Imaging of 4-AP–Induced, GABA<sub>A</sub>-Dependent Spontaneous Synchronized Activity Mediated by the Hippocampal Interneuron Network

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Sinha, Saurabh R. and Peter Saggau. Imaging of 4-AP–induced, GABA<sub>A</sub>-dependent spontaneous synchronized activity mediated by the hippocampal interneuron network. J Neurophysiol 86: 381–391, 2001. Under conditions of increased excitability, such as application of the K<sup>+</sup> channel blocker 4-aminopyridine (4-AP, 100 μM), interneurons in the hippocampal slice show an additional form of synchronized activity that is distinct from the ictal and interictal epileptiform activity induced by these manipulations. In principal neurons, i.e., pyramidal and granule cells, this synchronized interneuron activity (SIA) generates large, multi-component synaptic potentials, which have been termed long-lasting depolarizations (LLDs). These LLDs are dependent on GABA<sub>A</sub> receptor-mediated synaptic transmission but not on excitatory amino acid (EAA) receptors. Intracellular recordings from hilar interneurons have shown that depolarizing GABA<sub>A</sub> receptor-mediated synaptic potentials are also largely responsible for the synchronization of interneurons. The spatiotemporal characteristics of this interneuron activity have not been investigated previously. Using a voltage-sensitive dye and optical techniques that are capable of recording spontaneous synchronized activity, we have characterized the spatiotemporal pattern of SIA (in the presence of 4-AP + EAA receptor antagonists) and compared it with interictal epileptiform activity (in 4-AP only). Like interictal activity, SIA could be observed throughout the hippocampal slice. Unlike interictal activity, which originated in area CA2/CA3 and spread from there, SIA was most prominent in area CA1 and originated either there or in the subiculum. In CA1, interictal activity was largest in and near stratum pyramidale, while SIA was mainly located in s. lacunosum moleculare. Furthermore SIA was equally likely to propagate in either direction, and multiple patterns of propagation could be observed within a single brain slice. These studies suggest that hippocampal area CA1 has the highest propensity for SIA, that multiple locations can serve as the site of origin, and that interneurons located in s. lacunosum moleculare or interneurons that specifically project to this region may be particularly important for synchronized interneuron activity.

INTRODUCTION

Interneurons, defined as GABAergic nonprincipal nerve cells (Buckmaster and Somogyi 1996; Freund and Buzsáki 1996), comprise a diverse group of cells within the hippocampal formation. The primary role ascribed to interneurons in the hippocampus is as mediators of feedforward and feedback inhibition. In addition to inhibiting activity in principal neurons, these feedforward and feedback connections are important for the generation of certain forms of physiological synchronized activity such as the theta (Ylinen et al. 1995) and gamma rhythms (Whittington et al. 1995). Such rhythms are generally believed to be paced by sources external to interneurons and, most likely, external to the hippocampus, e.g., the medial septum for theta rhythm. In addition to these interactions with principal neurons, interneurons also show extensive interactions with other interneurons, forming an interneuron network (for review, see Freund and Buzsáki 1996). Under conditions of increased excitability, synchronized activity can arise within this hippocampal interneuron network; such activity may play a role in initiating seizure activity (Avoli et al. 1996; Köhling et al. 2000).

In hippocampal slices taken from adult rats (Avoli and Perreault 1987; Perreault and Avoli 1989) or guinea pigs (Michelson and Wong 1991), application of low doses of 4-aminopyridine (4-AP, 50–100 μM), which block mainly D-type K<sup>+</sup> channels (Storm 1988), induces two types of spontaneous field potentials: interictal epileptiform discharge and long-lasting depolarization (LLD). Recorded intracellularly, the LLD consists of a depolarization, lasting several hundred milliseconds, that is sensitive to antagonists of GABA<sub>A</sub> receptors such as bicuculline and picrotoxin. LLDs have also been recorded in the neocortex (Aram et al. 1991), entorhinal cortex (Avoli et al. 1996), and human neocortical and hippocampal neurons (Avoli et al. 1988). While both forms of activity observed during application of 4-AP are sensitive to the blockade of all synaptic transmission by application of low-Ca<sup>2+</sup> solutions or Cd<sup>2+</sup> (Perreault and Avoli 1992), only interictal epileptiform discharges are sensitive to excitatory amino acid (EAA) receptor blockers, i.e., blockers of ionotropic glutamate receptors.

In the presence of EAA receptor blockers, the intracellular correlate of the LLD in principal cells is a large, synchronous triphasic inhibitory postsynaptic potential (IPSP): an initial hyperpolarization mediated by GABA<sub>A</sub> receptors, followed by a depolarizing component, which is also mediated by GABA<sub>A</sub> receptors, and lastly a GABA<sub>B</sub> receptor-mediated hyperpolarization (Michelson and Wong 1991; Perreault and Avoli 1989). In area CA1 pyramidal cells, current-source-density analysis and local application of the GABA<sub>A</sub> receptor antagonist bicuculline

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culline have revealed that the depolarizing component of this triphasic IPSP originates in the apical dendrites (Avoli and Perreault 1987; Perreault and Avoli 1989). Depolarizing GABA_A receptor-mediated responses are common during development and have also been reported after strong stimulation (Perreault and Avoli 1988) or after iontophoreses of GABA in adult animals (Alger and Nicoll 1979, 1982; Andersen et al. 1980; Thalmann et al. 1981). For exogenously applied GABA, depolarizing responses are generally seen in the dendrites, although dendritic hyperpolarizing responses have also been reported. Similarly, prolonged or repeated application of GABA or activation of GABA_A receptors tends to give more depolarizing responses and can even convert a hyperpolarizing to a depolarizing response (Alger and Nicoll 1982; Andersen et al. 1980; Barker and Ransom 1978; Cerne and Spain 1997).

The precise mechanisms responsible for depolarizing GABA_A receptor responses are not known; however, several studies have suggested that HCO_3^- plays a role either by actual flux through GABA_A receptors or by more indirect routes (Lamsa and Kaila 1997; Staley et al. 1995; Taira et al. 1997). Investigations of high-frequency stimulation-induced long-lasting GABA-mediated depolarizations have suggested that nonsynaptic mechanisms such as elevations of extracellular K^+ concentrations or field effects (epaptic coupling) may also contribute (Bracci et al. 1999; Kaila et al. 1997; Smirnov et al. 1999).

The large, multicomponent IPSP, which is the intracellular correlate of the LLD, suggests synchronous activation of many GABAergic interneurons. Michelson and Wong (1991) recorded directly from hilar interneurons in the presence of EAA receptor blockers and either 4-AP or high [K^+]_o. The hilar interneurons showed large synchronized bursts of action potentials that correlated with large, synchronized IPSPs (LLDs) in pyramidal and granule cells. These bursts were due to a large depolarizing synaptic potential that was sensitive to GABA_A receptor antagonists and was not affected by antagonists of β-adrenergic or muscarinic receptors. More detailed analyses have elucidated some characteristics of the hilar interneurons involved in generating these synchronized IPSPs. Michelson and Wong (1994), found at least two subtypes of interneurons, which they referred to as types I and II. Synchronized bursting in type I interneurons was blocked by GABA_A receptor antagonists. In type II cells, bursting was not blocked by GABA_A receptor antagonists; instead, these cells were likely synchronized by the presence of gap junctions, which were suggested by dye coupling and spikes. In a more recent study (Forti and Michelson 1998), the characteristics of three subtypes of hilar interneurons with respect to generation of large synchronized IPSPs in the presence of 4-AP were investigated. Pyramidal-like stellate interneurons exhibited GABA_A receptor-dependent synchronized bursts that corresponded to the GABA_A receptor-mediated hyperpolarizing and depolarizing IPSPs in CA3 pyramidal cells. Spheroid and oviform interneurons showed bicuculline-resistant bursts that coincided with the GABA_A receptor-mediated component of the synchronized IPSPs recorded in CA3 pyramidal cells. Oviform cells were likely synchronized by nonsynaptic mechanisms such as gap junctions; spheroid cells were synchronized by a synaptic depolarization of unknown type, which was resistant to blockers of GABA_A, GABA_B, ionotropic and metabotropic glutamate, and β-adrenergic and muscarinic receptors.

Using field electrodes, Perreault and Avoli (1992) found that LLDs did not spread along the classic hippocampal pathways, i.e., the trisynaptic circuit, and in fact could travel across anatomical boundaries such as the hippocampal fissure. They also found that each subarea of the hippocampal slice (areas CA1 and CA3 and the dentate gyrus) were capable of independently generating LLDs. These findings are consistent with interneurons underlying the generation of LLDs. A more detailed characterization of the spatiotemporal characteristics of synchronized interneuronal activity could have important implications for the identity of the specific interneurons involved and possibly for the physiological or pathophysiological role(s) of this activity. Therefore in the present study, we have used optical recording techniques capable of recording transients of membrane potential associated with spontaneous synchronized activity with high spatiotemporal resolution to investigate synchronized interneuronal activity (SIA) in guinea pig hippocampal slices. SIA was induced and isolated by application of 4-AP and EAA receptor blockers. SIA was compared with interictal epileptiform activity induced in the same slice by application of 4-AP alone. The observed patterns of these two types of activity were quite distinct. The implications of these patterns of activity for the identity of the specific interneurons involved are discussed. Portions of this work have been presented before in abstract form (Sinha and Saggau 1996).

**METHODS**

Transverse hippocampal slices were prepared from 1- to 3-wk-old guinea pigs (wild type or albino, 100–150 g). The animals were anesthetized (methoxyflurane) and quickly decapitated. The brain was removed and placed in ice-cold artificial cerebrospinal fluid (ACSF), containing (in mM): 124.0 NaCl, 5.0 KCl, 2.0 CaCl_2, 1.2 MgCl_2, 26.0 NaHCO_3, and 10.0 d-glucose, saturated with 95% O_2-5% CO_2 to maintain a constant pH of 7.4. After allowing a few minutes for the brain to cool down, the hippocampi were dissected free. Slices (400 μm) were prepared from the middle third of the hippocampus on a vibrating tissue slicer (Vibratome 1000, TPI) and allowed to recover in ACSF at room temperature in ACSF for at least 1 h before use.

Slices were stained with the voltage-sensitive dye (VSD) RH-414 (Molecular Probes, Eugene, OR). The absorption maximum of this dye is at 538 nm, and the emission maximum is at 708 nm (for spectra, see Sinha et al. 1995). When applied to the external surface of a cell, this VSD responds to a depolarization with a decrease in its fluorescence intensity. Slices were stained with RH-414 for 15 min (25–50 μM in normal ACSF; stock solution: 4 mM in distilled water, stored in freezer for up to several months); slices were then washed for ≥15 min to remove excess dye. One slice at a time was then transferred to the recording chamber, and ≥20 min was allowed for the slice to acclimate.

The recording chamber was a temperature-controlled (31–32°C) submersion chamber located on the stage of an inverted microscope used for optical recording (described in the following text). The perfusion rate was ~5 ml/min. A low resistance (1–5 MΩ) glass microelectrode filled with filtered normal ACSF +1% Fast Green (Sigma, St. Louis, MO) to improve visualization was used to record extracellular field potentials. The electrode signal was amplified and low-pass filtered (100 or 1,000 Hz) by a Getting 5A microelectrode amplifier. It was further amplified to obtain an overall gain of 1,000–4,000. For evoked responses (most responses shown are spontaneous), a bipolar tungsten stimulation electrode was placed in the fimbria. Responses were evoked by applying 100- to 400-μA current pulses of 100- to 500-μs durations at rates <0.05 Hz.

The optical recording setup was based on an inverted microscope...
Neuramin. Stock solutions consisted of 10 mM CNQX in DMSO and were made fresh for every experiment. Picrotoxin (PTX) were obtained from Sigma. 4-AP was prepared as a stock solution and used in the following concentrations: 100, 250, 300 μM. Light from a tungsten lamp (12 V, 100 W, Xenophot HLX, Osram-Sylvania, Danvers, MA) was filtered to ensure that only 535/50 nm light was used for excitation. A photodiode matrix (PDM, 10 × 10 elements, MD-100, Centronics, Newbury Park, CA) and a custom-made amplifier were used to quantify the emitted light.

The amplifiers and procedures used to obtain the optical signals have been described in detail elsewhere (Colom and Saggau 1994; Sinha et al. 1995). Spontaneous activity was recorded in the following manner. Epochs of data 0.5–1 s in length were collected, and the field potential signal was displayed on-line. When the experimenter observed an event in the field potential, data collection was stopped. The data collected during the event and several epochs preceding and subsequent to the event were stored for later analysis.

All optical signals are displayed as change in fluorescence divided by resting fluorescence (ΔF/Φ). This corrects for variations in dye concentration, in illumination intensity, and in sensitivity of PDM elements. The normalized quantity ΔF/Φ is proportional to the change in membrane potential. Within the membrane potential range of interest, the relationship between membrane potential and ΔF/Φ is linear (Bullen and Saggau 1999; Ross et al. 1977). The amplitudes of the optical signals for all PDM elements covering similar regions of the slice were combined to obtain an average value. These regions were: stratum pyr. (elements covering s. pyramidale), s. oriens (elements covering s. oriens), s. rad. (elements covering the proximal apical dendrites, ~100–500 μm from center of s. pyramidale), s. lac. mol. (elements covering the distal apical dendrites, >500 μm from center of s. pyramidale to hippocampal fissure), and dentate (elements covering the dentate gyrus). All data are presented as means ± SE. Paired Student’s t-test was used to determine significance of results; *P < 0.05, **P < 0.01, NS, P > 0.05.

6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphono pentanoic acid (D-APV) were obtained from Tocris Neuramin. Stock solutions consisted of 10 mM CNQX in DMSO and 25 mM D-APV in ddH2O; both were stored in the freezer. 4-AP and picrotoxin (PTX) were obtained from Sigma. 4-AP was prepared as a 100 mM stock in ddH2O and stored in the refrigerator. PTX solutions were made fresh for every experiment.

RESULTS

Using the apparatus and procedures described above, VSD signals associated with evoked and spontaneous activity were recorded from hippocampal slices. The spatial resolution of the system was 200 × 200 μm/photodiode element; the temporal resolution was ~300 μs, although a lower resolution was used for most experiments (0.5–2 ms). To allow for comparison between interictal epileptiform activity and synchronized activity in the hippocampal interneuron network, for each slice, evoked and spontaneous interictal epileptiform activity were first recorded in the presence of 100 μM 4-AP only. Then spontaneous activity in the presence of EAA receptor antagonists (10 μM CNQX and 25 μM D-APV) in addition to 4-AP (4-AP + EAA antagonists) was recorded from the same slice.

To confirm that the activity recorded in the present experiments was the same as seen in previous studies, we investigated the pharmacology of the spontaneous activity by adding GABA_A receptor antagonists. The field potential was continuously recorded on a chart recorder during sequential exposure of slices (n = 7) to 4-AP, 4-AP + EAA antagonists, and 4-AP + EAA antagonists + PTX. In 4-AP, the frequency of spontaneous events was 0.21 Hz; in 4-AP + EAA antagonists, the frequency was 0.05 Hz; and in the presence of PTX, it was 0.00 Hz (range: 0–0.01; Fig. 1A). The frequencies were significantly different in all three conditions (P < 0.005). The blockade of the activity seen in the presence of EAA receptor blockers by PTX confirms its dependence on a GABA_A receptor-mediated event. To confirm that the effects of PTX were specific to the activity seen in 4-AP + EAA antagonists, PTX was applied to six slices bathed only in 4-AP. In these slices, PTX increased the size of the spontaneous epileptiform event but did not significantly reduce the frequency (Fig. 1B). Based on the similar pharmacological characteristics, we conclude that the activity we recorded in the presence of 4-AP + EAA antagonists is the same synchronized interneuron activity recorded by others under similar conditions. The spatiotemporal characteristics of this activity in various subregions of the hippocampal slices are given in the following text.

Evoked and spontaneous activity in the dentate gyrus

Because many of the previously published studies with synchronized interneuron activity were performed in the hilar region of the hippocampus, we first investigated the spatiotemporal pattern of SIA in the dentate gyrus. Data from a typical experiment are shown in Fig. 2. When a single stimulus was applied to the fimbria in the presence of 4-AP, activity first appeared in the hilus, the portion of the recording area nearest the stimulation site (Fig. 2B). This activity spread throughout the hilus and partially into the granule cell layer. A very similar pattern of activity was observed for spontaneous epileptiform activity in 4-AP (Fig. 2C). This was the reason for using fimbrial stimulation for the evoked responses: it generally resulted in responses that appear to be very similar to the spontaneous epileptiform activity. After addition of EAA receptor antagonists (4-AP + EAA antagonists), fimbrial stimulation did not evoke an observable signal in the dentate gyrus (data not shown). Spontaneous activity was still observed (Fig. 2D); however, the largest amplitude activity was seen in the distal dendritic regions of area CA1 that was partially included in the recording area.

Evoked and spontaneous activity in area CA3

In area CA3, activity evoked by fimbrial stimulation in 4-AP appeared first in the portion of the recording area nearest the site of stimulation (Fig. 3B). In contrast, spontaneous epileptiform activity induced by 4-AP could originate in any subregion of area CA3. From its site of origin, spontaneous activity spread around CA3 and into the hilus of the dentate gyrus and into area CA1 (see Figs. 2 and 4). This pattern of activity is demonstrated in Fig. 3C.

In 4-AP + EAA antagonists, stimulation of the fimbria caused a small VSD signal in the portion of CA3 nearest the fimbria, presumably due to antidromic activation of area CA3 pyramidal cells (data not shown). Spontaneous activity recorded under these conditions once again had a pattern distinct from the activity recorded in 4-AP only. This activity generally entered the recording area from area CA1 and traveled around area CA3 toward the dentate gyrus; the activity was largest in...
the apical dendritic regions. Spontaneous activity recorded in 4-AP was generally of greater or equal amplitude in the pyramidal cell layer than in the apical dendrites.

**Evoked and spontaneous activity in area CA1**

The recordings centering on either the dentate gyrus or area CA3 suggested that area CA1 may be the region of most interest as signals were generally largest in the portion of CA1 partially included in the recording area; moreover, spontaneous activity often seemed to enter the recording area from CA1. Data from a typical experiment in which activity was recorded in area CA1 are shown in Fig. 4. Again, evoked and spontaneous events in 4-AP were very similar to each other. Evoked activity entered the recording area from the side nearest area CA3 and spread across CA1 toward the subiculum; also, the activity was maximal in the cell body layer and the proximal dendrites (Fig. 4, B and C). In contrast to this pattern, spontaneous activity in 4-AP + EAA antagonists often entered the recording area from the subicular side of area CA1 (area CA1a) and spread toward CA3; also, this activity was maximal in the distal apical dendrites of area CA1 (Fig. 4D).

The velocity of spread of SIA was \( \sim 10–20 \text{ mm/s} \) (estimated from the times of peak activity recorded in different PDM elements). The direction of propagation of spontaneous activity was determined by comparing the onsets of optical signals recorded in portions of area CA1 near area CA3 to those nearer the subiculum. Data from nine slices are summarized in Fig. 5. Of all spontaneous events recorded in the presence of 4-AP \((n = 33; 2–5 \text{ events/slice})\), 97% traveled across CA1 from area CA3 toward the subiculum; the remaining 3% traveled in the opposite direction across CA1 (Fig. 5A). The results were similar if the percent of events per slice that traveled in a particular direction was considered (Fig. 5B): 97.8 \( \pm \) 2.4% (range 80–100%) traveled from CA3 toward the subiculum, while 2.2 \( \pm \) 2.4% traveled from the subiculum toward area CA3 \((P < 0.001)\). For spontaneous events recorded from the same slices in the presence of 4-AP + EAA antagonists \((n = 32; 2–5 \text{ events/slice})\), 41% traveled from area CA3 toward the subiculum; the remaining 59% traveled in the opposite direction. On a per slice basis, 41.7 \( \pm \) 17.7% traveled from CA3 to the subiculum and 58.3 \( \pm \) 17.7% traveled from the subiculum to CA3. Thus there was no significant preference for the direction of travel of the activity \((P > 0.05)\). In 4-AP + EAA antagonists, the spontaneous activity in several slices showed both directions of propagation, i.e., some events traveled from subiculum to CA3 and others from CA3 to subiculum (Fig. 5C). Thus this spontaneous activity cannot only originate in different regions across slices but also within the same slice. This indicates that multiple regions are capable of originating such activity.

To compare the spatial distributions of the various types of
activity, elements of the photodiode matrix were grouped according to the portion of the slice they covered. For spontaneous and evoked activity in 4-AP alone, there were no significant differences after normalization of amplitudes to the amplitude recorded in s. pyr. (data not shown). In both cases, the activity was largest in s. pyr. and s. rad.; i.e., in the cell body layer and the proximal apical dendrites. In comparing spontaneous activity in 4-AP to that in 4-AP + EAA antagonists, the amplitudes ($\Delta F/F$) were significantly smaller in all regions in the presence of the EAA receptor blockers (Fig. 6A). After normalization to the level of activity in s. pyr., the discrepancy in distributions became apparent: in the presence of EAA receptor blockers, activity was largest in s. lac mol. in area CA1 (Fig. 6B).

**DISCUSSION**

Using the techniques and procedures developed for optically recording infrequent spontaneous events, synchronized activity was studied in guinea pig hippocampal slices in the presence of the $K^+$ channel blocker 4-AP and EAA receptor antagonists. This activity was compared with spontaneous and evoked interictal epileptiform activity recorded in the same slices induced by 4-AP alone. The synchronized activity seen in the

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**FIG. 2.** Evoked and spontaneous activity in the dentate gyrus. A: schematic of hippocampal slice (left) showing location of stimulation and recording electrodes and the optical recording area (box). Also shown is an image of the recording area (right). The boxes indicate photodiode matrix (PDM) elements from which data are shown. B: evoked activity in 4-AP (arrow indicates time of stimulation). The field potential (top) and the optical traces from selected elements (bottom) are shown. C: spontaneous activity in 4-AP. D: spontaneous activity in 4-AP + excitatory amino acid (EAA) antagonists.

**FIG. 3.** Evoked and spontaneous activity in area CA3. A: schematic of hippocampal slice (left) and image of optical recording area with PDM selected elements (right). B: evoked activity in 4-AP (arrow indicates time of stimulation). C: spontaneous activity in 4-AP. D: spontaneous activity in 4-AP and EAA antagonists.
presence of the EAA receptor antagonists presumably corresponds to LLDs and SIA reported in previous studies. The pharmacological conditions we used to obtain the synchronized activity were similar to those used by other investigators (Avoli and Perreault 1987; Michelson and Wong 1991, 1994; Perreault and Avoli 1989, 1992); furthermore, in agreement with these previous studies, the synchronized activity was sensitive to the GABAA receptor antagonist picrotoxin. Last, the frequencies of such events was similar to that reported previously for spontaneous LLDs (0.04 Hz) (Perreault and Avoli 1992).

Thus the synchronized activity recorded in 4-AP EAA antagonists represents synchronized activity within the interneuron network and the consequent LLDs in the principal cells.

An important issue is the exact origin of the optical signals recorded in this study. The bath-applied VSD will stain all accessible cell membranes including interneurons, principal cells, and even glia although RH-414 has been shown to preferentially stain neurons over glia (for further discussion, see Sinha et al. 1995). Our recording technique provides a spatially averaged signal (each of the 100 photodiode matrix elements covers a 200 × 200 × 400 μm volume, where 400 μm is the thickness of the brain slice). Thus the actual signal seen by a matrix element is the weighted average of the activity in all the cells located in its recording area. Because the VSD molecules are located on the cell membrane, the weight is the surface area of the cell

$$\sum_i (\Delta V_{m,i} \times SA_i)$$

where $\Delta V_{m,i}$ represents the recorded signal, $\Delta V_{m,i}$ is the change in membrane potential in the $i$th structure in the recording area, and $SA_i$ is the surface area of this structure. During SIA, interneurons are firing action potentials ($\Delta V_{m_i} \sim 100$ mV for ~1 ms). On the other hand, principal neurons are undergoing LLDs, which, when recorded at the soma, are predominantly hyperpolarizing with a superimposed depolarizing component ($\Delta V_{m} \sim 10$ mV for

**FIG. 4.** Evoked and spontaneous activity in area CA1. A: fraction of all spontaneous events (in 4-AP or in 4-AP + EAA antagonists) that showed a particular direction of propagation are shown. B: the same data plotted as fraction of events/slice that showed a particular direction of propagation. C: multiple patterns of propagation observed in 4-AP + EAA receptor antagonists in a single slice. On the left is a spontaneous event that propagates from the subiculum toward area CA3. On the right is another event recorded from the same slice under the same conditions that propagates from area CA3 toward the subiculum.
antagonists was most prominent in area CA1. In the dentate gyrus, this activity was apparent in the hilus and even in PDM elements corresponding to the granule cell layer (Fig. 2D); however, the largest activity was always seen in the portion of s. lacunosum moleculare of area CA1 that was included in the recording area. In area CA3 (Fig. 3), this activity entered the recording area from area CA1. This is in contrast to the 4-AP-induced spontaneous interictal epileptiform activity, which always originated in area CA3 and then propagated into area CA1. Based on these observations, most experiments were conducted on area CA1.

Spatial pattern of activity in area CA1

In area CA1, several differences were found between spontaneous epileptiform activity and SIA (Fig. 4). The spatial distributions of these two types of activities were distinct (Fig. 6): epileptiform activity was largest in s. pyramidale and the proximal apical dendrites, whereas SIA was largest in the distal apical dendritic region. Epileptiform activity propagated from area CA3 to CA1 mainly synaptically via the Schaffer collaterals, i.e., the axons of the CA3 pyramidal cells, which synapse mainly in s. radiatum and oriens (Brown and Zador 1990); this activity then causes action potentials in the CA1 pyramidal cells. Any contribution by nonsynaptic mechanisms would also be expected to result in activity centered around s. pyramidale due to the small extracellular space found there and due to its proximity to the site of spike initiation (for a review of nonsynaptic mechanisms, see Jefferys 1995). Thus as observed experimentally, much of the activity during an interictal epileptiform event is expected to be located in the cell body layer and proximal dendritic regions.

There are several possible explanations for the observed spatial pattern of the synchronized interneuron activity. One possible reason is that the activity may be limited to a subgroup of interneurons. Interneurons of various morphologies occur throughout area CA1 (Freund and Buzsáki 1996). If the optical signal seen in the distal apical dendritic region mainly reflects action potentials in interneurons, then the spatial pattern would suggest that the interneurons located in the distal apical dendritic region (Lacaille and Schwartzkroin 1988a,b) have a higher propensity for this synchronized activity. It is also possible that the signal observed in this region is due to depolarization of the apical dendrites of CA1 pyramidal cells (LLDs) as a consequence of SIA. In this case, the spatial pattern would suggest that the interneurons that project their axons to the distal apical dendrites, irrespective of the location of their somata, have a special propensity for SIA. The possible identity of the interneurons involved in SIA is discussed in more detail in the following text.

In another scenario, the spatial pattern might be a possible artifact caused by the spatial-averaging inherent in this optical recording method. The small activity seen in the cell body layer and proximal dendritic regions could be a result of the spatial integration of bursts of action potentials in some or all of the interneurons in this area and of large hyperpolarizing IPSPs in pyramidal neurons. Due to their opposing polarities, these two responses could cancel each other out in the spatially averaged voltage-sensitive dye signal. The depolarization seen in s. lacunosum moleculare could still be due to firing of interneurons located there or could instead be due to depolar-

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**Fig. 6.** Spatial distribution of activity in area CA1. Comparison of spatial pattern of activity recorded in 4-AP alone and in 4-AP + EAA receptor antagonists. A: amplitude of activity recorded in the various subregions of the hippocampal slice. A schematic of a CA1 pyramidal cell is shown for reference. B: to allow for comparison of the spatial distribution of the different types of activities, the amplitudes in the various subregions were normalized to the amplitude of activity in s. pyr (the region centered around and including s. pyramidale).

~100–1,000 ms) (Perreault and Avoli 1992). It should be noted that the LLD are likely to have significantly different characteristics (amplitude, time course, and polarity) in the dendrites of the principal cells. The relative surface area of interneurons is much smaller than the pyramidal cells as there are many more principal cells than interneurons in the hippocampus (Brown and Zador 1990). Because of these issues, it is impossible to determine if the signals we recorded predominantly reflect activity in interneurons, in principal cells, or some combination of the two. This has important ramifications for interpreting the spatial pattern of the observed optical signals (discussed in the following text).

**Activity in the dentate gyrus and area CA3**

Even in experiments where the optical recording area was centered on either the dentate gyrus or area CA3, it became apparent that SIA observed in the presence of 4-AP + EAA.
izing responses in pyramidal cell dendrites. However, this scenario is highly unlikely for the following reason. The field potential recording, which represents a very different kind of spatially averaged signal (for review, see Johnston and Wu 1995), was also almost completely absent when recorded in s. pyramidal (data not shown). Because of the intrinsic differences between the optically recorded membrane potential transients and the electrically recorded field potentials, it is highly unlikely that spatial averaging would lead to the observed lack of SIA in the pyramidal cell layer and proximal dendritic regions in both types of recorded signals.

**Pattern of spread of spontaneous activity in area CA1**

The two types of spontaneous activity also showed distinct patterns of spread (Fig. 5, A and B). The epileptiform activity originated in area CA3 and propagated across CA1 toward the subiculum. This pattern is consistent with previous studies (e.g., Colom and Saggau 1994) and is most likely due to the relatively extensive recurrent excitatory connections in this region. The pattern of spread of the SIA was far more variable. Due to the fact that the recording area does not cover the entire brain slice, the actual site of origin could not be observed; instead, only its location relative to the recording area could be determined. Activity was almost equally likely to propagate in either direction across area CA1. Moreover, even in the same slice, both propagation patterns, from CA3 to the subiculum and from the subiculum to CA3, were observed (Fig. 5C). Based on this data, it cannot be directly determined if the site of origin was in some part of area CA1 not included in the recording area or if it was actually in another region of the slice. At least for activity propagating from area CA3 toward the subiculum, the site of origin was most likely in some part of CA1 outside the recording area. This is based on the observation that activity always entered from area CA1 when recording in area CA3 (Fig. 3). For activity propagating from the subicular side to CA3, however, the question whether the activity originates in some portion of area CA1 or in the subiculum remains open.

The observation of multiple patterns of propagation suggests that at least two different regions in the hippocampal slice can serve as the site of origin for SIA. Also, in preliminary experiments, we have found that such activity can be evoked by an electrical stimulus applied to various portions of s. lacunosum moleculare of area CA1, regardless of the exact location relative to area CA3 and the subiculum (McClure et al. 1998). The responses evoked by such a stimulus spread out from the site of stimulation and were blocked by the GABA_A receptor antagonist picrotoxin. Based on these preliminary observations regarding evoked SIA and the data presented in this paper, it is apparent that interneurons located in multiple areas of the hippocampal slice can serve as a “pacemaker” for the synchronized interneuron activity. This is consistent with the finding that long-lasting depolarizations can be initiated in any area of the slice when these areas are surgically isolated from the remainder of the slice (Perreault and Avoli 1992). It remains to be determined if certain regions or interneurons have a special propensity to be a “pacemaker.”

Perreault and Avoli (1992) used multiple field electrodes and lesioning of slices to investigate the spatiotemporal pattern of LLDs. In contrast to the results of the present study, their findings did not suggest any special propensity of area CA1 for synchronized interneuronal activity. In experiments where different subfields were isolated surgically, the frequency of events increased in area CA3 and decreased in area CA1 and the dentate gyrus. In field potentials recorded simultaneously in the cell body layer of the various subregions, the LLD was first recorded in area CA3 ~40% of the time, 31% occurred first in area CA1, and 2% in the dentate gyrus. In 17% of the cases, LLDs were recorded without measurable delay in the dentate gyrus and either area CA3 or area CA1. As these authors noted, it is difficult to determine the site of origin from this type of data as it can be easily confounded by cases where the activity originates at a site between the recording electrodes. This is especially true when the pathways by which the activity is propagating have been interrupted. While our experiments did not reveal the precise site of origin of SIA, the ability to simultaneously record from a large number of sites allowed us to obtain a more accurate description of the spatiotemporal characteristics of SIA. These recordings suggest a higher propensity for the generation of SIA in or near area CA1.

**Interneurons involved in synchronized activity in area CA1**

The identity of the interneurons that participate in the synchronized activity is not known; some hypotheses based on our experiments and previous studies are presented here. The spatial distribution of the activity in area CA1 (Figs. 4D and 6) suggests that interneurons located in the distal apical dendritic regions (L-M interneurons) or those that project to this region may be specially involved. L-M interneurons mediate feedforward inhibition in area CA1 (Lacaille and Schwartzkroin 1988b). In addition to their projection to pyramidal cells, they also project to other interneurons located in s. lacunosum moleculare. Such a projection to other interneurons would be a requirement for originating and propagating this synchronized activity. The extent to which these interneurons are connected to each other, another requirement for generating synchronized activity, is not known. The involvement of L-M interneurons also provides a possible explanation for the finding that LLDs, the correlate of synchronized interneuron activity, were observed to propagate between area CA1 and the dentate gyrus in the absence of area CA3 (Perreault and Avoli 1992). The processes of L-M interneurons cross the hippocampal fissure (Gulyás et al. 1996; Lacaille and Schwartzkroin 1988a) and thus provide a remaining synaptic connection between these two regions in a hippocampal slice after area CA3 has been surgically removed. Thus L-M interneurons are an attractive candidate for interneurons involved in SIA.

As mentioned in the preceding text, synaptic contact with other interneurons is an important requirement for cells that are involved in SIA. One such class of interneurons is immunoreactive for the calcium binding protein calretinin [referred to as interneuron-selective type 1, IS-1, by Freund and Buzsáki (1996)]. IS-1 interneurons make many synaptic contacts and direct dendro-dendritic contacts with other interneurons (Gulyás et al. 1996). Calretinin-immunoreactive interneurons are distributed throughout the hippocampal formation. If the synchronized interneuron activity actually occurs in all layers of area CA1, as discussed in the preceding text, then the calretinin-immunoreactive neurons may be the substrate. IS-2 neurons, which are immunoreactive for vasoactive intestinal pep-
tide, are another class of interneurons that project to other interneurons (Freund and Buzsáki 1996). They have their soma in the distal apical dendritic region; their dendrites are mainly located in s. lacunosum moleculare. Their axons project diffusely throughout the apical dendritic region.

For the reasons discussed in the preceding text, involvement of interneurons in other regions should not be excluded. Many other subpopulations of interneurons have axonal processes located in the distal apical dendritic region of area CA1 (Freund and Buzsáki 1996). These include O-LM cells (soma and dendrites in s. oriens and axonal projections in s. lacunosum moleculare), radial trilaminar cells (soma, dendrites and axons located throughout the various strata), bistratified cells (soma and dendrites in s. pyramidale or radiatum, axonal projection in s. oriens and radiatum).

Significance of synchronized interneuron activity

Synchronization of interneurons by extrinsic sources is believed to be important in the generation of various brain rhythms (Whittington et al. 1995; Ylinen et al. 1995). If synchronization of activity occurs within the interneuron network under physiological conditions, it may also be important in the generation of brain rhythms. The possibility that such synchronized activity may occur under physiological conditions was suggested by the observations of evoked LLD-like responses under physiological conditions in the hippocampal slice (Avoli et al. 1996). A pathophysiological role for the synchronized activity has been suggested based on the observation that in combined entorhinal cortex-hippocampal slices, LLDs often precede ictal epileptiform activity (Avoli et al. 1996). Thus LLDs were hypothesized to be a trigger for the transition from interictal to ictal activity by raising $[K^+]_o$, GABA$_A$ receptor-mediated depolarizations have also been suggested to underlie stimulus evoked ictal epileptiform activity (Köhling et al. 2000). Another possible mechanism by which LLDs could lead to ictal activity would be to simply synchronize neurons by simultaneously inhibiting them and thus making all of them available to participate in next discharge, which may allow that discharge to reach ictal status.

Beyond the possible involvement of SIA in epileptiform activity, investigation of SIA provides another window into the complex and diverse world of interneurons and their interactions with each other and with principal cells. Although isolated under relatively artificial conditions, the various mechanisms, interconnections and cells that form the substrates for SIA also exist in the hippocampal slice under less artificial conditions and in the hippocampus in vivo. These substrates are likely to play some role in the normal physiology of the hippocampus; thus understanding SIA and the mechanisms behind it will advance our understanding of the normal physiology of the hippocampus. Furthermore, it is important to note that during periods of intense activity, conditions can locally resemble those required for SIA e.g., elevation of $[K^+]_o$; therefore some form of SIA could occur under more physiological conditions.

REFERENCES


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