Local Circuit Abnormalities in Chronically Epileptic Rats After Intrahippocampal Tetanus Toxin Injection in Infancy

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Smith, Karen L. Chong L. Lee, and John W. Swann. Local circuit abnormalities in chronically epileptic rats after intrahippocampal tetanus toxin injection in infancy. J. Neurophysiol. 79: 106–116, 1998. In vitro slice experiments were undertaken in adult rats to investigate the physiological origins of a chronic epileptic condition that was initiated in infancy. A unilateral injection of a minute quantity of tetanus toxin into hippocampus on postnatal day 10 produced a severe convulsive syndrome characterized by brief but repeated seizures that lasted for 5–7 days. Hippocampal slices were then taken from these rats in adulthood because at this time previous studies have shown the occurrence of electrographic and behavioral seizures. Dramatic alterations in local circuit functioning were observed. In normal artificial cerebrospinal fluid (ACSF), spontaneous epileptiform network bursts were recorded in a majority (73%) of experimental rats. Network bursts occurred in area CA3 of both the injected and contralateral hippocampus. These consisted of intracellular depolarization shifts that were coincident with extracellularly recorded network bursts. Often they occurred at frequencies of 0.05–0.1 Hz and although variable in amplitude and duration, had all-or-none-like qualities. These events appeared to arise largely from local circuits in the CA3 subfield. Network bursts were rarely recorded in area CA1 and were never observed in the dentate gyrus. However in 31% of rats, a novel, higher frequency (2–8 Hz) field potential was recorded in area CA1. This was coincident with rhythmic, intracellularly recorded, inhibitory postsynaptic potentials (IPSPs). These summated IPSPs blocked action potential firing and reversed polarity near −75 mV. To understand the origins of network bursting in area CA3, comparisons were made of the fundamental neurophysiological properties of pyramidal cells in epileptic and control rats. Of the passive and active membrane properties examined, all appeared normal. Unusually prolonged bursts of action potentials were observed in a small subset of pyramidal cells. However on average the duration of intrinsic bursts were unaltered in the CA3 neurons analyzed from experimental rats. To explore the role that alterations in CA3 recurrent excitatory network excitability may play in epileptiform discharges, picrotoxin was bath applied. On blockade of γ-aminobutyric acid (GABA_A) receptors, slices from experimental rats underwent prolonged electrographic seizures that were up to 10 s in duration. In contrast, slices from control rats produced only brief 100-ms network bursts. These results suggest that a change in excitability within CA3 recurrent excitatory networks likely contributes to seizures in chronically epileptic rats. However, at the same time, this hyperexcitability is controlled to an important degree by functional GABA_A-mediated synaptic inhibition.

INTRODUCTION

Although studies in adult animals have convincingly demonstrated that severe seizures (i.e., status epilepticus) can lead to chronic epilepsy (Dudek et al. 1994; Lemos and Cavalheiro 1995; Tauck and Nadler 1985), relatively few studies have been conducted in immature animals. This is somewhat surprising because many individuals with intractable temporal lobe epilepsy have a history of severe febrile seizures in early life (Falcoer et al. 1964; French et al. 1993). It has long been suspected that such seizures damage the developing brain, particularly the hippocampus, and consequently produce an epileptic focus in the temporal lobe. However this issue has been and remains controversial. It is often argued that febrile seizures observed in infants and young children may be the product of a preexisting abnormality that renders the developing brain, particularly the hippocampus, unusually prone to seizures. It is further argued that this same condition may be responsible for chronic epilepsy later in life.

Because of limitations inherent in clinical studies, animal studies are essential to further an understanding of the consequences early-life seizures have on brain development. In this regard, a number of laboratories have examined the effects of kainic acid and pilocarpine-induced status epilepticus in immature rats (Cavalheiro et al. 1987; Okada et al. 1984; Tremblay et al. 1984). Results suggest that status epilepticus during postnatal weeks 1 and 2 does not produce chronic epilepsy or even alter seizure susceptibility later in life (Holmes and Thompson 1988; Okada et al. 1984; Staffstrom et al. 1992). On the other hand, studies of amygdaloid-kindled infant rats (Moshe and Albala 1982, 1983) and kindled infant rats (Moshe and Albala 1982, 1983) show that these animals have either a permanent enhancement in seizure susceptibility or unprovoked seizures in adulthood.

In adult rats, small quantities of tetanus toxin locally injected into hippocampus or neocortex have been shown to produce a chronic epileptic condition (Brener et al. 1991; Mellanby et al. 1977; Jefferys and Williams 1987). For instance, after hippocampal injection, spontaneous generalized seizures are observed for 6–8 wk. When in vitro slices are taken from the tetanus toxin-injected or contralateral hippocampus, epileptiform activity is recorded in the CA3 subfield (Jefferys 1989). Thus this model has substantial experimental utility because it permits examination of the cellular and biophysical mechanisms that underlie epileptiform discharging in chronic epilepsy. Although similar in vitro approaches have been taken in kindling (Traynelis et al. 1989) and status epilepticus (Bekenstein and Lothman 1993; Wuarin and Dudek 1996) models of chronic epilepsy, epileptiform discharges that presumably occur in vivo are rarely observed in slice preparations. Consequently, numerous in vitro slice studies of hippocampus and neocortex from tetanus toxin-injected adult rats have been conducted to un-

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understand the origins of abnormal discharging. Results from these studies appear to favor the notion that a deficit in synaptic inhibition plays a major role in the seizures occurring in these animals (Jordan and Jefferys 1992; Whittington and Jefferys 1994). Paradoxically, the amplitude of γ-aminobutyric acid (GABA<sub>A</sub>) receptor-mediated monosynaptic inhibitory postsynaptic potentials (IPSPs) was found to be normal or supernormal in slices from epileptic rats. However orthodromically elicited polysynaptic inhibition was profoundly reduced. This suggests that weakened excitation of inhibitory interneurons may be an underlying pathogenic mechanism.

To explore the consequences of early-life seizures on brain development, we developed a tetanus toxin model in infant rats (Lee et al. 1995). Previous results have shown that after a unilateral injection of tetanus toxin on day 10, rats develop frequent seizures (on average 1/h) that are typically 30 s to 2 min in duration and persist for 1 wk. Most commonly, these consist of repetitive wet dog shakes and wild running seizures. Electroencephalographic (EEG) recordings have revealed electrographic seizures that accompany these behaviors. Seizures arise not only from the injected hippocampus but from contralateral hippocampus and bilaterally in neocortex. Multiple independent epileptic foci characterize the recordings during the interictal period. In adulthood, the majority of these rats have a chronic focal epilepsy that persists beyond 6 mo and is likely a life-long condition (Anderson et al. 1995). Histological examination of the brains of these rats show no gross signs of pathology or apparent cell loss in the hippocampus or dentate gyrus (Lee et al. 1995). In adults, EEG recordings show that interictal and ictal discharges arise from the hippocampus both ipsilateral and contralateral to the site of tetanus toxin injection. Furthermore when slices are taken from adult rats that had tetanus toxin-induced seizures as infants, they exhibit spontaneous epileptiform discharges (Lee et al. 1995). Experiments reported here confirm and extend this latter observation and describe abnormalities in local circuit discharging in detail, as well as fundamental mechanisms that may contribute to their generation. Some of this work was presented in abstract form (Smith et al. 1995a).

**METHODS**

**Stereotoxic tetanus toxin injections**

Wistar rat pups (Harlan, Indianapolis, IN), 10 days of age, were anesthetized with an intraperitoneal injection of ketamine/xylazine (33 and 1.5 mg/kg, respectively). This dosage was supplemented with inhaled metofane (Pittman-Moore, Terre Haute, IN) when necessary. The surgical procedures used were approved by an institutional animal care committee and were in keeping with guidelines established by the National Institute of Health. First, a unilateral, intrahippocampal injection of tetanus toxin was performed as described previously (Lee et al. 1995). The tetanus toxin was a gift of the Massachusetts State Biological Labs. We assessed the potency of the toxin in mice by assaying its ability to produce paralysis of the right hind limb after injection in the gastronemius muscle. The minimal effective dose (MD<sub>50</sub>) that produced paralysis in all mice in a group was 0.25 ng.

To inject the toxin intrahippocampally, a rat was placed in a stereotoxic headholder, a midline incision was made and a small hole was drilled in the skull overlaying the right CA<sub>1</sub> hippocampal subfield. Two and one-half or five nanograms of tetanus toxin, dissolved in 20–40 nL of sterile saline, were slowly (4 nL/min) injected into the hippocampus via a 0.5-μL syringe fitted with a 31-gauge needle. The stereotactic coordinates for injections were AP, 2.1 mm; ML, 3.0 mm from the bregma; and DV, 2.95 mm from the dural surface. After an injection, the needle was left in place for 15 min to allow diffusion and reduce reflux up the needle track. Litter mates or age-matched pups, stereotaxically injected with the saline vehicle (n = 10) and untreated rats (n = 44) served as controls. During these procedures, all rat pups laid on a warmed metal plate, which maintained body temperature.

**Monitoring of behavioral seizures**

Rat pups were monitored for signs of behavioral seizures 1 h per day on 10 consecutive days after tetanus toxin injections. The types and severity of behavioral seizures were observed and the number of easily identified wild running seizures was counted. Rats that were observed to have two or more wild running seizures during these observation periods were selected for in vitro slice studies.

**In vitro slice procedures**

Hippocampal slices were prepared from rats once they reached young adulthood (n = 99; experiments, 45; litter mate or age-matched controls, 54). Rats ranged in age from 31 to 75 days for both experimental and control rats. Slices were prepared by methods previously described (Smith et al. 1995b; Swann et al. 1993). After anesthesia with metofane, the forebrain was removed and rinsed in oxygenated artificial cerebrospinal fluid (ACSF; see composition below). The right and left hemispheres were separated, and two researchers worked concurrently to prepare slices (500-μm thick) from the hippocampal contralateral and ipsilateral to the site of tetanus toxin injection. Three to five slices were taken from each hippocampus. Slices were usually obtained from the dorsal hippocampus. Slices from control rats were routinely placed along side slices from experimental rats so direct comparisons could be made under identical experimental conditions.

Slices were placed in an interface chamber, where they rested between an overlying humidified gas of 95% O<sub>2</sub>-5% CO<sub>2</sub> and a circulating perfusate. The chamber was constantly perfused (≈1 ml/min), with ACSF having the following composition (in mM): 123 NaCl, 3.5 KCl, 1.5 CaCl<sub>2</sub>, 1.5 MgSO<sub>4</sub>, 1.25 NaHPO<sub>4</sub>, 26.0 NaHCO<sub>3</sub>, and 10 glucose. The perfusate was bubbled with 95% O<sub>2</sub>-5% CO<sub>2</sub>. Temperatures was maintained at 32 ± 33°C. During the course of some experiments, the perfusate was switched to ACSF containing 20 μM of picrotoxin (Sigma Chemical, St. Louis, MO).

**Electrophysiological recordings**

After their preparation, the slices were allowed to recover for 1 h before electrophysiological recordings were undertaken. Standard electrophysiological techniques were employed. Intracellular and extracellular field recordings were made with glass micropipettes. Intracellular electrodes were filled with potassium acetate (4 M) and had resistances of 70–150 MΩ. Extracellular electrodes contained NaCl (2 M) and had resistances of 5–20 MΩ. Recordings were made routinely from the pyramidal cell body layer and were referred to a remote silver-silver chloride bath ground. Once a recording session began, slices from every rat were surveyed for spontaneous epileptiform activity. This was accomplished by placing field potential electrodes at various locations within the pyramidal cell body layer and granule cell body layer of the dentate gyrus. At each location, recordings were made for 5–10 min. If no activity was observed, the electrode would be moved and recordings commenced at a new location. Once epileptiform activity was observed, the discharges would be sampled in the nearby cell body layer.
to optimize (largest amplitude) recordings. In slices in which no spontaneous activity was recorded, a bipolar stimulating electrode was used to apply square wave (100 μs) constant current (10 to 100 μA) pulses (0.1 Hz) in an attempt to initiate spontaneous activity. However, this experimental maneuver was usually not needed and often had no clear effect on experimental outcome.

In experiments in which spontaneous epileptiform bursts were mapped in detail, field potential recordings were made simultaneously with three microelectrodes. One electrode was positioned at the site where network bursts were found to be of the largest amplitude. This was always in the CA3 subfield. The other two electrodes were systematically placed at 50-μm intervals along the pyramidal and granule cell body layers. In most experiments, recordings were made from 20 to 60 different sites. Discharges occurring at each of these locations that were synchronous with the large spontaneous epileptiform discharges recorded by the stationary electrode were analyzed. When the amplitude of burst discharges was measured, the peak of the slow envelope of the burst at each location of cell body layer was estimated. Previous studies have shown that the population spikes in such recordings are a product of this slower potential that is generated independently in the dendrites of CA3 hippocampal pyramidal cells (Swann et al. 1986).

All electrophysiological data were stored on tape for later analysis. Selected signals were collected and analyzed with software developed for a personal computer. Signals were digitized at 10–40 kHz. The faster digitizing rates were needed when the time course of action potentials was studied. When action potentials were examined, selected representative single spikes (n = 10) were analyzed. The first derivative with respect to time was also computed from these spikes. To obtain prespike baseline recordings, the A/D data acquisition board was triggered by an action potential and presentation of the spike was delayed for a selected interval by an analog delay line purchased from EG & G Reticon (RD5108 and -011). When IPSFs were analyzed in area CA1, membrane potential was altered by prolonged DC current steps. In these current-clamp recordings, bridge balance was monitored at each holding potential. When slow-time base recordings were displayed, they were played back onto a Gould rectilinear pen recorder.

Statistics

Comparisons of the number of slices displaying network bursts and high-frequency field potentials (HiFFs) in area CA3 from experimental and control rats and from injected and contralateral hippocampi were done by a χ² test analysis. All other statistical analyses were performed using a t-test for comparison of two independent means.

RESULTS

Behavioral seizures in infancy

Twenty-four to 48 hours after intrahippocampal injections of tetanus toxin on postnatal day 10, 42 of 45 rat pups displayed severe and repeated behavioral seizures. Previous EEG recordings in awake, behaving rat pups have verified that these behavioral events were concurrent with electrographic seizures in hippocampus and neocortex (Anderson et al. 1997; Lee et al. 1995). The seizures usually consisted of wild running episodes, prolonged wet dog shakes, or clonic forelimb activity. Often these would not occur in isolation, but as a constellation of behaviors. For instance, a series of unusually long wet dog shakes could precede the onset of wild running seizures. At other times, a wild running seizure might terminate with a lengthy series of wet dog shakes and forelimb clonic activity. These seizures were usually one-half to 2 min in duration. Seizures lasting more than 3 min were very uncommon. Over the 10 days of behavioral monitoring, up to 11 seizures were observed during the 1 h observation periods. On average, rats were observed to have 5.3 ± 2.6 (SD) seizures during this time. Rat pups having more than two seizures during these monitoring periods were chosen for in vitro slice studies. In adulthood, some rats were observed to have unprovoked seizures. However, the frequency of these events was usually low (Anderson et al. 1995) and, consequently, adult rats were not monitored for behavioral seizures before slicing.

Extent of epileptiform activity recorded in vitro: adult hippocampus

In all experiments, extracellular field recordings were used to survey spontaneous epileptiform activity arising from hippocampal slices taken ipsilateral and contralateral to the site of tetanus toxin injection. Similar recordings were made from slices taken from control rats. Epileptiform activity was never observed in recordings from 54 control rats. In contrast, spontaneous epileptiform bursts were routinely recorded in tetanus toxin-injected rats. Network bursts were recorded in slices from 73% (33/45) of experimental rats. (χ² = 56.1, degrees of freedom = 1, P < 0.0001 when compared with 0 bursts in slices from 54 control rats). Of slices showing epileptiform activity, 36% (27/74) were from the injected and 63% (47/74) were from the contralateral hippocampus. (χ² = 9.8, degrees of freedom = 1, P < 0.01: comparing frequencies in contralateral and injected hippocampus) These results are consistent with previous EEG recordings (Anderson et al. 1995) showing epileptic foci in both hippocampi of adult rats after tetanus toxin-induced seizures in infancy. We recorded from slices taken...
from rats between 31 and 75 days of age. No differences were seen between the frequency of network bursting in slices from juvenile (31–40 days) and adult rats (40–75 days).

Abnormal network discharging: area CA3

During simultaneous intracellular and extracellular field potential recordings from slices of tetanus toxin-injected rats, discharges occurred spontaneously that shared many of the features of network bursts described previously in acute in vitro models of epilepsy (Swann et al. 1986). Recordings in Fig. 1 show representative epileptiform discharges obtained from a slice of a hippocampus that was contralateral to the site of tetanus toxin injection. These discharges varied in frequency from animal to animal. In some instances, bursts were observed as infrequently as every 30 s. However, in others, like that in Fig. 1A, they were recorded every 5 to 10 s. Coincident with extracellular recordings of epileptiform bursting (2), individual neurons (1) would undergo an intense 10- to 30-mV depolarization that was up to 150 ms in duration. These ‘depolarization shifts’ could elicit a flurry of action potentials and tended to be all-or-none in nature. However, as shown in Fig. 1B, they could vary from event to event in both duration and amplitude. Within a slice, bursts varied 30–50 ms in duration. Across experiments, bursts measured 25–150 ms in duration. The synchronous nature of these discharges is depicted by simultaneous extracellular field recordings. In the pyramidal cell body layer, these consisted of a positive-going slow field potential on which multiple population spikes would ride (Fig. 1B2). As is evident from these recordings, the extracellular fields potentials varied in duration concurrent with simultaneous intracellular recordings.

Our initial recordings suggested that network bursts did not occur uniformly in a hippocampal slice but were largest in a circumscribed region of the CA3 subfield. To determine the location of the most intense network bursting and thus compare epileptiform discharging from animal to animal, we mapped discharges at ~50-μm intervals along the pyramidal cell body layer. Figure 2B summarizes the results of five separate experiments in which the peak amplitude of the slow envelope of the epileptiform burst was plotted against distance along the pyramidal cell body layer. Representative recordings from one of these experiments are shown in Fig. 2C and are referred to recording positions in the drawing in Fig. 2A. Epileptiform bursts were largest in the CA3 subfield as the pyramidal cell body layer courses between the upper and lower blades of the granule cell body layer. This region is commonly referred to as the hippocampal CA3C subfield. Discharges were substantially smaller in CA3A and CA3B (electrode positions 4, 5, and 6). In CA1 (position 7), they were even smaller and often absent. For example, in the five slices mapped systematically in Fig. 2, only one slice displayed a burst discharge in area CA1. Network bursts were always absent in the granule cell body layer of the dentate gyrus.

We next compared intracellular recordings in areas CA3A, CA3B, CA3C, and CA1 concurrent with epileptiform discharges recorded extracellularly in CA3C. Figure 3 shows the results from one of these experiments. Intracellular recordings of epileptiform bursting from area CA3C were usually larger than those in other areas of the CA3 subfield and area CA1. As depicted in Fig. 3A, middle and bottom, often only a burst of synaptic potentials was recorded in CA3B, CA3A (data not shown), and CA1, not a depolarization shift. On occasion, these synaptic events were large enough to elicit an action potential (left).

Abnormal network discharging: area CA1

During extracellular mapping of epileptiform bursting, recordings were also made of a novel extracellular event that

![Figure 2](http://jn.physiology.org/) Analysis of network bursts recorded extracellularly in pyramidal cell body layer in experimental rats. A: approximate sites (1–7) where extracellular field recordings in C were made. B: peak amplitude of burst at 31 locations in 5 separate slices is plotted. Data are normalized to largest potential recorded in each slice and plotted as mean ± SE on y-axis. Distance along pyramidal cells body layer from its end in hilus of dentate gyrus to CA3/subiculum interface is plotted on x-axis (tick marks = 200 μm). Electrode positions (1 to 7) in A are shown above graph in B. Data were obtained from 2 tetanus toxin-injected and 3 contralateral hippocampi. During all recording sessions, events recorded at each site were compared with simultaneous recordings made from a stationary electrode in cell body layer. This was located at site where network bursting was most robust.
Intracellular recordings from CA1 pyramidal cells were followed by a large prolonged afterhyperpolarization. We called them high-frequency field potentials, or HiFFs. The novel field events are shown in Fig. 4, where spikes decreased in amplitude and increased in duration. As shown in Fig. 4A1, these events prevented spontaneous action potential discharging and reversed polarity between -70 and -80 mV (Fig. 4, B and C). Their frequency was unaltered by changes in membrane potential. Thus they appeared to be large summed ipsps and likely the product of the synchronous discharging of inhibitory interneurons. Recordings were made from nine CA1 pyramidal cells. All revealed large irdps coincident with the occurrence of HiFFs. When recordings were obtained from four CA1 pyramidal cells in one slice, they all underwent similar irdps in concert with the generation of HiFFs.

**Intrinsic properties of CA3C pyramidal neurons**

Because epileptiform network bursting in area CA3C was so robust and occurred in slices from the majority of experimental rats, we focused our attention on understanding the biological origins of these epileptiform events. The passive and active membrane properties of individual neurons were compared in injected and contralateral hippocampus that underwent epileptiform bursts. These measurements were compared with those obtained from control rats. Results in Table 1 show that all measures made in injected and contralateral hippocampus from experimental rats were similar to those obtained from controls. These included resting membrane potential, input resistance, and measures of action potential amplitude, duration, and rates of rise and fall.

Further analysis of the recordings showed that in the injected hippocampus of experimental rats, 4 of 10 cells had intrinsic burst properties. This compares with 3 of 10 in contralateral hippocampus and 4 of 10 in slices from control rats. In the injected hippocampus, 3 of the 4 cells displayed unusually prolonged bursts.

In control rats, bursting cells often generated spontaneous bursts of two to four action potentials. The spike complex consisted of action potentials that decremented in amplitude and increased in duration as they rode the envelope of a slower depolarizing potential. During the course of these bursts, the also prominent in the subiculum (data not shown). Examples of recordings of the novel field events are shown in Fig. 4, trace 2. We called them high-frequency field potentials, or HiFFs. Intracellular recordings from CA1 pyramidal cells showed that simultaneous with HiFFs, individual pyramidal cells underwent a rapid hyperpolarization. When neurons were held slightly depolarized (2–5 mV) from the resting membrane potential, these hyperpolarizing potentials were quite large, often 5–10 mV in amplitude. As shown in Fig. 4A1, these events prevented spontaneous action potential discharging and reversed polarity between -70 and -80 mV (Fig. 4, B and C). Their frequency was unaltered by changes in membrane potential. Thus they appeared to be large summed irdps and likely the product of the synchronous discharging of inhibitory interneurons. Recordings were made from nine CA1 pyramidal cells. All revealed large irdps coincident with the occurrence of HiFFs. When recordings were obtained from four CA1 pyramidal cells in one slice, they all underwent similar irdps in concert with the generation of HiFFs.

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In control rats, bursting cells often generated spontaneous bursts of two to four action potentials. The spike complex consisted of action potentials that decremented in amplitude and increased in duration as they rode the envelope of a slower depolarizing potential. An example of a spontaneous intrinsic burst recorded from a slice taken from a control rat is shown in Fig. 5A. When bursting cells in slices from a control rat were injected with brief depolarizing current steps, a distinct threshold for burst generation was observed, during which a burst would occur in an all-or-none manner (see Fig. 5C). Bursts were followed by a prolonged afterhyperpolarization, which can be seen in the lower trace of Fig. 5A.

In experimental rats, intrinsic bursts were also generated in an all-or-none manner. Examples are shown in Fig. 5, B and D. Like bursts in slices from control rats, these events in experimental specimens also appeared to arise from an underlying slow depolarization. However, this event was unusually intense and prolonged (>100 ms) in three cells. As many as 8–10 action potentials rode the envelope of this depolarizing potential. During the course of these bursts, the spikes decreased in amplitude and increased in duration. As in cells, from control rats, the intrinsic bursts in epileptic rats were followed by a large prolonged afterhyperpolarization.
EPILEPSY INITIATED IN INFANCY

Fig. 4. Novel extracellular field potentials in area CA1 are coincident with rhythmic ipsips recorded intracellularly. Simultaneous intracellular (1) and extracellular (2) field recordings in A illustrate high-frequency field potentials recorded in CA1 pyramidal cell body layer, which were coincident with large inhibitory postsynaptic potential-like hyperpolarizing potentials recorded intracellularly (membrane potential, −64 mV; holding potential, −59 mV; input resistance, 40 MΩ; postnatal age, 39 days). B: results from same experiment in which amplitude (mean ± SE) of these potentials was plotted (y-axis) vs. membrane potential (x-axis). Amplitude is a linear function of holding potential and events reversed polarity near −76 mV. C: selected intracellular recordings (1) at different holding potentials. Only intracellular events recorded simultaneously with an extracellular field (e.g., trace 2) were analyzed.

(bottom, Fig. 5B). Figure 5D illustrates a prolonged intrinsic burst in a slice from an experimental rat that was evoked in an all-or-none manner by a brief depolarizing current step. A comparison of the duration of intrinsic bursts yielded average maximal duration of 82.5 ± 6.3 (SE) in slices from control rats, 70.0 ± 11.5 and 92.5 ± 11.1 in slices from contralateral and injected hippocampus from experimental rats respectively. These values did not differ from one another statistically (P > 0.05).

Local circuit inhibition and CA3 network hyperexcitability

Another series of experiments attempted to determine if epileptiform bursting in area CA3C of experimental rats might be attributed, at least in part, to enhanced excitability within CA3 recurrent excitatory networks. Network excitability in epileptic rats could be the product of changes in intrinsic excitability of individual pyramidal cells and/or hyperinteractivity via excessive or enhanced recurrent excitatory synapses. To test this hypothesis, picrotoxin was bath applied to slices from both experimental and control rats. We reasoned that if CA3 recurrent excitatory networks were hyperexcitable in epileptic rats, then elimination of GABA A IPSPs would result in epileptiform discharging that was far more intense than that seen in slices from control rats under the same conditions.

An example of recordings obtained is shown in Fig. 6. At the beginning of the top trace, spontaneous epileptiform bursting was recorded extracellularly in area CA3C in a slice from an experimental rat. Soon thereafter, picrotoxin was bath applied (↓). As the drug took effect, burst discharging grew markedly in amplitude and duration, and within 12 min an electrographic seizure (3) was recorded. Sections of the slower time base recordings 1–3 are shown below at a faster time base.

Figure 7 compares epileptiform discharges induced by picrotoxin in slices from experimental and litter-mate, saline-injected control rats. In slices from control rats, brief burst

TABLE 1. Comparison of the neurophysiological properties of CA3C pyramidal cells in experimental and control rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Tetanus Toxin-Injected</th>
<th>Contralateral</th>
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<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Vm, mV</td>
<td>−63.8 ± 4.2</td>
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<td>Rm, MΩ</td>
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<td>Spike duration, 1/2 amplitude, ms</td>
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<tr>
<td>Dmax, V/s</td>
<td>269.1 ± 46.4</td>
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<td>Dmin, V/s</td>
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<td>dV/dt, ratio</td>
<td>−1.83 ± 0.13</td>
<td>−1.89 ± 0.35</td>
<td>−1.84 ± 0.25</td>
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Except in n (number of cells) row, data are mean ± SD. Differences between all measures made in control and tetanus toxin-injected or contralateral hippocampus were not significant (P > 0.05).
duces a severe but transient convulsive syndrome in infancy also results in marked abnormalities in local circuit functioning in the hippocampal slices taken from rats once they reach adulthood. Two forms of network abnormalities are observed. In area CA3, spontaneous epileptiform network bursting was common. Less frequently, but in a significant number of rats, we also recorded spontaneous rhythmically occurring IPSPs in CA1 pyramidal cells. At least conceptually, these two types of network discharges could oppose each other in terms of hippocampal functioning in chronic epilepsy. Although network bursting in area CA3 would be expected to contribute to seizures observed in these animals, the abnormal IPSPs recorded in area CA1 may counter or prevent the spread of epileptiform activity from area CA3 to subiculum and the entorhinal cortex.

The developing hippocampus and chronic epilepsy

This is the first experimental model of epilepsy in which seizures in early life are reported to result in abnormalities in local circuit activity in adulthood. In general, previous studies employed two different types of models, status epilepticus and kindling, in an attempt to examine the effects of seizures in early life on the developing brain. In terms of models of status epilepticus, 2-wk-old rats are very sensitive to the kainate and pilocarpine. Nonetheless, these seizures fail to produce noticeable histological damage to hippocampus and rats neither become chronically epileptic nor have a lowered threshold for experimentally induced seizure (Caivalheiro et al. 1987; Okada et al. 1984; Stafstrom et al. 1992; Wasterlain and Shirasaka 1994). In contrast to these findings are results from exposure to hypoxia in infancy (Jensen et al. 1992). Rat pups that have seizures in response to hypoxia mature to have a lowered threshold to convulsants. Alterations in seizure susceptibility were also reported after kindling in infant rats. Brief but repeated seizures in 2-wk-old rats lower seizure threshold in adulthood (Moshe and Albala 1982, 1983). Other studies have shown that amygdala kindling in kittens produces a chronic form of severe epilepsy (Shouse et al. 1990, 1992). As adults, these cats display spontaneous focal seizures and have 10–20 unprovoked seizures per day. Thus even though studies of status epilepticus suggest that a single seizure, even with a prolonged time course (several hours), has little if any lasting effect on the developing CNS, the kindling model suggests that brief and repeated seizures may have severe long-lasting consequences.

Considering these findings, it is perhaps not surprising that tetanus toxin-injected rat pups that have many seizures in the week after intrahippocampal injections would be chronically epileptic in adulthood. In kindling studies, rat pups that experience only three generalized convulsions had increased vulnerability to kindled convulsions in adulthood (Moshe and Albala 1982). Recent long-term video monitoring of tetanus toxin-injected rat pups suggests that they experience ~100 wild running seizures during the week after tetanus toxin injections (our unpublished observation). Thus the severity of the convulsive syndrome experienced by tetanus toxin-injected rat pups greatly exceeds that experienced in kindling studies.
Abnormal network bursting in area CA$_{3C}$: localization

The recordings from in vitro slice preparations reported here fully support the notion that severe early-life seizures can result in a chronic epileptic syndrome and are consistent with recent EEG recordings from awake-behaving adult rats (6 mo of age) that were injected with tetanus toxin as infants.

Eighty-five percent were found to have interictal discharge, and 58% demonstrated unprovoked electrographic seizures (Anderson et al. 1995). Interictal discharges were recorded in the injected and contralateral hippocampus in vivo (Anderson et al. 1995), as they were observed in this study. Similar multifocal discharging was observed in adult rats injected with tetanus toxin (Brener et al. 1991; Hawkins and Mellanby 1987). The presence of abnormal network bursting arising in a focus in contralateral hippocampus could be a product of a "kindlinglike" process initiated by seizures that secondarily generalize from the epileptic focus in the injected hippocampus. However it is also possible that tetanus toxin is retrogradely transported (Habermann and Erdmann 1978) to the contralateral hippocampus via commissural fibers and that the transported toxin produces the second focus in the contralateral hippocampus.

Regarding the mechanisms that underlie epileptogenesis in contralateral hippocampus, results suggest that network bursts occur to a greater extent there than in the injected hippocampus. Tetanus toxin is known to preferentially block the release of the inhibitory neurotransmitters, e.g., GABA (Bergey et al. 1983, 1987; Williamson et al. 1992). However, at higher concentrations it can also suppress glutamate release (Albus and Habermann 1983; Schiavo et al. 1992). It seems plausible that at the injection site in area CA$_3$, the toxin levels may be sufficiently high to suppress excitatory amino-acid synaptic transmission. In keeping with this notion is our observation that the toxin transiently suppresses the background EEG in the injected hippocampus (Lee et al. 1995). If glutamate release is compromised locally, it might hinder the processes contributing to epileptogenesis in injected hippocampus. However this would be expected to occur to a lesser extent in the contralateral hemisphere.

Perhaps one of the more surprising aspects of the results presented here is that epileptiform activity was largest in a rather restricted area of CA$_3$, subfield, area CA$_{3C}$. This was true for both ipsilateral and contralateral hippocampus. Thus the cellular abnormalities responsible for network bursting do not appear to be uniformly distributed in the hippocampus or even in the CA$_3$ subfield. Previously, our laboratory conducted extensive mapping of network burst discharging in hippocampal slices that were taken from normal adult rats.
and treated with a GABA$_A$ receptor antagonist (Swann et al. 1986). The results suggested that burst discharges were quite uniform in amplitude within a CA$_3$ subfield. That is, unlike results presented in Fig. 2, field potentials in normal rats are similar in size and wave form whether recorded in subfields CA$_{3A}$, CA$_{3B}$, or CA$_{3C}$. The localized nature of bursting in our model would suggest that the underlying network abnormalities are localized to a subregion of area CA$_3$. The reason for such a regionalized difference is unknown at this time. In contrast to findings in our model of early-onset epilepsy, Empson and Jefferys (1993) reported that in hippocampal slices from adult rats injected with tetanus toxin, epileptiform bursts were generated uniformly in all regions of the CA$_3$ subfield. However an earlier paper (Jefferys 1989) that analyzed times of onset of bursts suggested they were initiated in between CA$_{3B}$ and CA$_{3C}$.

Abnormal network bursting in area CA$_{3c}$: potential mechanisms

SYNAPTIC INHIBITION. One potential mechanism that could be responsible for network bursting in area CA$_3$ is the chronic suppression of synaptic inhibition. Experiments conducted did not address this issue directly. However, results in Figs. 6 and 7 suggest that GABA$_A$ mediated synaptic inhibition is present and effective in hippocampi from experimental rats. Indeed, inhibitory synaptic transmission mediated by GABA appears to be an important in controlling excitability and preventing the generation of unusually intense electrographic seizure discharges in these animals. These results also suggest that abnormalities within CA$_3$ recurrent excitatory networks contribute importantly to hippocampal seizures in this chronic model of early-onset epilepsy. However, they cannot completely rule out a defect in local circuit inhibition as another contributing factor to seizure generation.

In contrast with these findings, Jefferys and coworkers (Jordan and Jefferys 1992; Whittington and Jefferys 1994) studied the mechanisms that underlie a chronic epilepsy that persists for 6–8 wk after an intrahippocampal tetanus toxin injection in adulthood. Results suggest that a selective loss of GABA$_A$-mediated synaptic inhibition underlies epileptiform discharging. However, GABA synapses themselves appear normal because monosynaptic ipsps are unaltered. Instead, it appears that excitatory activation of inhibitory interneurons may be substantially reduced in epileptic rats. Whether or not an additional enhancement of activity within recurrent excitatory networks may also contribute to these discharges, as we suggest in the tetanus toxin model of early-onset epilepsy, apparently has not been explored.

INTRINSIC PROPERTIES OF CA$_{3C}$ PYRAMIDAL CELLS. The intrinsic properties of CA$_3$ pyramidal cells in epileptic rats were quite comparable with those obtained from control rats. At the time of tetanus toxin injection (postnatal day 10), when this epileptic syndrome was initiated, the electrophysiological properties of CA$_3$ pyramidal cells are markedly different from those of adulthood. For instance, previous studies in our laboratory reported the duration (at one-half-maximal amplitude) of action potentials in immature bursting cells to be $\sim 0.90$ ms (Smith et al. 1995b), compared with $0.54 \pm 0.13$ ms in tetanus toxin-injected hippocampus from adult epileptic rats, which is identical to that in control rats. Similar age-related differences are seen when the rates of rise and fall of action potentials are compared, but no differences are seen between these measures in experimental and control rats (Table 1). This leads us to conclude that the epileptiform discharges that likely occur throughout the life of the epileptic rat did not interfere with the normal maturation of at least some of the basic biophysical properties of hippocampal pyramidal cells.

A few pyramidal cells in slices taken from the injected hippocampus had unusually prolonged intrinsic bursts (Fig. 5). Hyperrexcitability of individual pyramidal cells could be responsible for network bursting in epileptic rats. At the present time, however, we discount this as the major mechanism underlying hippocampal seizures in these rats. While bursts shown in Fig. 5, B and D, are impressive, the average duration of the events were not statistically different from those recorded in control rats. Moreover, pyramidal cells in contralateral hippocampus had intrinsic burst durations that were not different from those recorded in control rats. However, slices of contralateral hippocampus were more likely to generate network bursts than those taken from the injected hippocampus. If an intrinsic hyperrexcitability of individual cells is to produce network bursting, then our recording should have shown a higher frequency of bursting cells or more intense bursting in contralateral hippocampus. This was not the case. However, our analysis was limited because of the small number of cells examined. Future studies will need to address this issue in greater detail.

RECURRENT EXCITATION. Another mechanism that could account for hippocampal seizures in this chronic model is an enhancement of recurrent excitatory synaptic transmission in area CA$_3$. Early in life, indeed at the time of tetanus toxin injection, the hippocampus has a marked propensity for seizures (Swann and Brady 1984). For instance, when slices are taken from normal infant rats (9–19 days of age) and exposed to GABA$_A$ receptor antagonist, they undergo prolonged electrographic seizures. These discharges, which are blocked by excitatory amino-acid receptor antagonist (Brady and Swann 1988; Gomez-DiCesare et al. 1997), are like those seen in slices from experimental rats reported here (Figs. 6 and 7) after picrotoxin application. Thus at least superficially, recurrent excitatory networks that mediate seizures in area CA$_3$ in infant and chronically epileptic rats share some basic physiological properties. The developmental critical period of seizure susceptibility was shown to correspond to a time of massive outgrowth of recurrent excitatory collaterals from CA$_3$ hippocampal pyramidal cells (Gomez-DiCesare et al. 1997). This outgrowth is followed by axonal remodeling where 50% of axon branches appear to be pruned. In many areas of the CNS, especially the visual system, this type of axonal remodeling has been shown to be dependent on action-potential based neuronal activity (Katz and Shatz 1996). Synchronous activiation of synapses has been shown to prevent their age-dependent remodeling (Stryker and Strickland 1984). Thus it is conceivable that the synchronous discharges of seizures could prevent axon pruning during maturation of CA$_3$ pyramidal cells and lead to a hyperinnervate and hyperrexcitable CA$_3$ subfield (Swann 1995). Tests of this hypothesis must await anatomic studies of intracellularly filled cells and paired recordings from pyramidal cells in epileptic rats.
Local circuits and synaptic inhibition: area CA1

During field potential mapping of epileptiform bursts, potentials of higher frequency and smaller amplitude were unexpectedly recorded in area CA1 (Fig. 4). Intracellular recordings show that these are correlated and likely produced by synchronous generation of large IPSPs in CA1 hippocampal pyramidial cells. Similar events have not been reported in rats treated in adulthood with tetanus toxin. The origins of these events are unknown at this time. A previous in vitro study of tissue obtained during human epilepsy surgery reported similar intracellular events (Schwartzkroin and Knowles 1984). However, a follow-up study in primates suggested that these events could be recorded in slices from nonepileptic neocortical tissue (Schwartzkroin and Haglund 1986). In our studies, we have never recorded such events in slices of control rats. Thus we feel that they are associated with an underlying epileptic syndrome. As mentioned earlier, these events could be a compensatory response of the hippocampus in an attempt to prevent the spread of seizure discharges to entorhinal cortex and thus limit seizure generalization to the rest of the nervous system. How inhibitory local circuitry is altered to produce these events and why they are observed in a minority of slice preparations are unknown at this time.

The frequency of these events is very similar to that of the hippocampal theta rhythms (4–10 Hz). Interneurons have been implicated in the generation of synchronous oscillations in the CNS (Traub et al. 1996). Moreover, theta activity has been correlated with the firing of hippocampal inhibitory interneurons and the generation of IPSPs in pyramidial cells (Mody and Soltesz 1993; Buzsáki et al. 1983). The rhythmicity of theta activity is thought to be controlled by neurons of the medial septum. Projections from the septum directly to populations of inhibitory interneurons are thought to be necessary for the generation of hippocampal theta (Freund and Antal 1988). On this basis it is not surprising that rhythmic activity like theta is rarely recorded in vitro and then only under very specialized conditions. The occurrence of similar events in slices of hippocampus of epileptic rats could be purely coincidental or it could be that patterns of local circuit connectivity or properties of interneurons are altered during development to generate physiological events reminiscent of those recorded in vivo during hippocampal theta rhythms. Whether or not the rhythmic ipsp recorded in epileptic rats and theta activity share underlying mechanisms must await further study. However, they likely subserve quite different functions in the normal and epileptic brain.

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