of population spikes as observed in control rats (i.e., 1-2 population spikes), and the remaining 35% had only three population spikes. These results suggest that kainate-treated rats have a partial to full recovery in the number of population spikes by 7 days after treatment.

An increase in the number of population spikes was usually observed with a concomitant increase in field PSP duration. A decrease in inhibition could prolong the field PSP. Therefore, we calculated the change of the field PSP duration as a measurement of a loss of inhibition. In 24% (4 of 17) of kainate-treated rats, the duration of the field PSP did not change 1-8 days after treatment (Fig. 3.11A). Single stimuli evoked prolonged field PSPs (i.e., $\geq$ 2 times the initial field PSP length) 1 day after treatment in 76% (13 of 17) of kainate-treated rats (Figs. 11B and 11C). In these 13 rats, 54% (7 of 13) had a persistent increase of field PSP length throughout the testing period (Fig. 3.11B). The remaining 46% (6 of 13) of rats had a partial to full recovery of initial length by 4-8 days after treatment (i.e., $\geq$50% recovery, Fig. 3.11C).

Paired-pulse stimulation of the perforant path was used to determine local inhibition. To measure this inhibition, the paired-pulse index was calculated by taking the ratio of the amplitude of the population spike from the test pulse by the conditioning pulse. A paired-pulse index $\leq$1 was used to determine that

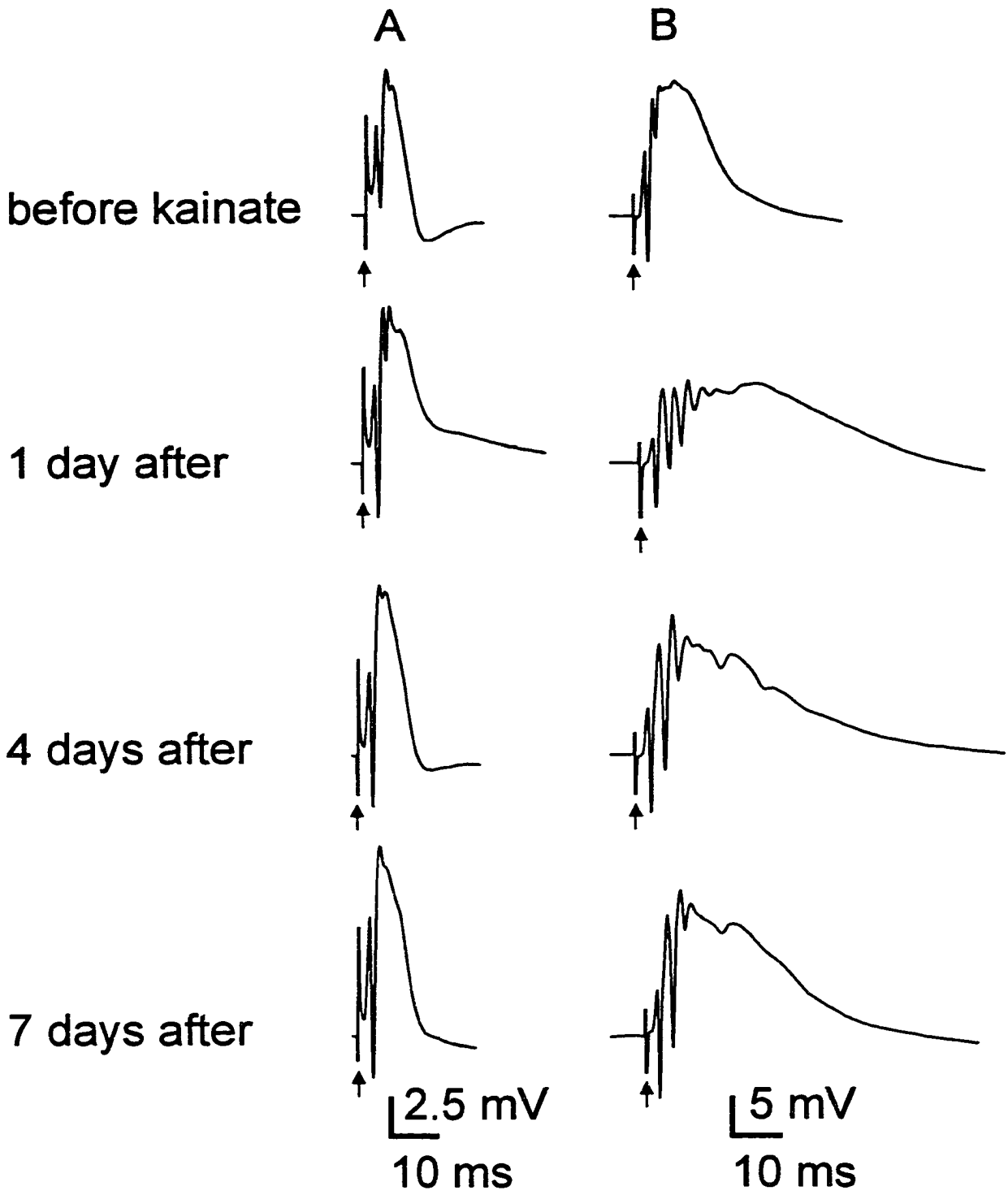

Figure 3.10: Number of population spikes produced from perforant path stimulation during the first week after kainate treatment. (A) In 47% of treated rats, the difference was not significantly different than controls in the number of population spikes 1-8 days after treatment. (B) In the remaining 53% of treated rats, several population spikes were observed 1-4 days after treatment (i.e., $\geq$3 population spikes). By 7-8 days after treatment, all rats had $\leq$ 3 population spikes.

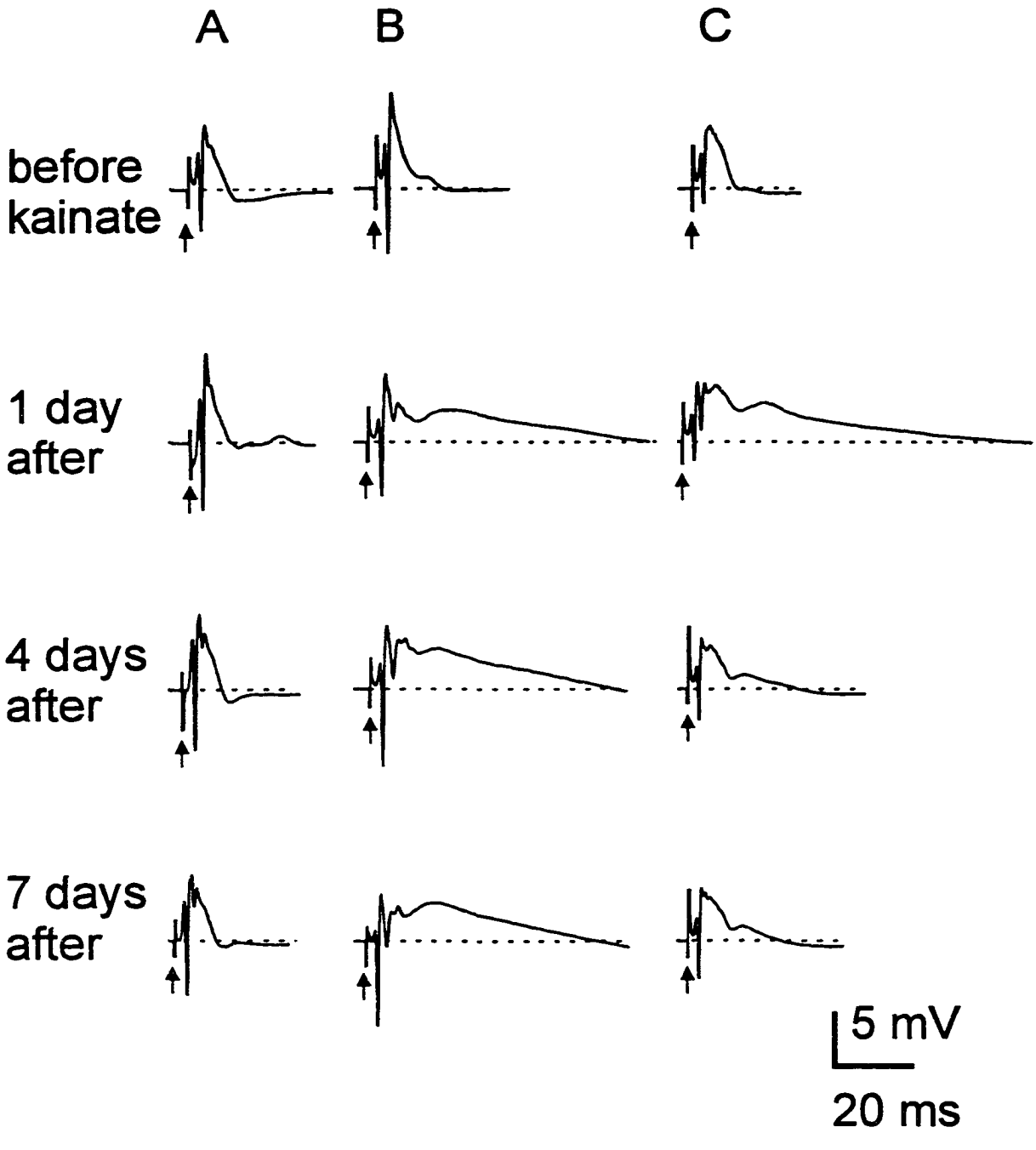

Figure 3.11: Prolonged evoked field PSPs in the dentate gyrus during the first week after kainate treatment. (A) In 4 of 17 treated rats, single stimuli produced responses with an uniform length of field PSP throughout the testing paradigm. (B) Single stimuli produced elongated field PSPs from 1-8 days after treatment in 7 of 17 treated rats. (C) Partial to full recovery of the length of the field PSP length was observed in the remaining 6 of 17 treated rats.

inhibition was intact (see Materials and Methods). All controls had a paired-pulse index ≤1, suggesting a normal inhibitory response (see Fig. 3.9B). Similarly, paired-pulse stimulation produced inhibition in 65% (11 of 17) of kainate-treated rats 1-8 days after treatment (Fig. 3.12A). The mean paired-pulse indices ranged between 0.00–0.56 for these 11 animals. The remaining 35% (6 of 17) of kainate-treated rats displayed paired-pulse facilitation 1 day after treatment (Fig. 3.12B). This facilitation was large in three rats (range: 2.08-3.17) and modest in the other three rats (range: 1.14-1.68). By 7-8 days, however, 4 of the 6 kainate-treated rats with facilitation 1 day after treatment, had a paired-pulse index <1, suggesting a normal inhibitory response.

The mean responses of the three measures we used for inhibition are shown in Figure 3.13. In controls, the differences in means of number of population spikes, field PSP lengths, and paired-pulse index were not significant between pretreatment and 1, 4, and 7-8 days after treatment (P>0.7, ANOVA, Fig. 3.13 left side of histograms). However, the number of population spikes was significantly increased the first day after kainate treatment compared to controls and before and 4-8 days after treatment (P<0.05, Student-Newman-Keuls, Fig. 3.13A). By 4-8 days after treatment, a significant reduction of the number of population spikes was observed, although there was still a significant increase compared to before treatment (P<0.05). Kainate-treated rats also had significantly longer field PSPs 1 day after treatment compared to before and 4-8 days after treatment (P<0.05, Student-Newman-Keuls, Fig. 3.13B). The length of the field PSP had recovered significantly 4-8 days after treatment;

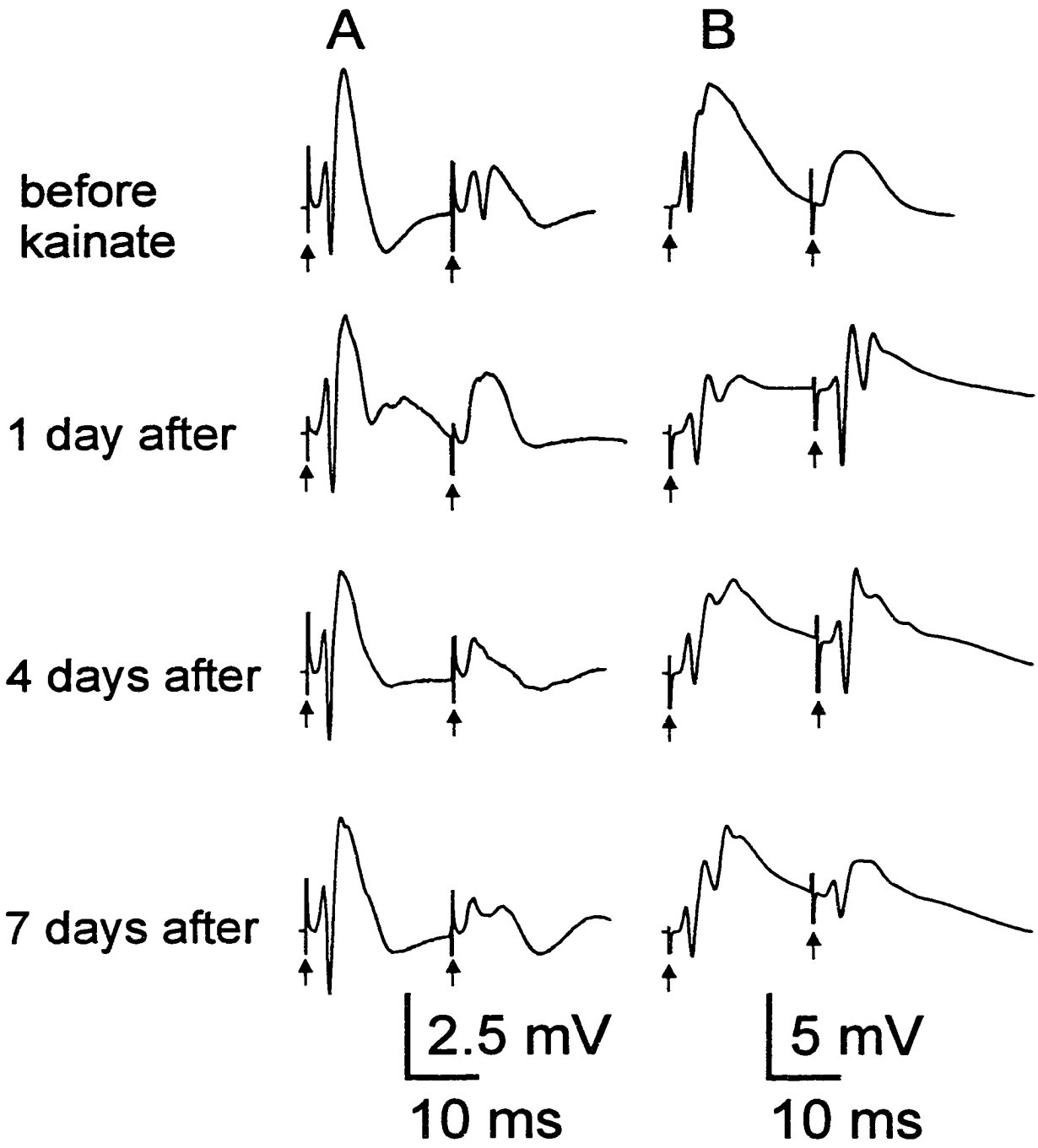

Figure 3.12: Paired-pulse responses in kainate-treated rats during the first week after treatment. (A) In 65% of treated rats, paired-pulse stimuli produced attenuated responses throughout the testing paradigm. The paired-pulse indices ranged from 0.00 - 0.56. (B) Paired-pulse stimuli produced facilitated responses 1-4 days after treatment in the remaining 35% of rats. Partial to full recovery of paired-pulse responses was observed in 88% of rats at 7-8 days after treatment. By 7-8 days, however, 4 of the 6 kainate-treated rats with a facilitated response 1 day after treatment, had a paired-pulse index <1.

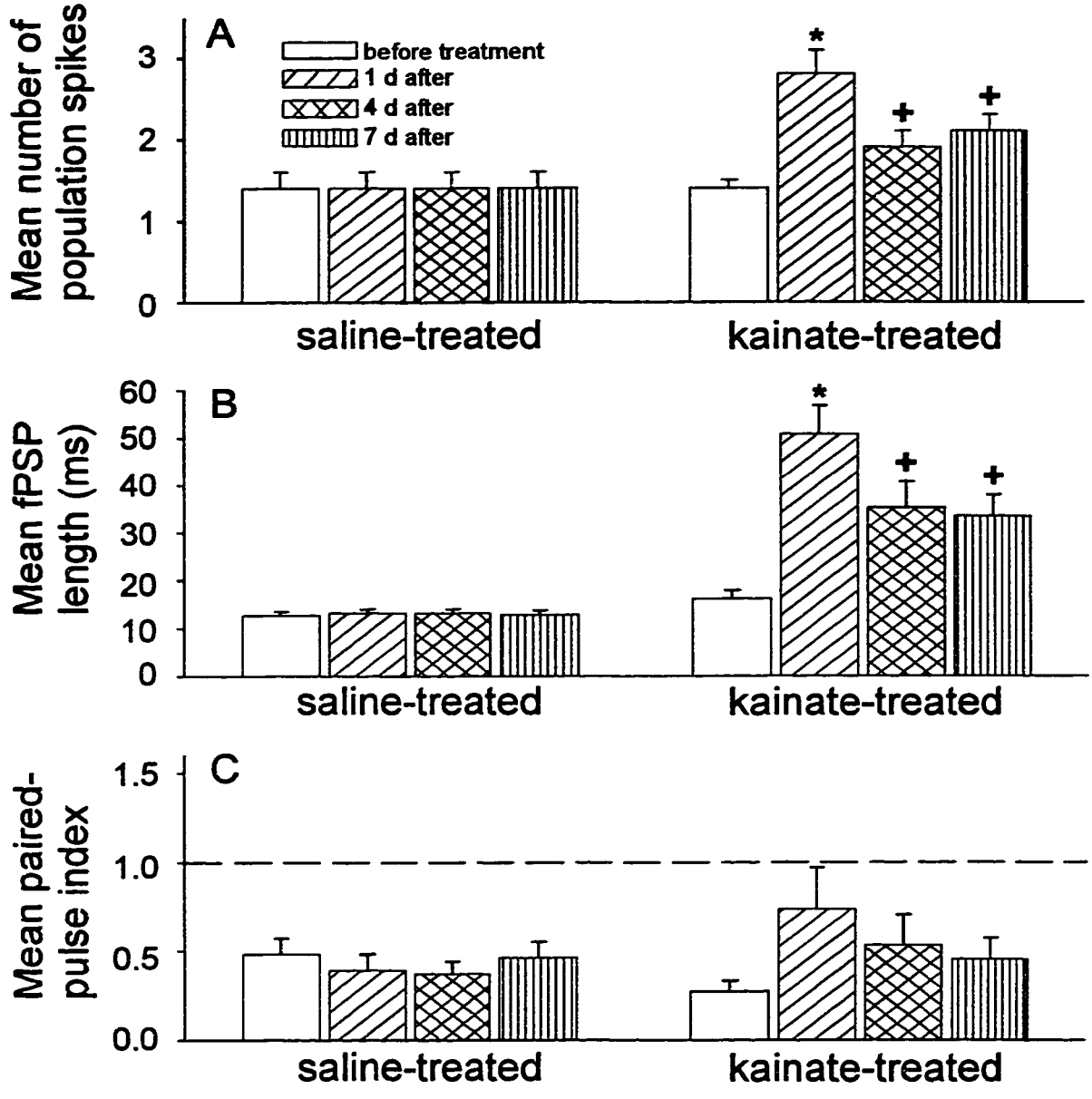

Figure 3.13: The mean number of population spikes and field PSP lengths of kainate-treated rats were significantly different from controls. (A) Kainate-treated rats had significantly more population spikes 1 day after treatment compared to before, 4 and 7-8 days after treatment. By 4-8 days after treatment, a partial recovery of the number of population spikes was observed. (B) Similarly, kainate-treated rats had significantly longer field PSPs 1 day after treatment compared to before, 4 and 7-8 days after treatment. However, a partial to full recovery of the field PSP duration was observed 7-8 days after treatment. (C) Both kainate-treated and control rats had an inhibitory response at before and 1, 4, and 7 days after treatment. At 1 days after treatment, kainate-treated rats had an increase in the mean paired-pulse index; however, this difference was not significant. The asterisk represents significant differences over all data points ($P<0.05$, Student-Newman-Keuls). The plus symbol represents significant differences from all except between those data points.

however, the field PSP duration was not necessarily equal to before treatment. (P<0.05). Analysis of variance between saline- and kainate-treated rats showed that the differences of paired-pulse indices were not significant (P=0.171, Fig. 3.13C). Although some kainate-treated rats had a facilitated paired-pulse response at 1, 4, and 7-8 days after treatment, the mean differences were not significant from the before treatment response (P>0.5). These data suggest that inhibition initially decreased following kainate treatment when comparing the number of population spikes, field PSP duration, and paired-pulse index. This loss of inhibition, however, recovered relatively rapidly (i.e., by 7 days after kainate treatment).

Relationship between the percent of recovery and mean Timm score.

From in vivo experiments, Sloviter (1992) observed that kainate-treated rats had a loss of inhibition that was later restored months after treatment, when robust mossy fiber sprouting was present. This result led to the hypothesis that significant mossy fiber sprouting, months after kainate treatment, leads to restored or increased inhibition in the dentate gyrus. Therefore, we decided to assess if the recovery of inhibition in our study was associated with mossy fiber sprouting in the inner molecular layer at 7 days after kainate treatment. The two measurements that had a significant change after kainate treatment (i.e., number of population spikes and field PSP) were used for this analysis. Pearson's correlation coefficient was used to determine the relationship between the amount of recovery versus mean Timm score. To ascertain the amount of

recovery, the before treatment response was subtracted from the responses at 1 and 7 days after treatment. These differences were then used to produce the percentage of the response at 7 days from 1 day after treatment (Fig. 3.14A). The amount of Timm reaction product in the inner molecular layer of the dentate gyrus was determined throughout the septotemporal axes of the hippocampi of each rat (see Materials and Methods). Only those rats used for histology at 7 days after kainate treatment were utilized for this analysis (n=9).

The responses measured for recovery of the field PSP duration and the mean Timm score are shown in Table 3.2. This analysis was dependent upon amount of recovery in the length of the field PSP; therefore, only rats with a change between the first day after treatment and before treatment were used. The percent of recovery of the field PSP was found to be *negatively* correlated to the amount of Timm staining in the inner molecular layer of the dentate gyrus (Pearson's r = -0.88, Fig. 3.14B). This association was found to be highly significant (P=0.0036, linear regression). Six of eight rats had little to no Timm staining 7 days after kainate treatment (range: 0.0-0.55 Timm score). These six rats also had a large amount of restored inhibition. The two remaining kainate-treated rats had the greatest amount of mossy fiber sprouting in the inner molecular layer 7 days after treatment (i.e., Timm score >1), and also had the least amount of recovery. The responses measured for recovery in the number of population spikes are shown in Table 3.3. Six of nine rats had no recovery in the number of population spikes 7 days after kainate treatment. However, there was no correlation between the percent of recovery of the number of population

Table 3.2: Lengths of field post-synaptic potentials and mean Timm scores used for the linear regression analysis in figure 3.14.

Rat #	Timm score	PSP before	PSP 1 d	before-1d length	PSP 7 d	before-7d length	% of field PSP recovery
24	1.26	9.50	12.20	2.70	13.50	4.00	-48
9B	0.00	10.70	36.80	26.10	15.70	5.00	81
11B	0.00	9.50	9.50	0.00	11.00	1.50	divided by zero
19A	0.07	15.00	43.90	28.90	27.20	12.20	58
19B	0.00	10.30	50.90	40.60	15.80	5.50	86
21B	0.11	10.90	80.60	69.70	36.30	25.40	64
23B	0.55	9.80	43.70	33.90	30.20	20.40	40
26A	1.05	17.30	70.00	52.70	58.10	40.80	23
26B	0.42	11.90	79.00	67.10	23.70	11.80	82
						Pearson's r =	-0.88

PSP: field post-synaptic potential (ms)
1 d: one day after kainate treatment
7 d: seven days after kainate treatment

Table 3.3: Numbers of population spikes and mean Timm scores used for the linear regression analysis.

Rat #	Timm score	# of PS before	# of PS 1 d	before-1d	# of PS 7 d	before-7d	% of # of PS recovery
24	1.26	1	2	1	2	1	0
9B	0.00	1	2	1	3	2	-50
11B	0.00	1	2	1	2	1	0
19A	0.07	2	2	0	3	1	-50
19B	0.00	2	3	1	1	-1	67
21B	0.11	1	3	2	3	2	0
23B	0.55	1	3	2	1	0	67
26A	1.05	1	2	1	2	1	0
26B	0.42	1	3	2	1	0	67
						Pearson's r =	0.13

PS: population spikes
1 d: one day after kainate treatment
7 d: seven days after kainate treatment

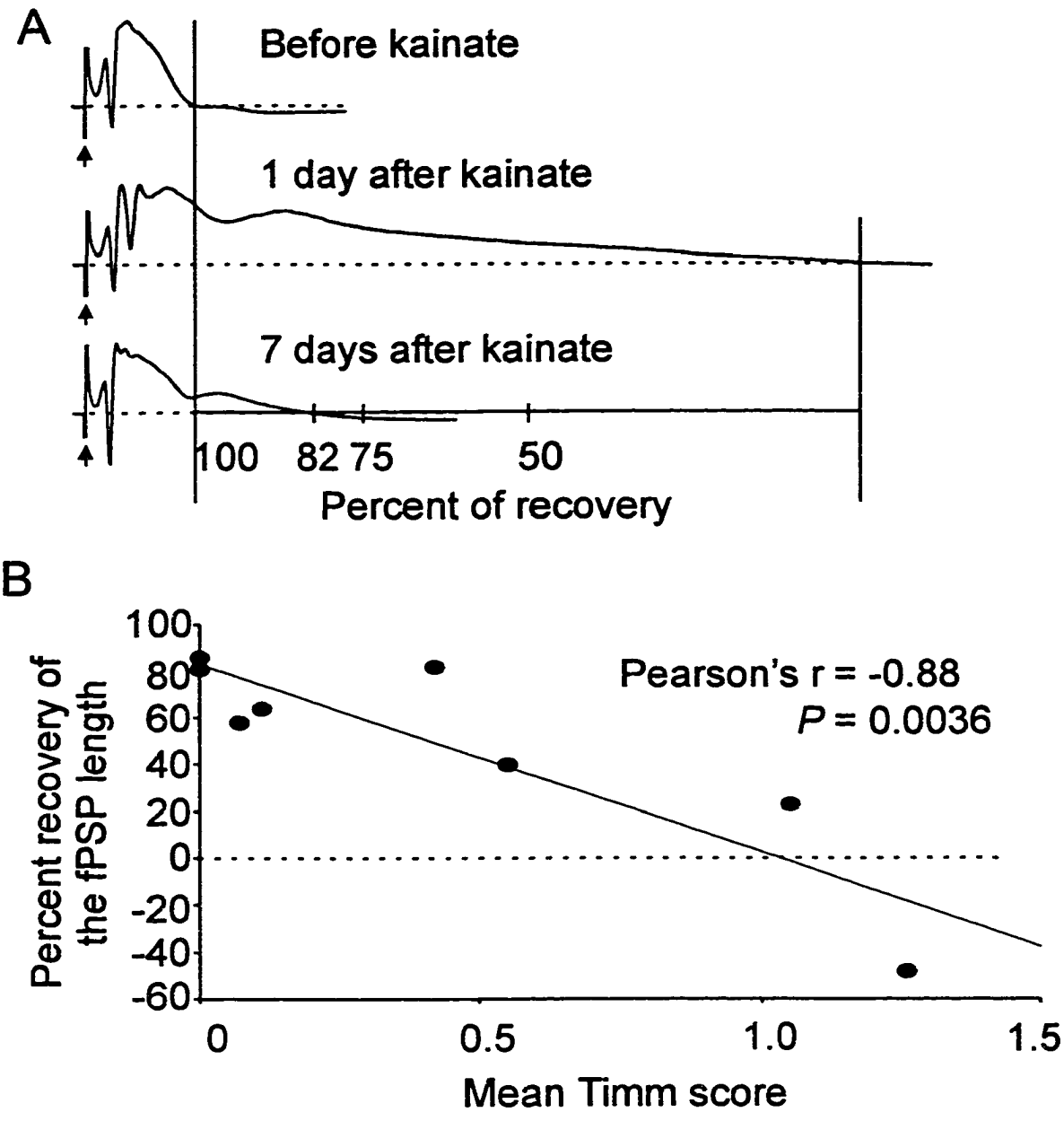

Figure 3.14: Graphical representation of how the amount of recovery was measured. In the top trace, the original field PSP duration is shown. This length was subtracted from the field PSP duration at 1 and 7 days after treatment. One day after treatment, the duration of the field PSP lengthened. This abnormal increase in length was used to determine that amount of recovery. The bottom trace shows that at 7 days after treatment, the abnormal length of field FPSP recovered by 82%. (B) At 7 days after treatment, the relationship between the percent of recovery and the mean Timm score was negatively correlated (Pearson's r = -0.88). This relationship was highly significant (P=0.0036).

spikes and the amount of Timm staining (Pearson's r = 0.13, P=0.75, data not shown). Therefore, these results suggest that mossy fiber sprouting in the inner molecular layer is not the primary mechanism for the restoration of inhibition in the dentate gyrus.

DISCUSSION

The primary findings of this study are summarized as follows:

1. Rats treated with multiple low doses of kainate experienced electrographic status epilepticus (i.e., ictal activity for $\geq$30 min) during the injection series. This electrographic status epilepticus consisted of non-convulsive ictal activity with episodes of behavioral motor seizures. Immediately following the behavioral seizures, non-convulsive ictal activity resumed instead of a post-ictal negativity, which is consistent with status epilepticus.

2. One day after kainate treatment, field responses to single stimulation of the perforant path had a modest increase in the number of population spikes and a large increase in the field PSP duration. These physiological changes, however, showed partial to full recovery by 4-8 days after treatment. Normal inhibitory responses were observed from paired-pulse stimulation in most kainate-treated rats. By 7-8 days after treatment, only a few rats had a small decrease in inhibition. These observations suggest

that the functional changes in the septal hippocampus last for a few days after kainate treatment, and that they alone may not be crucial for epileptogenesis.

3. The transient pathophysiological changes were associated with permanent structural modifications in the dentate gyrus. These changes included both hilar neuron loss and modest Timm staining in the inner molecular layer at 7 days after kainate treatment. Despite these histological alterations, recovery of the number of population spikes and field PSP length began within 4 days after kainate treatment.

4. As early as 7 days after kainate treatment, the dentate gyrus of most rats had nearly normal inhibitory responses and little Timm staining in the inner molecular layer. When a linear regression was performed to determine the relationship between recovery of inhibition and mean Timm score at 7 days after kainate treatment, the association was negatively correlated. This correlation showed that kainate-treated rats with the most abnormal Timm staining had the least amount of recovery of inhibition. Therefore, these results suggest that mossy fiber sprouting in the inner molecular layer is not the primary mechanism for the recovery of inhibition.

5. In the present study, kainate-treated rats had a temporary reduction in inhibition (i.e., partial to full recovery was observed at 4-8 days after treatment). Two rats did not show reduction in inhibition immediately following kainate treatment, and these rats were observed to have spontaneous motor seizures months after treatment. Therefore, loss of inhibition may not be necessary to induce epileptogenesis, at least in the septal hippocampus. These findings argue against the "dormant basket cell" hypothesis, which states that surviving inhibitory neurons (i.e., basket cells) are possibly non-functional after injury due to the loss of excitatory inputs that normally evoke inhibition.

Seizure frequency during the first week after kainate treatment

During 24-h video monitoring, motor seizures (i.e., class III/IV/V) were differentiated between other behaviors by using the scale designed by Racine (1972). A silicon-intensified target camera with automatic gain control was utilized to ensure that behavioral seizures were not missed (for further details see Hellier and Dudek, 1999). The 24-h video monitoring in the present study, however, suggests that 81% of kainate-treated rats had no spontaneous motor seizures during the 1-week testing period. In the remaining 19% of animals (n=5 of 26), two rats experienced 1-2 motor seizures within the first 27 h after treatment, which presumably reflects the influence of remaining kainate. We treated rats with multiple low-doses of kainate to induce electrographic status epilepticus. This protocol used systemic injections, which may allow a slower

diffusion of kainate to the brain compared to a single venous, intrahippocampal, or intraventricular injection (e.g., Cavalheiro et al., 1982). Using our protocol, the exposure of kainate may persist for more than 24 h after the initial injection series. Therefore, we consider that these two rats with seizures directly after kainate treatment (i.e., within the first 27 h after treatment, but not during the next 6 days) would not be defined as being epileptic. The other three rats with seizures had their first ictal activity 5 or 7 days after treatment. The seizure frequencies during the seven consecutive 24-h periods ranged between 0.006-0.095 seizure/h (i.e., $\leq$ 1 seizure/100 h; Fig. 3.7). These seizure frequencies from 24-h video monitoring during the first week are much lower compared to those observed in rats many months after kainate treatment (i.e., $\geq$ 1 seizure/2 h; see Hellier and Dudek, 1999). Although 88% of kainate-treated rats have a latent period greater than 1 week, these data suggest that 12% (3 of 26) of kainate-treated rats have a latent period to the first spontaneous seizure as short as 5-7 days.

In a previous study with 6-8 h/wk of direct monitoring, only one of 98 (1%) rats was observed to have a seizure during the first week after kainate treatment. The mean seizure frequency for these rats with kainate-induced epilepsy, when examined similarly months later, was calculated to be 0.18 seizures/h (i.e., 1 seizure/5 h; Hellier et al., 1998). In another study using 24-h video monitoring, the seizure frequency of rats many months after kainate treatment was determined to be 1.9 seizures/h (i.e., 2 seizures/h; Hellier and Dudek, 1999). Therefore, 24-h video monitoring reveals 10.9 times more

seizures compared to 6-8 h/wk of direct observation. This result may account for the difference in the number of rats observed to have a spontaneous seizure during the first week after kainate treatment.

Comparison of permanent pathophysiological events in kainate-treated rats and human temporal lobe epilepsy

Interictal spikes of kainate-treated rats resembled those seen in humans with temporal lobe epilepsy. Immediately after kainate treatment, many of the animals had a low frequency of interictal spikes, while other rats had many interictal spikes at a frequency as high as 0.56 Hz (Fig. 3.8). This range in individual interictal frequency is similar to those recorded in humans with temporal lobe epilepsy (Quesney et al., 1993). Although we sampled spontaneous electrographic activity for 2-min intervals, we may have underestimated the actual frequency of these events. Interictal spikes and sharp waves have been shown to be state dependent in humans with temporal lobe epilepsy (e.g., become more distributed and change morphologically during sleep, Drury, 1996). Therefore, longer sampling periods to determine the frequencies of interictal spikes during different behavioral states are necessary and would be a subject for additional studies. Nonetheless, in the animals that were allowed to survive (n=8), spontaneous interictal events and motor seizures were observed until the kainate-treated rat had died or was euthanized (i.e., 2-6 months after treatment), suggesting that permanent pathophysiological changes had occurred.

These interictal spikes may be caused by a loss of inhibition because they were present 1 day after kainate treatment when most rats had an increase in population spikes and field PSP length. These events, however, persisted in 94% of rats throughout the 1-week testing period when inhibition was shown to have a partial to full recovery (Fig. 3.13). Similarly, in the eight rats that were allowed to survive, interictal spikes were seen as long as many months after kainate treatment when inhibition has been restored (Sloviter, 1992). Therefore, interictal spikes may not be caused by a loss of inhibition, but instead could be responses from potentiated synapses that were created by tetanization during the kainate-induced status epilepticus (e.g., Staley et al., 1998).

Relationship between histological changes and transient function in the dentate gyrus

Seven days after kainate treatment, rats had fewer hilar neurons and abnormal Timm staining per section compared to controls. The differences in neuron population and Timm staining were significant only at the temporal end of the hippocampus (Fig. 3.6). Transient electrographic abnormalities, however, were recorded near the septal end of the hippocampus where cell loss and Timm staining in the inner molecular layer were observed but were not significant. Previous studies have shown that both hilar neuron loss and abnormal Timm staining were significant throughout most of the septotemporal axis in rats with kainate-induce epilepsy (Buckmaster and Dudek, 1997). The rats used in the

current study, however, were euthanized 7 days after kainate treatment (i.e., many months earlier than in the Buckmaster and Dudek investigation). These data suggest that neuron loss in the septal and middle portions of the hippocampus may be gradual, and that neuron loss may not be time-locked with kainate treatment. Another possibility is that Buckmaster's animals were many months older than ours were, and this alone (i.e., aged rats) could account for both the discrepancies in neuron counts and the amount of Timm reaction product in the inner molecular layer. Nonetheless, we have the same general results that show a significant decrease in hilar neuron population but little Timm staining 7 days after treatment (Fig. 3.6). Furthermore, transient electrographic abnormalities were recorded near the septal end of the hippocampus where moderate neuron loss and Timm staining were observed.

Neuronal loss in the hippocampus has been hypothesized to cause basket cells (i.e., inhibitory neurons) to become dormant (Sloviter, 1991), suggesting why feedback inhibition is decreased a few days after kainate treatment (Sloviter, 1992). To test if inhibition decreased in the present study, dentate granule cell synchronization and hyperexcitability were quantified by the number of population spikes, field PSP duration, and paired-pulse inhibition at 1, 4, and 7 days after kainate treatment. These responses were used for analyzing hyperexcitability because with a loss of feedback inhibition, granule cells presumably will continue to fire, and therefore, produce multiple population spikes and an elongated field PSP as well as paired-pulse facilitation. Although some neurons may not be at the threshold needed to evoke an action potential,

they are capable of eliciting an excitatory PSP, which can possibly add to both the length of the field response and paired-pulse facilitation. The differences in population spikes, field PSP duration, and paired-pulse index were primarily observed only the first day after kainate treatment (Fig. 3.13). These responses were beginning to recover at 4 days after kainate injections, and some responses were completely recovered by 7 days after treatment. Therefore, the transient hyperexcitability may not be solely caused by the lack of excitatory input to the basket cells, but may also be due to other physiological mechanisms.

Paired-pulse stimulation may be an inaccurate measure of inhibition in kainate-treated rats

Paired-pulse stimulation has been the mainstay test for both feed-forward and recurrent inhibition. Feedback inhibition is quantified by presenting two stimuli with a 20-40 ms interstimulus interval, and it is hypothesized to test $GABA_A$-mediated inhibition (i.e., the fast component of inhibition). A previous study using an interstimulus interval of 40 ms showed that anesthetized rats had paired-pulse facilitation 3 days after kainate treatment, and that inhibition recovered 2 months later when significant mossy fiber sprouting in the inner molecular layer was present (Sloviter, 1992). We used the paired-pulse paradigm with an interstimulus interval of 20 ms to test inhibition in freely-behaving rats before and 1, 4, and 7 days after kainate treatment. The present

data showed that if paired-pulse inhibition was decreased, it mostly recovered when little or no mossy fiber sprouting in the inner molecular layer had occurred, which is in disagreement with Sloviter's results (1992). This discrepancy may be because our animals were not anesthetized and we stimulated at a much slower frequency (0.05 Hz) to ensure that tetanization did not occur. We also tested the recovery of inhibition much earlier than two months after kainate-treatment (i.e., at 7-8 days after treatment).

One of the main problems we encountered with paired-pulse stimulation is that many kainate-treated rats had elongated field PSPs. This elongated field PSP could be masking the response from the second stimulation by inactivating voltage-sensitive ion channels, depolarizing and desynchronizing neurons. In extracellular recordings from hippocampal slices of control rats, unreported personal observations have shown that stimulation of the perforant path (i.e., 10-100 uA) produced elongated field PSPs and multiple population spikes when the slices were bathed in bicuculline (a $GABA_A$ antagonist). In these same sections, paired-pulse stimulation would evoke both normal and facilitated inhibitory responses. Therefore, multiple population spikes may be a better representation for the loss of inhibition in kainate-treated rats because they are also produced during epileptiform bursts. In the present study, we may have underestimated the number of population spikes because of a lack of a refined definition for identifying a population spike. Latent spikes that had lost their fast ascending and fast descending components were not included in the analysis. This change of population spike shape may be caused by desynchronizing

neurons or because of accommodation (Staley, personal communication). Therefore, we may have had more population spikes at 1 day after kainate treatment, and this is why it appears that the multiple population spike measure may not have had as complete a recovery at 7 days after treatment as the length of the field PSP. These studies, however, incorporated three measures of inhibition in order to address concerns about the paired-pulse technique.

CONCLUSION

Rats treated with multiple low doses of kainate developed a chronic epileptic state months after treatment (n=8). During the first week after treatment, however, transient pathophysiological changes were observed in most kainate-treated rats (i.e., a modest increase in the number of population spikes and a large increase in the field PSP duration at 1 day after treatment). Normal if not stronger inhibitory responses from paired-pulse stimulation were also observed in most rats at 7-8 days after kainate treatment. Permanent histological changes consisted of hilar neuron loss and very little Timm staining in the inner molecular layer, suggesting that the structure of the dentate gyrus was altered by the kainate-induced status epilepticus. The recovery of inhibition was negatively correlated with the mean Timm score at 7 days after kainate treatment. This negative correlation showed that kainate-treated rats with the most abnormal Timm staining had the least amount of recovery of inhibition. Therefore, these results suggest that mossy fiber sprouting in the inner

molecular layer is not the primary mechanism for the recovery of inhibition, and argue against the hypothesis that mossy fiber sprouting is only restorative.

DISSERTATION CONCLUSIONS

From behavioral observations and in vivo experiments, we found that kainate-treated rats have several behavioral, histologic, and electrographic similarities with human temporal lobe epilepsy. First, electrographic status epilepticus was the initial injury that led to epileptogenesis in rats treated with multiple low-dose injections of kainate, which is similar to some humans with temporal lobe epilepsy. Many months after treatment, kainate-treated rats had spontaneous motor seizures (i.e., became epileptic). These spontaneous motor seizures: 1) increased in frequency for the first 4 months after the onset of seizures, 2) occurred more often during periods of inactivity, and 3) appeared to be chronic. Immediately after kainate treatment, rats exhibited interictal spikes in the dentate gyrus. These electrographic abnormal events continued until the animal died. During the first week after treatment, however, transient pathophysiological changes were observed in most kainate-treated rats (i.e., a modest increase in the number of population spikes and a large increase in the field PSP duration at 1 day after treatment). Finally, histological changes consisted of hilar neuron loss and little Timm staining in the inner molecular layer, suggesting that the structure of the dentate gyrus was altered by the kainate-induced status epilepticus, but the final stage of hippocampal sclerosis had not yet been reached. From these experiments, several new questions have developed and a few are addressed below:

1. Do seizures beget seizures, and thereby induce epileptogenesis?
2. Does serotonin have a role in controlling seizures during active behavior in rats with kainate-induced epilepsy?
3. What mechanisms are responsible for the recovery of inhibition during the first week after kainate treatment?

Chapter One:

The presence of a latent period of weeks supports the hypothesis that the initial damage from the kainate-induced status epilepticus is by itself insufficient to be the basis for chronic epileptogenesis. Along with the latent period, the increase in seizure frequency during the months following the first chronic seizure emphasizes the progressive nature of temporal lobe epilepsy. These data support but do not prove the idea that "seizures beget seizures". It has been suggested that epilepsy is a progressive syndrome, however, no evidence has shown this to be true in either human temporal lobe epilepsy or in animal models. If epilepsy is a progressive syndrome, there are at least two hypothetical explanations for this phenomenon. One hypothesis is that "seizures beget seizures" (i.e., the seizures after a precipitating injury contribute to subsequent injury and more seizures); another hypothesis is that anatomical changes (e.g., mossy fiber sprouting) continue to develop for several months or years after the initial insult independent of the seizures. An investigator could test these two hypotheses using anti-epileptic drug administration to kainate-treated rats with long-term implanted electrodes. The logic is: if epilepsy is a progressive

syndrome due to synaptic reorganization independent of the seizures, then seizure frequency should increase over time even when anti-epileptic drugs are used to block seizures, because synaptic reorganization should continue to develop. One would anticipate a linear increase in seizure frequency prior to drug treatment. During drug administration, seizure frequency should decrease, but return to pre-drug linear frequencies after the discontinuation of anti-epileptic drug therapy. This result would suggest that epilepsy is a progressive syndrome and that blocking the seizures with anti-epileptic drugs does not stop the progressive worsening of the seizures.

Chapter Two:

Data from 24-h video monitoring showed that rats with kainate-induced epilepsy had significantly more spontaneous motor seizures during inactive behaviors (i.e., little to no volitional movement, including apparent sleep) compared to activity (i.e., apparent volitional movement, as in walking, grooming, eating, etc.). One possible reason for this difference in seizure frequency as a function of behavioral state could be the general release of the neuromodulator serotonin. Serotonin is released during repetitive activity and may modulate inhibition during these behaviors, and in turn raise seizure threshold. If this were correct, then one could hypothesize that rats with kainate-induced epilepsy have a decrease in seizure frequency during inactivity while serotonin re-uptake inhibitors were being administered (e.g., fluoxetine has been shown to be an anticonvulsant in animal models). To test this hypothesis, one could 24-h video

monitor rats with kainate-induced epilepsy prior to and after serotonin re-uptake inhibitor treatment, and then calculate the differences in seizure frequency. One could also look at seizure-like activity in hippocampal slices from these kainate-treated rats (post-fluoxetine treatment) during bath application of serotonin, and would anticipate spontaneous seizure activity in artificial cerebrospinal fluid with high extracellular K^+ and bicuculline to decrease with the additional application of serotonin. These results would suggest that serotonin modulates inhibition and may lead to additional experiments to test this more specifically.

Chapter Three:

When a linear regression was performed to determine the relationship between recovery of inhibition and mean Timm score at 7 days after kainate treatment, the association was either negatively correlated or not correlated. These results suggest that mossy fiber sprouting in the inner molecular layer is not the mechanism for the recovery of inhibition. One hypothesis for the transient hyperexcitability is that the number of presynaptic $GABA_B$ receptors produced in basket cells is temporarily increased immediately following kainate treatment. The logic is: if $GABA_B$ receptors increase immediately after kainate treatment, then one would expect that the electrographic responses would be slightly hyperexcitable as observed in this study because some of the $GABA_B$ receptors would be presynaptic and thereby reduce the amount of GABA being released (i.e., disinhibition). An investigator could test this hypothesis by performing autoradiography on the $GABA_B$ receptor in hippocampi at pretreatment and

1, 4, and 7 days after kainate treatment to determine if there is a significant change. Furthermore, one could also look at synaptically-evoked responses in hippocampal slices (at pretreatment and 1, 4, and 7 days after kainate treatment) during bath application of a presynaptic $GABA_B$ agonist (e.g., baclofen). At one day after kainate treatment, one would anticipate mostly normal responses to peforant path stimulation in artificial cerebrospinal fluid. With the additional application of baclofen, however, the responses would become hyperexcitable because the release of GABA would be altered. These results would suggest that presynaptic $GABA_B$ receptors modulate inhibition immediately after kainate treatment, and may lead to additional experiments to test this more specifically.

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