Seizure-Like Events in Disinhibited Ventral Slices of Adult Rat Hippocampus

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Borck, Cornelius and John G. R. Jefferys. Seizure-like events in disinhibited ventral slices of adult rat hippocampus. J. Neurophysiol. 82: 2130–2142, 1999. Epileptic discharges lasting 2–90 s, were studied in vitro in slices from the ventral hippocampus of adult rats, in which inhibition was blocked acutely with bicuculline methiodide (BMI, 5–30 μM) and potassium (K\(^+\)) raised to 5 mM. These seizure-like events (SLEs) comprised three distinct phases, called here primary, secondary, and tertiary bursts. Primary bursts lasted 90–150 ms. Secondary bursts lasted a further 70–250 ms, comprising a short series of afterdischarges riding on the same depolarization as the primary burst. Finally a train of tertiary bursts started with a peak frequency of 5–10 Hz and could last >1 min. Slices from the ventral hippocampus showed significantly higher susceptibility to SLEs than did dorsal slices. SLEs proved sensitive to α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) receptor antagonists. They were insensitive to N-methyl-D-aspartate (NMDA) receptor antagonists; 50 μM D-2-amino-5-phosphonopentanoic acid (D-AP5) did block the transient secondary bursts selectively. SLEs were restricted to the hippocampus proper even if the entorhinal cortex was present. Entorhinal bursts could last <2 s and were only coupled with hippocampal bursts in a minority of slices. Reentry of epileptic bursts occasionally occurred during interictal discharges, but not during the later stages of SLEs. Full-length SLEs always started in CA3 region and could be recorded in minislices containing CA3 plus dentate hilus. Ion-sensitive microelectrodes revealed that interictal discharges were followed by short (2–3 s) [K\(^+\)]\(_o\) waves, peaking at ~7.5 mM. SLEs were always accompanied by increases in [K\(^+\)]\(_o\), reaching ~8.5 mM at the start of tertiary bursts; [K\(^+\)]\(_o\) then increased more slowly to a ceiling of 11–12 mM. After the end of each SLE, [K\(^+\)]\(_o\) fell back to baseline within 10–15 s. SLEs were accompanied by significant increase in synaptic activity, compared with baseline and/or interictal activity, estimated by the variance of the intracellular signal in the absence of epileptic bursts and action potentials (0.38 mV\(^2\), compared with 0.13 mV\(^2\), and 0.1 mV\(^2\), respectively). No significant increases were observed in the interval preceding spontaneous interictal activity. These studies show that focal assemblies of hippocampal neurons, without long reentrant loops, are sufficient for the generation of SLEs. We propose that a key factor in the transition from interictal activity to SLEs is an increase in axonal and terminal excitability, resulting, at least in part, from elevations in [K\(^+\)]\(_o\).

INTRODUCTION

Over the last decades the understanding of epileptic discharges has grown enormously. Many factors involved in the generation and synchronization of bursts have been identified from the study of animal models. Blocking synaptic inhibition provides one of the most extensively studied models of epilepsy. Application of penicillin, picrotoxin, or bicuculline, to slices from adult rodents, blocks GABA\(_A\)-mediated inhibition and induces brief synchronized population bursts, often followed by a series of shorter secondary bursts (afterdischarges), lasting a few hundred milliseconds overall (Hablitz 1984; Schwartzkroin and Prince 1977; Traub et al. 1993b).

The current understanding of single-channel physiology and intrinsic bursting properties of pyramidal cells makes clear that a seizure is not just an enormously prolonged single burst; the conductances involved have time constants much shorter than the duration of ictal (seizure) events. An intermediate type of epileptic activity, between the single bursts and the full-blown seizure, is the epileptic discharge with afterdischarges (or secondary bursts), which has been studied in some detail in brain slices in which GABAergic inhibition has been blocked by drugs (Traub and Jefferys 1994b; Traub et al. 1993a,b, 1987; Wong et al. 1986). As shown by experiments and computer simulation studies, afterdischarges are generated when the initial excitation becomes strong enough to activate voltage-dependent Ca\(^{2+}\) and K\(^+\) currents, which form an intrinsic oscillator in pyramidal cells. This intrinsic oscillator mechanism continues to operate as long as a sufficient excitatory drive is provided, in this case by N-methyl-D-aspartate (NMDA) receptors. Oscillations are coupled between pyramidal neurons by the excitatory connections between them (Traub and Jefferys 1994a; Traub et al. 1993a,b).

Ictal events lasting tens of seconds are less well understood. One reason for this lack of understanding is that seizure-like events (SLEs) have been reported only sporadically for several epilepsy models in vitro (Anderson et al. 1990; Lee et al. 1995; Lewis et al. 1990; Louvel et al. 1994; Merlin and Wong 1997; Stashkeff et al. 1993; Swann et al. 1993; Traynelis and Dingle 1989).

In slices taken from immature hippocampus (10–16 day-old rats), disinhibition with penicillin caused prolonged synchronized discharges with a series of afterdischarges lasting up to 30 s (Smith et al. 1995; Swann and Brady 1984). These neonatal SLEs are generated by a large and prolonged depolarization of so-called “generator cells,” providing the required excitatory drive. The neonatal hippocampus is also more susceptible to chronic epileptic activity. Injecting tetanus toxin into the hippocampi of neonates can lead to permanent changes in epileptogenesis (Lee et al. 1995), much more reliably than...
the same treatment in adults (Hawkins and Mellanby 1987; Whittington and Jefferys 1994).

High extracellular potassium concentrations (>8.5 mM KCl) induce epileptic discharges in vitro and can result in SLEs (Jensen and Yaari 1988; Traynelis and Dingledine 1988, 1989). These high-[K+]_o ictal events occur in CA1 and most likely rely on nonsynaptic mechanisms (Haas and Jefferys 1984; Jefferys 1995; Jefferys and Haas 1982; Taylor and Dudek 1982), but they are triggered by a synaptic input from CA3 that shows continuous firing of rhythmic interictal activity (Traynelis and Dingledine 1988).

Entorhinal cortex slices can generate SLEs in response to several convulsants, including 4-aminopyridine and low Mg^{2+} (Jones 1989; Lopantsev and Avoli 1998). Several authors have suggested that reentrant activity, for instance between entorhinal cortex and hippocampus, may be required for ictal activity (Lothman 1994; Paré et al. 1992). Others suggest that ictal activity from CA3 may restrain ictal bursts starting in entorhinal cortex (Barbarosie and Avoli 1997).

Here we show that disinhibition, in combination with slightly elevated potassium levels, caused ictal-like events in the CA3 region of ventral hippocampal slices from adult rats. These events lasted up to 90 s and comprised three morphologically distinct phases. One of the characteristics of these long-lasting ictal-like events is a long series of afterdischarges at a frequency of ~2–5 Hz with increased levels of synaptic activity between them. We propose a prominent role of this synaptic “noise” as a mechanism for the generation of the series of afterdischarges.

**METHODS**

Adult male Sprague Dawley rats (Harlan OLAC, Bicester, UK, and Charles River, Margate, UK), weighing 250–300 g, were anesthetized by intraperitoneal injection of either 200 mg/kg of ketamine (Janssen-Cilag), or a mixture of midazolam (HynoNovel, Roche Products, Welwyn Garden City, UK) and fluanisone plus fentanyl citrate (Hypnorm, Janssen-Cilag, Sauderton, UK) to final doses of 4.1 mg/kg midazolam HCl, 8.2 mg/kg fluanisone, and 0.26 mg/kg fentanyl citrate. The rats were then killed by cervical dislocation. The recording system included an Axoprobe amplifier (Axon Instruments, Burlington, CA), Digitimer amplifiers and filters (Digitimer, Welwyn, UK), and a CED 1401 (MSDOS) computer system running SIGAVG and SPIKE2 (Cambridge Electronic Design, Cambridge, UK).

**RESULTS**

**Morphology of SLEs in ventral slices**

Recordings in CA3 from slices bathed in BMI and 5 mM KCl showed long-lasting discharges with a distinct three-phase firing pattern. These phases will be called here primary, secondary, and tertiary bursts (Fig. 1). The entire discharge usually lasted 10–30 s, but could last as long as 90 s; all but the first 100–500 ms consisted of rhythmic tertiary bursts.

The three phases of the SLE had the following characteristics. Primary bursts always presented as single bursts lasting 90–150 ms. Intracellular recordings revealed a series of action potentials riding on an underlying paroxysmal depolarization shift. The depolarization always outlasted the firing and thereby the primary burst. The primary burst was immediately followed by a short series of 2–6 afterdischarges at 20–30 Hz, lasting 30–70 ms each and 70–250 ms overall. These secondary bursts rode on the tail of the same paroxysmal depolarization shift (PDS) as the primary burst, each starting another depolarizing wave. All the principal neurons of the firing network were synchronized through primary and secondary bursts, with no impaled cell failing to participate in bursting. Secondary bursts ended when the membrane potential returned to the pre-PDS baseline potential (Fig. 1A, expanded in B).

At the end of the secondary bursts, and sometimes after a delay of 20–500 ms, started the third phase of SLEs, a train of rhythmic tertiary bursts, each of which comprised a depolarization with a few action potentials at its start (Fig. 1C). Tertiary bursts differed from secondary in that they were longer, each lasting 50–75 ms; slower, at a peak frequency of 3–7 Hz (thereby leaving discharge-free intervals of 50–300 ms); and they started from, or slightly hyperpolarized to, resting potential. A minority of recordings showed a small under-
lying depolarization under the entire train of tertiary burst of \( \approx 10 \) mV. This contrasts with the sustained depolarization seen during SLEs in neonatal hippocampal slices (Smith et al. 1995).

Each train of tertiary bursts gained its peak frequency either immediately or within the first second. It then gradually slowed down until the firing broke off at a frequency of 0.5–3 Hz; SLEs with higher peak frequencies stopped at higher frequencies. The principal cells of the firing network were synchronized throughout the entire train of tertiary bursts. All impaled cells participated in each burst. SLEs differed in overall length by nearly 100-fold. The shortest duration for the entire cycle of primary, secondary, and tertiary bursts was 0.5–0.7 s. Most SLEs lasted 5–20 s. Short SLEs differed from longer ones only in the length of the train of tertiary bursts; primary and secondary bursts and the individual tertiary bursts did not show any differences.

In 3 of all 80 slices tested (from 35 rats), the SLEs showed a more complex firing pattern with afterdischarges (secondary bursts) attached to many of the tertiary bursts. However, the three distinct phases still remained clearly distinguishable. In these cases each tertiary burst was usually followed by one afterdischarge (maximum 4). These more complex tertiary bursts lasted slightly longer (~100 ms) and were generated at a lower frequency (peak of 3.5 Hz).

Most of the SLEs shown here were elicited with single pulse stimulation (0.2 ms, 10–100 V, delivered to the perforant path in the dentate gyrus less than once every 10 min). SLEs of the kind described above were not unique to electrically evoked responses. Touching the slice with the stimulating electrode, while placing it in the perforant path region, often elicited an SLE without any electrical pulse. However, fully spontaneous SLEs were rare. During the entire series of experiments (80 slices from 35 rats), 13 spontaneous SLEs were observed of which 5 were recorded (Fig. 1D). Spontaneous SLEs resembled evoked SLEs in all respects: the three distinct phases, shapes, amplitudes, and duration. One atypical spontaneous SLE started with accelerating interictal bursting running directly into a train of tertiary bursts without primary or secondary bursts (Fig. 1E).

**Success rate of the model**

With the use of 5 mM KCl and 10–30 \( \mu \)M BMI (later experiments showed 5 \( \mu \)M to be sufficient), SLEs could be recorded in 63 of 80 (78.8%) slices from the ventral hippocampus. These SLE-positive slices came from 33 rats of 35 used (94.3%); in some experiments SLEs could only be evoked in some of the slices from the same rat, hence the lower success rate for the slices compared with that for rats. We suspect that the source and strain of rat may affect the success rate, but have not explored this systematically. The present series used Sprague Dawley rats from two separate suppliers and yielded broadly similar results.

The occurrence of SLEs depended on the slightly elevated potassium concentration and the preparation of the slices from the ventral half of the hippocampus. The success rate fell to 30.0% for ventral slices in 3 mM KCl. Of the dorsal slices only 27.3% showed long-lasting activity in 5 mM KCl and none in 3 mM KCl (Table 1). The difference in the success rate between dorsal and ventral slices, bathed in 5 mM KCl, was highly significant (\( \chi^2 \) test, \( P = 0.0001 \)). Furthermore, in two experiments (included in Table 1) ventral and dorsal slices were cut from the same rat, using one hippocampus for ventral slicing and the other for dorsal. In these experiments, all eight ventral slices of the same rats produced reliable SLEs, but only one dorsal slice (out of 7) produced a single SLE.

**Glutamate receptors and SLEs**

We, and others, have previously shown that secondary bursts in disinhibited slices depend on NMDA receptor activation and
can be blocked by antagonists such as D-AP5 (Lee and Hablitz 1990; Traub et al. 1993a,b). Here we applied 50 mM D-AP5 to determine its effects on SLEs (Fig. 2). The control responses reveal the sequence of primary, secondary, and tertiary bursts described above. D-AP5 abolished the secondary bursts (most evident in the expanded inserts in the figure; 8 of 13 slices from 9 rats), but the tertiary bursts survived (12 of 13 slices from 9 rats). On wash out, the secondary bursts recovered. There was a progressive, gradual reduction in the length of the tertiary bursts throughout the experiment of Fig. 2, but that process was unaffected by the application of D-AP5. The tertiary bursts differed from the secondary in not being sensitive to NMDA receptor antagonists; they also did not depend on secondary bursts for their initiation.

Tertiary bursts were blocked by 10–20 mM NBQX, an antagonist at a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors, along with all spontaneous epileptic activity. However, single afferent stimuli still evoked epileptic responses, lasting 50–70 ms and resembling interictal discharges (data not shown). Presumably the evoked EPSP sufficed to displace the Mg²⁺ block of the NMDA receptor and trigger the equivalent of a low-Mg²⁺ burst (Traub et al. 1994). Antagonists at both NMDA- and AMPA/kainate receptors were required to block the evoked interictal epileptic discharges (data not shown) (see also Psarropoulou and Descombes 1998).

Initiation and propagation of bursts

In addition to SLEs, disinhibition induced typical, spontaneous interictal bursts at a rate of 1–30 per minute in all slices. Intercellular discharges comprised an initial burst (50–150 ms) and up to four secondary bursts. However, most of the spontaneous activity showed either no secondary burst, or just one. These interictal bursts lasted 50–450 ms overall, closely resembling the primary and secondary bursts of the SLE. Intracellular recordings confirmed the similarities for primary and secondary bursts between interictal and ictal activity. A few impaled cells showed single action potentials preceding the bursts by up to 10 ms. These action potentials probably took part in the buildup of synchronization (Chamberlin et al. 1990; Ives and Jefferys 1990).

Multiple extracellular recording electrodes were placed in 29 slices to identify the initiation site of epileptic events. These multichannel recordings revealed that spontaneous interictal activity started in CA3 of all slices, and spread from there along the pyramidal cell layer, usually recruiting CA1 and the dentate gyrus (Fig. 3, A and B). They also revealed that all tertiary bursts started in CA3c (Fig. 4B). This was the case even in the 14 slices where interictal activity started in CA3a or CA3b, so that in these cases the initiation site shifted to CA3c on the transition to tertiary bursts.

TABLE 1. Incidence of SLEs under different conditions

<table>
<thead>
<tr>
<th>Slices</th>
<th>Total</th>
<th>With “ictus”</th>
<th>Total</th>
<th>With “ictus”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal slices</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mM KCl</td>
<td>16</td>
<td>0 (0.0)</td>
<td>5</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>5 mM KCl</td>
<td>33</td>
<td>9 (27.3)</td>
<td>5</td>
<td>3 (60.0)</td>
</tr>
<tr>
<td>Ventral slices</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mM KCl</td>
<td>20</td>
<td>6 (30.0)</td>
<td>5</td>
<td>3 (60.0)</td>
</tr>
<tr>
<td>5 mM KCl</td>
<td>80</td>
<td>63 (78.8)</td>
<td>35</td>
<td>33 (94.3)</td>
</tr>
</tbody>
</table>

Rates compared for dorsal versus ventral slices and for 3 mM versus 5 mM KCl. Events lasting >500 ms were counted as “ictus.” Values in parentheses are percentages.

TABLE 2. Main features of the primary, secondary, and tertiary bursts tabulated for all the SLEs recorded in this series

<table>
<thead>
<tr>
<th></th>
<th>Primary Bursts</th>
<th>Secondary Bursts</th>
<th>Tertiary Bursts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burst duration, ms</td>
<td>90–150</td>
<td>30–70</td>
<td>50–75</td>
</tr>
<tr>
<td>Frequency of bursts, Hz</td>
<td>15–30</td>
<td>1–6</td>
<td>0.5–7</td>
</tr>
<tr>
<td>Number of bursts</td>
<td>1</td>
<td>1–6</td>
<td>2–100+</td>
</tr>
<tr>
<td>Overall length, ms</td>
<td>90–150</td>
<td>70–250</td>
<td>250–90,000</td>
</tr>
</tbody>
</table>

FIG. 2. Differential sensitivity of secondary bursts to N-methyl-D-aspartate (NMDA) receptor antagonists. A: extracellular recordings of control SLE evoked in CA3 as described in Fig. 1 show primary, secondary, and tertiary bursts. The primary and secondary components are expanded in the inset to the bottom right. B: addition of 50 µM D-2-amino-5-phosphonovaleric acid (D-AP5) abolished the secondary bursts but not the primary or tertiary. Note the substantial gap between the primary and tertiary components. C: secondary bursts partially recovered on wash out of the D-AP5. There was a gradual rundown of the duration of the tertiary section throughout the experiment, at a rate that was unaffected by the D-AP5. Similar results were obtained with another NMDA receptor antagonist, 50 µM ketamine.
Horizontal slices of ventral hippocampus can include adjacent areas including the entorhinal cortex (EC). Such “combined” slices can preserve at least some of the long-range connections between hippocampal and parahippocampal regions (Iijima et al. 1996; Stanton et al. 1987; Walther et al. 1986). Both the hippocampus and the entorhinal cortex showed spontaneous interictal activity in these slices. Bursts in the entorhinal cortex appeared as negative extracellular fields, occurred more slowly, and were longer (0.5–1.0 s) than hippocampal interictal discharges.

The interactions of bursting in hippocampus and entorhinal cortex created complex firing patterns. The higher frequency of bursting (3–30 min⁻¹) meant that the hippocampus usually led the entorhinal cortex (0.5–3 min⁻¹; Fig. 3A). The time for burst propagation from CA3, through CA1, to the entorhinal cortex varied between 50 and 180 ms (Fig. 3C), indicating that either a “buildup” of activity was needed to generate an entorhinal burst, or a slow propagation via a succession of synaptic relays. The next few hippocampal bursts then fell into a refractory period in the entorhinal cortex, resulting in successful invasions by only every second to fifth hippocampal burst (Fig. 3A).

The majority, but not all, of the recorded bursts started in the hippocampus. Some bursts originated from the entorhinal cortex and propagated from there into the hippocampus (Fig. 3D).

SLEs are generated within the CA3 network of the hippocampus proper

SLEs could be elicited in the combined hippocampal/entorhinal cortex slice (Fig. 4) in the same way as in hippocampal slices by stimulating the perforant path. Recordings of SLEs from the hippocampus of combined slices showed the typical
three-component pattern described above (Fig. 1) for slices from the hippocampus only. Although the stimulus evoked a typical burst in the entorhinal cortex with several afterdischarges, the entorhinal cortex did not participate in the later stages of SLEs (Fig. 4A). Bursts in the entorhinal cortex lasted up to 2 s, so that they stopped while the hippocampus continued to fire tertiary bursts. On some occasions afterdischarges in the entorhinal cortex could outlast the shortest SLEs in the hippocampus. There were no obvious signs of any interactions between the two structures during tertiary bursts. SLEs spread through all parts of the hippocampus, usually including CA1 and the dentate gyrus (Fig. 4). No spontaneous SLEs were captured with the multichannel recordings in the combined slice preparation, due to their rare occurrence.

Multichannel recordings from the combined slices revealed that the SLEs described in Fig. 1 were a special feature of the hippocampus. Although SLEs invaded the entire hippocampus, each burst was strictly phase-locked through the entire structure. At the relatively slow firing rate of tertiary bursts (3–7 Hz), the intervals between the bursts were longer than the individual bursts plus the propagation time along the participating cells. The maximum time lag due to the propagation of the bursting activity through the hippocampus was 10 ms, whereas intervals between the individual bursts usually lasted ~100 ms. Thus there was a silent period throughout the hippocampus lasting many tens of milliseconds before the next tertiary burst started in CA3c (Fig. 4B), providing evidence against reentry of epileptic activity as a possible mechanism for the prolongation of SLEs in this model.

**Minimal amount of tissue required for SLEs**

The hippocampus alone could sustain SLEs (Fig. 5C, cut 1, which removed the entorhinal cortex, compared with Fig. 5B, the intact combined slice). Progressively smaller minislices were made to determine how much of the hippocampus was required for SLEs. The next cut removed CA1 by a cut along the hippocampal fissure and through CA2 perpendicular to the pyramidal cell layer, leaving a minislice containing CA3 and...
the dentate gyrus (Fig. 5D, cut 2). In all four minislices of this type (from 4 different rats) SLEs could be evoked by perforant path stimuli. These SLEs showed no differences in form or duration from those in normal hippocampal slices. This means that, although nearly all SLEs in intact hippocampal slices invaded CA1, activity in CA1 played no obvious role for the generation and maintenance of SLEs.

Slices were further reduced by cutting away the dentate gyrus from beneath the c-shaped line of the layer of granule cell bodies (Fig. 5E, cut 3), leaving a minislice of the complete CA3 region and most of the hilus. A protective ACSF was used for this cutting procedure, to exclude further damage to the slice with the laborious preparation (see METHODS). Recordings from this type of minislice showed the typical interictal activity as described for normal hippocampal slices. Furthermore, these slices showed full SLEs following single stimuli (Fig. 5E, cut 3). In one case, touching the slice with the stimulating electrode in the mossy fiber region was sufficient to evoke an SLE. Both the minislices of this kind generated SLEs, showing that the CA3-hilar network is sufficient to generate and maintain an SLE.

The smallest minislice had a further cut along a line connecting the edges of the outer and inner blades of the granule cell bodies. This removed the dentate gyrus, the hilus, CA4, and adjacent parts of CA3 (Fig. 5F, cut 4). All four minislices of this type (from 2 rats) failed to show SLEs. These CA3 minislices also did not show spontaneous interictal activity, indicating that the network surviving this cutting procedure was not sufficient for epileptic synchronization.

Role of potassium levels

A slightly elevated $K^+$ concentration in the ACSF favored the occurrence of SLEs. It has long been known (Heinemann et al. 1977; Moody et al. 1974; Prince et al. 1973; Sypert and Ward 1971) that epileptic activity leads to increases in extracellular potassium concentration ($[K^+]_o$), the result of ion fluxes during action and synaptic potentials and during after-hyperpolarizations mediated by a range of potassium conductances (Nichols and Lopatin 1997; Sah 1996). Changes in $[K^+]_o$ in the extracellular space were monitored directly, using potassium-sensitive microelectrodes (Fig. 6). $[K^+]_o$ rose during both interictal events and SLEs (Fig. 6, A and B, respectively). Closer inspection of the onset of the $[K^+]_o$ increase showed that it rose at the same rate for both the interictal and ictal events (Fig. 6C), but $[K^+]_o$ stopped rising at a mean of 7.5 mM (maximum of 8.2 mM) during interictal events. In contrast, during events that would develop into SLEs, $[K^+]_o$ reached a mean of 8.5 mM (>$90\%$) were over 8.0 mM) at the time the tertiary bursts started (Fig. 6D). $[K^+]_o$ continued to rise during the tertiary phase of SLEs and reached a maximum of 10–12 mM $\sim$2–3 s into the discharge. Subsequently $[K^+]_o$ decreased gradually to $\sim$1 mM below its peak by the end of the SLE and then decayed back to baseline over 10–15 s (Fig. 6B). Longer SLEs differed from shorter not in the peak $[K^+]_o$, but only in the duration of the $[K^+]_o$ plateau.

Spontaneous SLEs resembled those evoked by stimulation in every respect. $[K^+]_o$ reached the same peak values and showed a similar plateau with small oscillations. When the SLE started with a marked break between secondary and tertiary bursts, $[K^+]_o$ initially dropped, but with the beginning of the train of tertiary bursts $[K^+]_o$ showed the typically slower rise to a plateau of 10–12 mM (Fig. 6E). In these cases, $[K^+]_o$ had reached a high level (>9 mM) during primary and secondary bursts, so that it remained above 8 mM during the trough, suggesting that this level may be necessary for triggering tertiary bursts. During interictal activity, $[K^+]_o$ never reached values $>$8.2 mM (Fig. 6, A and D).

Synaptic activity during SLEs

Intracellular recordings showed that all impaled cells that had the characteristics of pyramidal cells participated in every interictal burst and in every SLE. During the intervals between epileptic discharges, impaled cells were comparatively quiet; spontaneous action potentials occurred only occasionally and usually preceded another epileptic discharge. Close inspection of the intracellular traces at higher gain revealed low-frequency spontaneous synaptic events of the size and duration of small excitatory postsynaptic potentials (EPSPs; <10 per second, <1 mV). This background activity changed during SLEs, where intracellular traces displayed an enormous increase in spontaneous activity (Fig. 7, A, B, and E). The periods between the depolarization shifts underlying the tertiary bursts appeared as sweeps of frequent and large EPSPs (>20 per second, >1 mV). The increased synaptic activity during tertiary bursts gave the intracellular recordings of SLEs a much more “noisy” appearance. Increased synaptic “noise” accompanied all SLEs.

Continuous intracellular recordings showed baseline activity at a few EPSPs per second, which did not change before interictal bursts, suggesting that spontaneous EPSPs had no obvious role in the initiation of interictal bursts. Usually 1–3 EPSPs occurred during the 500 ms preceding an interictal burst, but they remained isolated events and did not show any temporal summation (Fig. 7D). Each interictal burst was followed by a long (400–600 ms) afterhyperpolarization (AHP) of the impaled cell. No spontaneous EPSPs were recorded for 600 ms after interictal bursts, presumably because the entire network was hyperpolarized and inactive. When baseline synaptic activity resumed, the next interictal burst usually followed within 5–20 s.

In contrast with the pause of spontaneous synaptic activity after each interictal burst, intracellular traces during SLEs showed many EPSPs occurring between tertiary bursts and after the end of the SLE, before gradually declining to baseline activity (Fig. 7, E and F). Spontaneous interictal bursting took even longer to resume after an SLE, usually 1–2 min. Another difference of interictal and ictal activity was the absence of AHPs after SLEs. Cells remained close to resting potential or slightly hyperpolarized. The lack of a clear AHP may be one reason why spontaneous synaptic activity continued after the end of SLEs.

Synaptic noise was quantified as the power (or variance) of the intracellular trace for a selected interval. For the calculation of the noise levels during tertiary bursts, 200-ms epochs were selected during the isoelectric intervals between tertiary bursts; epochs of 500 ms were used for measurements of the baseline and preinterictal power. The frequency of tertiary bursts did not allow longer epochs for power measurements during SLEs. The means of synaptic power were then compared for baseline and preinterictal power. The frequency of tertiary bursts did not allow longer epochs for power measurements during SLEs. The means of synaptic power were then compared for baseline activity, activity preceding interictal bursts, and the activity during SLEs for each intracellular recording individually (Fig. 7H).
SLEs were characterized by substantial increases in the frequency and size of the EPSPs between tertiary bursts. The intervals between any given pair of tertiary bursts were filled by a series of spontaneous synaptic events, most of them. Usually the level of synaptic activity during tertiary bursts was several times the baseline activity, and always was significantly greater (P < 0.015, Wilcoxon signed rank test for all the experiments of this kind; Fig. 7H). There was no significant increase of the noise level during the 500 ms preceding interictal bursts. Unfortunately, no spontaneous SLEs were observed during intracellular recordings, giving no evidence about the role of spontaneous synaptic activity in the generation of a spontaneous SLE.

**DISCUSSION**

Seizures are a hallmark of the epilepsies. They differ from brief interictal spikes in their temporal and spatial extent. Theories of the transition of brief bursts into full seizures have to account for both aspects: the sustained firing over long periods of time and the recruitment of large brain areas. Presently there is no single compelling theory for the transition of interictal bursts into seizures (Jefferys 1998). One key debate centers on the question of whether the recruitment of large brain areas into the bursting is necessary for a prolonged firing. One concept, the reentry theory of seizures, argues that the formation of a spatially extended loop of reverberating epileptic activity is the crucial step for sustaining a seizure discharge (Lothman 1994; Lothman et al. 1991; Paré et al. 1992). The opposing concept argues for sustained focal firing of small assemblies of neurons that then may entrain larger structures (Traub et al. 1996), or for multiple focal seizure generators interacting with no net phase lag; in effect coupled oscillators rather than reentrant circuits (Beldhuis et al. 1993; Bradin et al. 1997). Seizures in vivo will inevitably involve more tissue and
structures beyond those contained in a hippocampal slice. Although the slice cannot provide the whole story of epilepsy as a clinical problem, it does show that small aggregates of a few thousand neurons can sustain events lasting as long as seizures in vivo.

Seizure-like activity lasting 2–90 s was found here in slices of ventral hippocampus and followed a distinct firing pattern with three phases comprising: a primary burst lasting 90–150 ms, a short series of secondary bursts, riding on the same depolarization shift, and lasting 70–250 ms, and a train of tertiary bursts lasting the remainder of the SLE. Individual tertiary bursts were very uniform. They lasted 50–75 ms and were generated rhythmically at frequencies that varied between SLEs from 0.5–7 Hz and slowed gradually during each SLE.

This pattern of seizure-like activity differs from that in models such as high-K\(^+\) (Jensen and Yaari 1988; Traynelis and Dingledine 1988), where long discharges preferentially arose in CA1 and resembled the nonsynaptically synchronized discharges found in low-Ca\(^{2+}\) (Haas and Jefferys 1984; Jefferys 1995; Jefferys and Haas 1982; Taylor and Dudek 1982). The SLEs described here more closely resembled those we have reported in brief for 4-aminopyridine (Traub et al. 1996), or others have reported in CA3 for rapid kindling (Stasheff et al. 1993) and low-Mg\(^{2+}\) in relatively thick hippocampal slices (Anderson et al. 1986). They also resembled epileptic events recorded from implanted electrodes in vivo in several epilepsy models (Bragin et al. 1997; Finnerty 1993; Paré et al. 1992) and in epileptic patients in preparation for the surgical removal of an epileptic focus (Spencer et al. 1992; Spencer and Spencer 1994).

Two conditions favored the generation of SLEs here. One was a slightly higher [K\(^+\)]\(_o\). The concentration used was 5 mM
rather than 3 mM and was much less than the 8 mM used in the high-\([K^+]_o\) model (Chamberlin et al. 1990; Jensen and Yaari 1988; Traynelis and Dingledine 1988). The biochemists who first developed the brain slice preparation used 5–6 mM \([K^+]_o\) on the grounds that this brought the metabolic rate and ion balance of the brain slice in vitro back to values found in the intact brain in vivo (Gibson and McIlwain 1965). In the present context, it may be that the slightly increased \([K^+]_o\) causes the recovery of part of the spontaneous activity found in vivo (Paré et al. 1998). If so, then the need for 5 mM \([K^+]_o\) for SLEs may reflect a requirement for network excitability closer to that found in vivo than in vitro. The second condition for reliable SLEs was for the use of ventral hippocampal slices.

What is special about the ventral hippocampus?

The SLEs described here occurred much more reliably and were longer in slices from ventral hippocampus than from dorsal. The septotemporal origin of hippocampal slices is ignored for most electrophysiological studies. However, several studies have identified septotemporal variations in hippocampal function and anatomy. The dorsal part is implicated more strongly in spatial information processing than the ventral part (Jung et al. 1994). Anatomic differences exist in connectivity for the two ends of the hippocampus (Amaral and Witter 1989; Bernard and Whead 1994; Cavazos et al. 1992; Li et al. 1994; Witter 1986), and in the proportions of certain classes of neurons (Buckmaster et al. 1994). The ventral hippocampus has a higher seizure susceptibility in several models in vivo and in vitro (Bragdon et al. 1986; Elul 1964; Gilbert et al. 1985; Lee et al. 1990; Racine et al. 1977). Elevated potassium in the ACSF produced a significantly higher rate of interictal bursting in ventral slices than dorsal, which may be directly relevant to the present observations (Bragdon et al. 1986; Gilbert et al. 1985). There appears to be a greater density of several modulatory pathways in the ventral part of the hippocampus (Jung et al. 1994; Lee et al. 1990). Perhaps the key factor is the density and efficacy of local excitatory connections between CA3 pyramidal cells, but no data are available to compare dorsal and ventral hippocampus.

The longitudinal hippocampal slice preparation is of special interest in this context, because it includes the entire CA3 region from all along the septotemporal axis of the hippocampus. Disinhibition in such slices showed the typical epileptic bursts with afterdischarges (Traub et al. 1993a). However, the propagation of these afterdischarges was not always uniform and depended on the stimulation site, with a tendency of more afterdischarges at the temporal pole. Together with the present observations, these studies show that the ventral end of the hippocampus is more prone to epileptic activity than the dorsal, for reasons that remain unclear.

Minimal aggregate of neurons required for SLEs

The entorhinal cortex was not required for the SLEs found here, although it clearly can sustain prolonged epileptic discharges under some circumstances (Jones 1989; Walther et al. 1986; Wilson et al. 1988). The entorhinal cortex and hippocampus can be synchronized in several kinds of epilepsy, including low-\([Mg^{2+}]_o\) in slices (Wilson et al. 1988), tetanic commissural stimulation in vivo (Bragin et al. 1997), and intracranial recording of clinical seizures in humans (Spencer and Spencer 1994); in each of these cases the hippocampus could sustain seizure-like discharges independently of activity in the entorhinal cortex. In marked contrast, a study on the mouse combined slice preparation exposed to 4-aminopyridine showed that interictal activity from the CA3 region prevented seizure-like discharges from being initiated in the entorhinal cortex (Barbarosie and Avoli 1997). In the present study, multichannel recordings showed that SLEs started in CA3 and invaded CA1 and dentate [the latter presumably by pathways described recently (Li et al. 1994; Muller and Misgeld 1991; Scharfman 1994)]. There was no evidence of reentrant activity via CA1 or dentate. Rather, there were periods of tens to hundreds of milliseconds between successive tertiary bursts, during which there was no population activity in any part of the slice. Cutting minislices revealed unequivocally that the minimum aggregate required was the CA3b/c regions with CA4 and the hilus attached.

Although reentrant epileptic activity can reverberate around long pathways under some conditions, and we cannot exclude the possibility that such a process can help sustain seizure-like epileptic activity in some cases (Lothman 1994; Lothman et al. 1991; Paré et al. 1992), the evidence here is that it is not necessary for SLEs.

What drives tertiary bursts?

Many features that enable the disinhibited hippocampal slice to generate brief interictal bursts will also be involved in tertiary bursts, i.e., the fast, AMPA receptor–mediated, recurrent excitatory connections between, and the intrinsic burst properties of, the CA3 pyramidal cells. Most of these components are too brief to explain the long duration of SLEs. Additional factors have to be involved. They must become activated before the start of the tertiary bursts and must persist during the entire SLE. We have previously shown that NMDA receptor antagonists fulﬁl this role for secondary bursts in disinhibited slices (Traub et al. 1993a,b). The lack of effect of NMDA receptor antagonists on tertiary bursts in the present study shows that this does not explain SLEs. Two candidate mechanisms suggest themselves.

Merlin et al. have shown that group I mGluR agonists can, over a period of 1–2 h, transform brief interictal bursts in picrotoxin to SLEs similar to those reported here (Merlin et al. 1995; Taylor et al. 1995). This transformation depends on protein synthesis (Merlin et al. 1998) and is mediated by mGluR activation even after the group I agonist has washed out (Merlin and Wong 1997). The underlying change appears to be an autopotentiation of group I mGluRs (Merlin 1999). It may be that the rats in the present study had naturally high levels of mGluR function in their ventral hippocampi, and that the SLEs here shared mechanisms with those uncovered in guinea pig slices by group I mGluR agonists.

Two factors slow enough to sustain SLEs have been observed here. They are the rise in extracellular potassium and the increase of spontaneous synaptic activity. The \([K^+]_o\) increase during the primary and secondary bursts needed to reach >8.5 mM if an SLE was to be triggered.

The idea that fluctuations in \([K^+]_o\) could play a role in synchronizing epileptic bursts has a long and honorable history (Green 1964; Heinemann et al. 1977; Moody et al. 1974;
Prince et al. 1973; Sypert and Ward 1971). Increasing [K\(^+\)]\(_o\) will have many effects, as discussed in Traub and Dingledine (1990). Several effects of increased [K\(^+\)]\(_o\) can be excluded, including the following: greater involvement of NMDA receptors (Traynelis and Dingledine 1988), because \(\alpha\)-AP5 did not block the tertiary bursts, and reduced inhibition (Korn et al. 1987; McBain 1994; Thompson and Gähwiler 1989), because inhibition is already blocked. Although the rise in [K\(^+\)]\(_o\)], will increase the excitability of pyramidal cell somata and dendrites (Traynelis and Dingledine 1988), we believe that other effects are more significant to the SLEs seen here. Specifically, these are the increase in synaptic “noise” and the loss of AHPs we observed between tertiary bursts.

AHPs decrease with increasing [K\(^+\)]\(_o\)] due to the lower driving force for K\(^+\) efflux (Alger and Williamson 1988). The difference between \(\sim7.5\) mM after an interictal burst and \(\sim9\) mM at the end of the secondary bursts of the SLE (Fig. 6) would represent a shift in K\(^+\) reversal of \(\sim5\) mV and could explain the difference in AHP amplitudes at these stages (Fig. 7). Reducing AHP amplitude is a good way of accelerating interictal discharges (Traub and Dingledine 1990).

The second major feature of the tertiary burst phase was the increase in background synaptic activity. Elevated potassium concentrations increase spontaneous transmitter release (del Castillo and Katz 1954) and synaptic excitability (Goh and Sastry 1985) and also increase the incidence of ectopic axonal action potentials (David et al. 1993; Kapoor et al. 1993; Poolos et al. 1987). In the present case the increase in synaptic activity then boosts the excitability of the network of CA3 pyramidal cells to such an extent that events similar to the primary burst occur much more frequently than interictal bursts, resulting in a continuous train of tertiary bursts. This is supported by evidence for ectopic spikes in the rapid kindling model (Stasheff et al. 1993), realistic computer simulations of the high-[K\(^+\)]\(_o\)] model (Traub and Dingledine 1990), and realistic computer simulations of SLEs induced by 4-aminopyridine and bicuculline (Traub et al. 1996).

Microdissection experiments showed that the hilus needed to be attached to CA3 for SLEs to occur. Strowbridge has shown that exciting hilar cells can potentiate spontaneous synaptic potentials to a considerable extent (Strowbridge and Schwartzkroin 1996). The significance of the hilus for the present phenomenon would be as a source of spontaneous synaptic excitation, both interictically and, boosted by increased [K\(^+\)]\(_o\)], between tertiary bursts.

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