Voltage Imaging Reveals the CA1 Region at the CA2 Border as a Focus for Epileptiform Discharges and Long-Term Potentiation in Hippocampal Slices

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Submitted 11 May 2007; accepted in final form 29 June 2007

Chang PY, Taylor PE, Jackson MB. Voltage imaging reveals the CA1 region at the CA2 border as a focus for epileptiform discharges and long-term potentiation in hippocampal slices. J Neurophysiol 98: 1309–1322, 2007. First published July 5, 2007; doi:10.1152/jn.00532.2007. Voltage-sensitive-dye imaging was used to study the initiation and propagation of epileptiform activity in transverse hippocampal slices. A portion of the slices tested generated epileptiform discharges in response to electrical shocks under normal physiological conditions. The fraction of slices showing epileptiform responses increased from 44 to 86% when bathing [K⁺] increased from 3.2 to 4 mM. Regardless of stimulation site in the dentate gyrus and hippocampus, discharges generally initiated in the CA3 region. After onset, discharges abruptly appeared in the CA1 region, right at the CA2 border. This spread from the CA3 region to the CA1 region was saltatory, occurring before detectable activity in the intervening CA2 and CA3 regions. Discharges did eventually propagate smoothly through the intervening CA3 region into the CA2 region, but on a slower timescale. The surge in the CA1 region did not spread back into the CA2 region, but spread through the CA1 region toward the subiculum. Tetanic stimulation, theta bursts, and GABA_A receptor antagonists failed to alter this characteristic pattern, but did reduce the latency of discharge onset. The part of the CA1 region at the CA2 border, where epileptic responses emerged with relatively short latency, also expressed stronger long-term potentiation (LTP) than the rest of the CA1 region. The CA2 region, where discharges had long latencies and low amplitudes, expressed weaker LTP. Thus the CA1 region at the CA2 border has unique properties, which make this part of the hippocampus an important locus for both epileptiform activity and plasticity.

INTRODUCTION

Brain regions vary considerably in their capacity to participate in different forms of epileptic activity (Clark and Wilson 1999; McCormick and Contreras 2001; Prince 1978; Traub and Miles 1991). Epileptic discharges generally initiate at specific locations and, after onset, spread in preferred directions along preferred pathways (Demir et al. 1998; Traub and Miles 1991). Thus the spatiotemporal pattern of seizure activity provides a map of underlying neural circuitry with a proclivity for abnormal electrical activity. In humans and animals, cortical epilepsy can begin with abnormal activity at a seizure focus, followed by synchronization, and subsequent spread throughout the cortex (McCormick and Contreras 2001; Prince 1978; Traub and Miles 1991). A detailed understanding of the spatial patterns of discharge onset and spread will aid in understanding epilepsy by identifying specific neural circuit elements with unique roles in seizures.

Brain slices have served as useful in vitro models in studies of the mechanisms underlying the genesis and expression of epilepsy (Clark and Wilson 1999; McCormick and Contreras 2001; Prince 1978; Traub and Miles 1991). Imaging electrical activity in brain slices offers an approach to the mapping of neural circuitry that generates epileptiform activity in vitro. A number of efforts have been made to use voltage-sensitive dyes and imaging techniques to follow the spatiotemporal pattern of epileptiform activity in slices from various seizure-prone brain regions, including hippocampus (Albowitz and Kuhnt 1991; Colom and Saggau 1994; Nakagami et al. 1997; Sekino et al. 1997), piriform cortex (Demir et al. 1998), and neocortex (Albowitz et al. 1990; London et al. 1989; Sutor et al. 1994; Tsau et al. 1998; Wadman and Gutnick 1993). This approach has illuminated some of the neural circuitry that generates and sustains this abnormal activity. Voltage imaging has revealed both varied (Colom and Saggau 1994) and highly focal (Demir et al. 1998) sites of onset, and revealed sites where electrical activity plays a critical role before discharge onset (Demir et al. 1999). Measurements of propagation velocities suggested different roles for different axonal systems in the conduction of discharges (Demir et al. 2001). Furthermore, conduction can proceed in either a wavelike (Albowitz and Kuhnt 1991; Demir et al. 1998) or a saltatory (Wadman and Gutnick 1993) manner, indicating that neural circuits can support discharge conduction by different mechanisms.

In the present study voltage imaging was used to investigate the genesis and evolution of epileptiform discharges in slices prepared from the rat hippocampus. This investigation revealed a highly stereotypical pattern in the initiation and propagation of epileptiform discharges, indicating that different hippocampal subfields can make distinct contributions to epileptiform activity. Discharges usually began in the CA3 region, but as these discharges evolved they showed a striking preferential spread to the CA1 region at the CA2 border. By contrast, discharges were weak and delayed in the CA2 region. Tetanic stimulation (TS), theta burst stimulation, and γ-aminobutyric acid type A (GABA_A) receptor blockade all left this fundamental pattern unchanged. Furthermore, theta bursts applied to various sites in the CA1 and CA2 regions induced strong long-term potentiation (LTP) in the CA1 region at the CA2 border, but little LTP in the CA2 region.

METHODS

Slice preparation

Male Sprague–Dawley rats 1 to 2 mo old (100–250 g) were decapitated under CO2-induced narcosis, in accordance with the...
guidelines of the National Institutes of Health, as approved by the Animal Care and Use Committee of the University of Wisconsin–Madison. The brains were then removed and chilled in ice-cold cutting solution consisting of (in mM) 124 NaCl, 3.2 or 4.0 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 1 CaCl₂, 6 MgSO₄, and 10 glucose, bubbled with 95% O₂-5% CO₂. Transverse hippocampal slices 350 to 400 μm thick were cut with a tissue slicer (HR2, Sigmam Elektronik, Hüffenhardt, Germany), selecting sections midway along the dorsal–ventral axis. A cut at the CA2 or CA3 regions was made in experiments on LTP to block epileptiform activity. Slices were stored in a vial of 95% O₂-5% CO₂–bubbled artificial cerebrospinal fluid (ACSF) for 1 h. ACSF was identical to the cutting solution but contained 2.5 mM CaCl₂ and 1.3 mM MgSO₄. Slices were stained with one of three different voltage-sensitive dyes: 0.2 mg/ml RH155, 100 μM RH414 (Molecular Probes, Eugene, OR), or 0.02 mg/ml RH482 (NK3630, Hayashibara Biochemical Laboratories, Okayama, Japan) (Chang and Jackson 2003; 2006; Momose-Sato et al. 1999). Staining was accomplished by incubation in a solution of dye in 95% O₂-5% CO₂–bubbled ACSF for 1 h. During staining and perfusion slices were continuously perfused with 95% O₂-5% CO₂–bubbled ACSF at a flow rate of 2–2.5 ml/min in a submerged recording chamber and maintained at 29–31°C. All chemicals (other than voltage-sensitive dyes) were purchased from Sigma (St. Louis, MO).

Electrophysiology

Stimulating and field potential (FP) recording electrodes were fabricated from borosilicate glass capillaries (1.15 mm ID, 1.50 mm OD) and filled with ACSF. Stimulating electrodes had tip diameters of 10–30 μm; recording electrodes had tip diameters of approximately 1 μm with resistances ranging from 2 to 5 MΩ. Slices were stimulated with 0.2-ms current pulses delivered by a monopolar constant-current stimulus isolator (Model A365, World Precision Instruments, Sarasota, FL). The theta burst stimulation protocol used to induce LTP consisted of three theta bursts separated by 10 s. Each theta burst consisted of 10 bursts at 5 Hz, with each burst consisting of six pulses at 100 Hz. The tetanic stimulation (TS) protocol consisted of a 100-Hz train for 1 s. Field potentials were recorded with an Axopatch 200B amplifier or an Axopatch 1C amplifier (Axon Instruments/Molecular Devices, Foster City, CA).

Voltage imaging and data analysis

The instrumentation follows Wu and Cohen (1993) and is very similar to the commercial photodiode array system Neuroplex-II from RedShirtImaging LLC (Fairfield, CT) (Demir et al. 1998; Jackson and Scharfman 1996). A set of 464 optical fibers was bundled into a hexagonal array, and each fiber was coupled to a photodiode. Signals were amplified to 5 V/nA of photocurrent, low-pass filtered with a four-pole Bessel filter at 500 Hz, multiplexed, and digitized at a frame rate of 1.63 or 10.0 kHz with eight expansion boards (MSXB 002-02), one DAP3200e/214, and one DAP5200 data acquisition board (Microstar Laboratories, Bellevue, WA).

In experiments using the absorbance dyes RH155 and RH482, an upright Reichert Jung Diastar microscope (Leica, Deerfield, IL) was illuminated from below with a 100-W tungsten-halogen bulb driven by a Keppco ATE-36-30DM power supply (Flushing, NY). Illuminating light passed through a 702 ± 18-nm band-pass filter (Omega, Brattleboro, VT), and transmitted light was collected with a Zeiss Fluar ×5 objective lens or a ×10 Olympus UPlanApo objective lens. The center-to-center distance between neighboring photodiode fields was 134 or 67 μm for the ×5 and ×10 objectives, respectively. In experiments with the fluorescent dye RH414 epifluorescence illumination was used, with a 520 ± 45-nm band-pass excitation filter (Chroma, Brattleboro, VT), a 590-nm dichroic mirror, and a 610-nm long-pass emission filter (Chang and Jackson 2003). Optical signals (ΔI/I or ΔF/F) were presented as the change in transmitted/fluorescent light divided by the resting light intensity.

A movable mirror directed images to a charge-coupled detector (CCD) camera for capture by the computer with a frame grabber (Data Translation, Marlboro, MA). Alignment between CCD images and photodiode array images was performed using test images of pinholes. The alignment was further checked by comparing the map of resting light intensity against the CCD image.

Data acquisition, signal processing, and analysis were performed with a Pentium 4 computer running a C++ program written in this laboratory (Chang 2006). Additional analysis and plotting were performed with the program Origin (Microcal, Northampton, MA). We used a binomial temporal filter with a width of 1.2 ms to reduce noise in the optical traces. The baseline of each trace was corrected by fitting the time before the stimulus and 300 ms after the stimulus to a third-order polynomial and subtracting this function from the entire trace. Stimulus artifacts were removed from displayed FP recordings. Peak signals were used to create color-encoded maps of responses and of LTP. The colors in pixels between centers of diodes were linearly interpolated. A Gaussian spatial filter with a space constant of half the interdiode distance reduced noise in these maps. LTP was defined as the increase in peak amplitude of optical signals, and LTP data were used only if the preinduction baseline remained stable for ≥20 min (Chang and Jackson 2006).

The latency was measured as the time to reach half-maximum amplitude. Arithmetic means of measurements are presented with the SD, except where otherwise noted. Tests for statistical significance are indicated as used.

RESULTS

Voltage imaging of epileptiform activity in hippocampal slices

Hippocampal slices stained with voltage-sensitive dye were stimulated at various locations and the absorbance or fluorescence was imaged over time to follow the progress of electrical responses through the tissue. CCD camera images were used to identify the hippocampal subfields. The precise delineation of the CA2 region is especially important for this study, and we note that this region along with its borders with the CA1 and CA3 regions are clearly identifiable by the following criteria. In the stratum pyramidale, the pyramidal neurons of the CA2 region have larger cell bodies but a lower packing density than in the CA1 region. The border between the CA2 and the CA3 regions is recognized by the difference in the organization of pyramidal cell bodies. The CA2 region could also be recognized by inflections in the otherwise smoothly arcing stratum pyramidale. Finally, the stratum lucidum, which contains the mossy fiber pathway, provided an additional distinguishing feature for the CA3–CA2 boundary. With our optical system the stratum lucidum could often be recognized as a dark strip adjacent to the stratum pyramidale. It became fainter from the CA3c to the CA3a subregions, but did not enter the CA2 region. These distinctive features together with careful alignment between the photodiode and CCD images (METHODS) enabled precise anatomical localization of optical signals.

Hippocampal slices were initially tested in control ACSF with a stimulus applied in the stratum molecular of the dentate gyrus. In a fraction of these slices a stimulus of ±100 μA elicited an epileptiform discharge. These responses had an all-or-none character, with stimulation at the threshold intensity triggering epileptiform activity about 50% of the time (Fig. 1). The threshold stimulus current varied considerably from
slice to slice. In 3.2 mM K⁺, 44% of 25 slices produced discharges with stimulation ≤100 μA and in 4 mM K⁺, 86% of seven slices produced discharges. There was a slightly greater tendency for epileptiform activity in slices from younger rats but the mean age of animals yielding epileptogenic slices was not significantly different from the mean age of animals from which slices showed no epileptiform responses. Spontaneous discharges were never observed under these control conditions.

Figure 1 presents the basic features and spatial distribution of optical signals recorded in one experiment. An initial nonepileptiform response could be seen in the dentate gyrus and, with threshold stimulation, these responses were identical regardless of whether the slice showed an epileptiform response. This response reflected the direct activation of neurons and synapses by the stimulus (Jackson and Scharfan 1996). Subtracting the subthreshold response from the suprathreshold response removed the direct response to yield a map of the regions participating in an epileptiform response (Fig. 1Ci). This map highlights two important locations of electrical activity during an epileptiform response. The first is the CA3 region, where the epileptiform discharge initiated, and the second is the CA1 region. The CA2 region had relatively weak epileptiform responses compared with the CA1 and CA3 regions. Optical traces and field potential signals clearly demonstrated the all-or-none character of epileptiform activity (Fig. 1D).

**Inhibitory contributions and sharp wave-ripple bursts**

Because strong stimulation triggered these discharges in control ACSF without any pharmacological treatment or epilepsy-inducing manipulation, we considered the possibility that the observed events were sharp wave-ripple bursts (Cohen et al. 2006; Maier et al. 2003; Papatheodoropoulos and Kostopoulos 2002; Ylinen et al. 1995). To test this hypothesis we applied gabazine (SR 95531), a GABA_A-receptor antagonist (Fig. 2). This drug has very different actions on sharp wave-ripple bursts and epileptiform activity, inhibiting the former but enhancing the latter. Gabazine failed to inhibit these events in our slices (Fig. 2A), but significantly reduced their latency in the CA3 and the CA1 regions (Fig. 2, B and D). Additionally, spontaneous discharges also occurred in the gabazine-treated slices, suggesting that these events were epileptiform discharges rather than sharp wave-ripple bursts.
In Fig. 2, the only noticeable changes in amplitude were in the dentate gyrus and the subiculum (Fig. 2Aiv). Blocking GABA<sub>A</sub> receptors led to the appearance of a slow component in the dentate gyrus and facilitated the propagation of epileptiform activity into the subiculum (Fig. 2C).

The function of inhibitory synapses was assessed in slices with a cut between the CA3 and the CA1 regions to prevent epileptiform activity in the CA1 region. Nonepileptiform responses were then evoked by stimulation of the stratum radiatum of the CA1 region. Application of the GABA<sub>A</sub>-receptor antagonist bicuculline (10 µM) increased these nonepileptiform responses in the stratum radiatum at a distance of 200 µm from the site of stimulation by 39 ± 21% (n = 7). Thus the epileptiform activity in control ACSF cannot be due to a complete absence of inhibition in our slices, although some degree of interneuron loss remains a possibility.
**Spatiotemporal pattern of epileptiform activity**

Epileptiform events usually initiated within the CA3 region (28 of 29 slices). Figure 3 displays a representative result demonstrating the propagation pattern we saw in most experiments. Figure 3B shows a typical distribution of peak responses with peak amplitude encoded as color, for a discharge triggered by stimulation in the dentate gyrus. Mapping the latency from the time of stimulation to the time at half-maximum amplitude revealed the initiation site of the epileptiform activity in the CA3 region (Fig. 3C, yellow arrow).

Surprisingly, the epileptiform response appeared abruptly in the CA1 region next to the CA2 region before spreading to the CA2 region, even though the CA2 region was closer to the initiation site (Fig. 3C, white arrow). We observed this sequence in 27 of 29 slices. Signals at four locations provided a further illustration of this temporal pattern (Fig. 3D). The latency from initiation in the CA3 region until emergence in the CA1 region was significantly shorter than the latency from initiation until appearance in the CA2 region (6.0 ± 1.2 vs. 10.3 ± 1.7 ms, mean ± SE, n = 15, P < 0.02, paired t-test).

**FIG. 3.** Initiation and propagation of epileptiform activity. A: CCD image highlighted as in Fig. 1A. Sites are similar to those in Fig. 1A but site 3 is in the CA2 region. Signals from locations 1–4 are displayed in D. Dye: RH482; [K⁺] = 4 mM. B: map of peak amplitude encoded as color according to the scale in the bottom left corner. A stimulus (20 µA) applied in the stratum moleculare of the DG triggered epileptiform activity. Responses were large in the CA3 and CA1 regions but weak in the CA2 region. C: map of latency encoded as color. Responses in the DG had the shortest latency. Epileptiform discharge began in the CA3 region (yellow arrow), and then emerged in the CA1 region at the CA2 border (white arrow). Latency was defined as the time from stimulus to half-maximum amplitude. D: optical signals recorded in the DG, CA3, CA2, and CA1 regions illustrate the temporal sequence of epileptiform activity (sites indicated in A). Four traces were superimposed below to highlight relative latencies. Notice that the epileptiform response appeared in the CA1 region before the CA2 region. Traces are averages of 10 trials. Black arrows indicate the time of stimulation. Inset: latencies from CA3 to CA1 and CA2 regions (mean ± SE). Latency for the CA1 region was significantly shorter than for the CA2 region (n = 15, P < 0.02, paired t-test). E: temporal sequence of maps at 3.68-ms intervals, with optical signal amplitude encoded as color, shows the spatiotemporal pattern of an epileptiform discharge. Color scale is as in B, with the first frame taken immediately before the stimulus. Yellow arrows in frames 7 and 8 indicate the initiation site in the CA3 region and the white arrows in frames 8 and 9 indicate the site of abrupt emergence in the CA1 region near the CA2 border.
initiation and propagation (Fig. 4C). In each case, the epileptiform activity consistently originated in one area of the CA3 region, emerged in the CA1 region at the CA2 border, propagated through the rest of the CA1 region, and sometimes propagated into the subiculum.

**Velocity and mode of discharge propagation**

A detailed analysis of the velocity and evolution of the discharge waveform can provide insight into the mechanism of discharge propagation (Demir et al. 1998; Golomb and Amitai 1997; Hoffman and Haberly 1993; Miles et al. 1988; Traub et al. 1987). We observed that as discharges propagated, they preserved their overall shape within each subfield but changed their shape on passage from the CA3 region to the CA1 region (Fig. 5; traces were normalized to emphasize temporal relationships). In the CA3 region discharges rose rapidly in an initial phase, but rose further at a slower rate before reaching a peak. In the CA1 region, discharges rose more rapidly, reaching a peak without the later slow phase seen in the CA3 region (compare traces 1–4 with traces 6–8 in Fig. 5C). Within each subfield the shape was well preserved, thus indicating a wave-like propagation within that region (Golomb and Amitai 1997).

To determine the velocity of discharge conduction, we plotted the time to reach half-maximum amplitude versus distance for a series of sites along the trajectory of propagation. Fitting this plot to a line yields velocity (Demir et al. 1998; Golomb and Amitai 1997; Miles et al. 1988; Traub et al. 1987). Discharges elicited by dentate gyrus stimulation propagated

![Diagram](https://example.com/diagram)

**FIG. 4.** Stimulation of different sites evokes epileptiform discharges with similar spatiotemporal characteristics. Stimulation in the DG and CA3 and CA1 regions evoked epileptiform activity with similar initiation and propagation patterns. Three different examples are shown: A (RH155; [K⁺] = 4 mM), B (RH155; [K⁺] = 4 mM), and C (RH482; [K⁺] = 3.2 mM). i: CCD images of slices. ii–v: color maps display the spatial distributions of peak amplitude in each of these 3 examples. Red arrows indicate stimulation sites; white arrows indicate locations with large epileptiform responses. Notice that the spatial distributions of epileptiform responses are similar regardless of the stimulation sites in each experiment. Color scales were normalized to the maximum peak amplitude in each slice.

This temporal pattern was counterintuitive because epileptiform activity initiating in the CA3 region should pass through the CA2 region before it reaches the CA1 region. A temporal series of color maps illustrates the spread of electrical activity in greater detail (Fig. 3E). The first frame of this series was the map immediately before the stimulus, with subsequent frames at 3.68-ms intervals. The site of onset can be clearly identified in the CA3 region in frames 7 and 8 (yellow arrows). The site of first appearance of epileptiform activity in the CA1 region can be identified in frames 8 and 9 (white arrows). Only well after this appearance in the CA1 region did the epileptiform activity invade the CA2 region from the CA3 region.

We observed the same stereotypical pattern regardless of whether epileptiform activity was triggered by stimulating the dentate gyrus or the CA3 or CA1 regions (Fig. 4). For each slice studied, the pattern evoked by stimulation at different locations was very similar. Stimulation in the stratum molecular of the dentate gyrus, the infrapyramidal blade, the suprapyramidal blade, or the crest all evoked similar patterns of
with a slightly higher velocity in the CA3 region ($164 \pm 97 \text{ mm/s, } n = 9$) than in the CA1 region ($145 \pm 62 \text{ mm/s, } n = 9$). Both of these velocities were slower than the apparent action potential conduction velocity, as inferred from the propagation of nonepileptiform responses evoked by stimulation of the stratum radiatum in the CA1 region ($201 \pm 72 \text{ mm/s, } n = 12$). These results are all consistent with a mechanism of discharge propagation through recurrent excitatory collaterals, as posited by Traub (1987).

**Tetanic stimulation induces epileptiform activity and reduces the onset latency**

Tetanic stimulation (TS) has been shown to induce epileptiform activity in hippocampal slices (Stasheff et al. 1985, 1989). Application of TS to the dentate gyrus induced epileptiform discharges with the same stereotypical pattern of initiation and propagation as seen in slices exhibiting epileptiform responses without prior TS. In one example, 25 $\mu$A was initially subthreshold and failed to evoke epileptiform activity (Fig. 6Bi). After a single TS, the same 25-$\mu$A stimulus evoked epileptiform responses in the hippocampus and subiculum (Fig. 6Bii). The spatiotemporal pattern was the same as that for discharges evoked in untetanized slices. The epileptiform activity initiated in the CA3 region and then appeared abruptly in the CA1 region at the CA2 border before spreading through the CA1 region and into the subiculum. The spatial distribution of optical signals before and after TS shows that the differences in responses were most prominent in the CA3 and CA1 regions (Fig. 6Ci). Optical signals at four locations further demonstrated the all-or-none character of epileptiform activity induced by TS (Fig. 6D, black and red traces).

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**FIG. 6.** Tetanic stimulation induces epileptiform activity and reduces the latency. A: CCD image labeled and highlighted as in Fig. 1A. Signals from locations (1–4) are displayed in D. Dye: RH155; [K+] = 4 mM. B: spatial distributions of responses in 3 conditions. i: spatial distribution of peak amplitudes evoked by stimulation ($25 \mu$A in the DG) below the threshold for epileptiform activity. White arrows indicate locations with prominent epileptiform responses in ii and iii after induction with tetanic stimulation (TS, 1 s, 100 Hz). ii: after one TS, a single stimulus as in i evoked epileptiform activity. iii: after 3 TSs, a $25 \mu$A pulse evoked activity with a spatial distribution similar to that in ii. C: difference maps between responses in B, i, ii, and iii. i: difference map between Bi and Bii shows that one TS produced large changes in responses in the CA3 and CA1 regions. Responses in the subiculum and part of the stratum moleculare also increased. ii: difference map between Bii and Biii shows that 3 more TSs produced no further changes in peak amplitudes. D: optical signals at the 4 locations indicated in A show the effects of TS. Initially, there was no response in the hippocampus and the SB (black traces at locations 2–4). After one TS, the response in the DG increased slightly (location 1, red trace), and epileptiform activity spread to sites 2–4. Three more TSs produced no significant further change in amplitude at any location, but reduced the latency of epileptiform discharges by 9 ms. Signals were averages of 5 trials. E: bar graph of changes in latency in the DG, CA1 region, and CA3 region after TS or theta bursts. Reduction in the latency of epileptiform activity in the CA3 and CA1 regions was significant, but no significant changes were seen in the DG ($n = 8; P < 0.02$ by $t$-test for both CA3 and CA1).
The application of additional TS produced little if any further changes in the epileptiform activity induced by the first TS. When three more TSs were applied, a subsequent stimulus of 25 μA evoked an epileptiform response (Fig. 6Bi) that showed a distribution similar to that mapped in Fig. 6Bii. The spatial distribution of differences between the maps shown in Fig. 6Bi, ii and iii confirmed that there were no significant changes in amplitude after these three additional TSs (Fig. 6C). Examination of optical signals at four locations revealed that although the amplitudes of responses remained the same and the stereotypical pattern did not change, the latency to discharge onset in the hippocampus and the subiculum was reduced by 9 ms. In eight slices, the onset latencies in the CA3 and CA1 regions were significantly reduced after three more TSs by about 8 ms (Fig. 6E, t-test, P < 0.02, n = 8). The reduction in onset latency could be due to strengthening of excitatory connections or weakening of inhibitory connections in the dentate gyrus or CA3 region.

To test whether theta bursts can affect the spatiotemporal pattern of epileptiform activity in disinhibited slices, we blocked GABA<sub>A</sub> receptors with gabazine (Fig. 7). Theta bursts induced a small amount of LTP of responses in the dentate gyrus, but elsewhere in the hippocampus the amplitudes of the epileptiform discharges failed to change (Fig. 7A). In gabazine, theta burst stimulation reduced the latencies of discharge onset in the CA3 and CA1 regions by 2–3 ms (Fig. 7B and C). A FP recording in the stratum pyramidale of the CA3 region also showed a reduction in discharge latency (Fig. 7D). The latency for the direct responses in the dentate gyrus remained essentially unchanged (Fig. 7D). These results suggest that when excitatory synapses in the dentate gyrus become stronger, the buildup of electrical activity leading to discharge onset is accelerated. It is likely that both TS and theta bursts enhance epileptiform activity through a similar mechanism involving LTP at excitatory synapses. Because theta bursts reduced the onset latency only 2–3 ms when gabazine was present, compared with 8 ms in control ACSF, LTD of inhibitory synapses may also play a role in accelerating the onset of epileptiform activity.

**Stronger LTP in the CA1 region at the CA2 border**

Epileptiform activity was especially prominent at the edge of the CA1 region bordering the CA2 region. Discharges generally emerge at this site with a short latency and large amplitude. The prominence of epileptiform discharges at this location raises questions about the neuronal excitability and circuitry at this site. We therefore tested the capacity of this region to generate LTP under conditions where epileptiform activity was blocked by severing Schaffer collaterals/commissional fibers with a cut in the CA2 region. Theta bursts were applied to the stratum radiatum at various locations in the CA1 region to induce LTP. Figure 8 shows a typical LTP experiment. The spatial distributions of peak amplitude before and 60 min after TBs are displayed in Fig. 8A, ii and iii, respectively. Figure 8Aiv shows the spatial distribution of LTP, taken as the difference between the signals mapped in Fig. 8A, ii and iii. This map shows that at the CA2 border the CA1 region expressed stronger LTP than at other locations in the CA1 region.
LTP map (white arrow). When the stimulating electrode was placed closer to the CA2 region (725 ± 9262 m; Fig. 9B), the LTP expressed at the border started to overlap with another LTP expression area near the stimulating electrode. When the stimulating electrode was 525 ± 9262 m away (Fig. 9C), the region between the stimulating electrode and the CA2 region expressed stronger LTP than the region on the other side of the stimulating electrode. With a stimulation current of 50 ± 9262 A, the area expressing strong LTP was usually close to the stimulating electrode (Chang and Jackson 2006). However, when the stimulating electrode was close to the CA2 region, theta bursts with low current intensity induced strong LTP in this same border zone where epileptiform activity first emerges in the CA1 region (Fig. 9D).

Figure 10A displays the summary profile of LTP induced by moderate stimulation currents (50 – 75 ± 9262 A) in the stratum radiatum of the CA1 region (n = 18). These results demonstrated that the capacity to express LTP at the edge of the CA1 region close to the CA2 border is higher than elsewhere in the CA1 region.

To investigate LTP induction with stimulation in the CA2 region, a cut was made in the nearby CA3a region. This preserved the CA2 region but still blocked the spread of epileptiform activity from the CA3 region. A stimulating electrode was placed in the stratum radiatum of the CA2 region to evoke action potentials in the Schaffer collaterals/commissural fibers that propagate orthodromically into the CA1 region. In this experimental configuration, theta bursts still induced stronger LTP in the CA1 region at the CA2 border (Fig. 9, E and F displays two examples from six such experiments). Figure 10B shows the summary profile of LTP along the stratum radiatum in the CA2 and the CA1 regions, with LTP induced by moderate stimulation currents applied to the CA2 region (40 – 80 ± 9262 A). This plot shows that the CA1 region at the CA2 border again has a higher capacity for LTP. Furthermore, LTP was generally much weaker in the adjacent CA2 region and this, in turn, corresponds well with the very low amplitude and long latency of epileptiform activity in this region (see Fig. 3).

**DISCUSSION**

This study has documented a distinctive pattern in the onset and propagation of epileptiform discharges in the hippocampal slice. The highly reproducible and stereotypical patterns reported here build on our understanding of the functional...
circuitry of the hippocampus and offer insights into both normal and pathological activity in this brain region. It is well established that the CA3 pyramidal cells innervate one another through their associational connections. CA3 pyramidal cells also innervate CA1 pyramidal cells through Schaffer collaterals in an ordered and spatially distributed pattern (Ishizuka et al. 1990). The recurrent excitation between CA3 pyramidal neurons gives the CA3 region a strong proclivity for synchronous epileptiform discharges. Just as the CA3 region is an important hot spot for seizure activity, the CA2 region is relatively resistant to damage in human epilepsy (Houser 1999; Leranth and Ribak 1991; Margerison and Corsellis 1966; Sloviter et al. 1991). This parallels our observation of weaker optical signals in the CA2 region during discharges and long latencies to discharge onset. Thus the onset and strong participation of the CA3 region, weak participation of the CA2 region, and strong participation of the CA1 region constitute an interesting parallel with human epilepsy.

FIG. 9. TB stimulation in the CA1 or CA2 regions induced strong LTP in the CA1 region at the CA2 border. A–D: 4 separate experiments with different stimulation sites in the CA1 region illustrate how the same area that initiates epileptiform activity in the CA1 region at the CA2 border also expressed strong LTP. First column: CCD images of slices with labels as in Fig. 8A. Second column: spatial map of amplitudes before TBs. Third column: spatial map 40 min after TBs. Fourth column: difference as a map of LTP. White arrows indicate sites close to the CA2 border expressing strong LTP. Stimulus currents were A, 80 μA; B, 80 μA; C, 60 μA; D, 40 μA. E and F: 2 examples with stimulation in the stratum radiatum of the CA2 regions (red arrows). Responses were evoked by 60- and 40-μA pulses in E and F, respectively. Responses in the CA2 region were weak. After TBs, the CA1 area close to the CA2 border expressed strong LTP, but little LTP was evident in CA2 region. Similar results were observed in a total of 6 slices. Dashed lines delimit the border between the CA2 and CA1 regions. Dye: RH482 and [K⁺] = 3.2 mM in both experiments.
bursts (Korn et al. 1987; Rutecki et al. 1985; Staley et al. 1998). An appreciable frequency was seen at $[\text{K}^+] = 6.5$ mM (Rutecki et al. 1985), and even $5$ mM produced some spontaneous epileptiform events (P. Rutecki, personal communication). Thus our observation of evoked discharges at $3.2$ and $4$ mM $[\text{K}^+]$ may be related to the spontaneous discharges at higher concentrations, especially considering that increasing $[\text{K}^+]$ from $3.2$ to $4$ mM doubled the fraction of slices exhibiting this behavior. Finally, we note that a significant fraction of CA3 pyramidal cells produces bursts of action potentials in an all-or-none fashion in response to brief electrical stimulation in control saline (Wong and Prince 1981). Thus we can conjecture that the discharges reported in the present study are triggered when an electrical stimulus activates a critical number of intrinsically bursting CA3 pyramidal cells.

In studies of hippocampal slices, it is very common to separate or remove the CA3 region to prevent interference from unwanted epileptiform discharges, even under control conditions without GABA$_A$-receptor antagonists (Ireland et al. 2004; Muller and Lynch 1990; Raymond and Redman 2006). This would suggest that epileptiform activity in the CA3 region occurs fairly often in many different laboratories even under control conditions. The absence of epileptic activity in the CA3 region of a healthy animal may reflect stronger tonic inhibition in vivo. The slightly higher $[\text{K}^+]$ in our experiments than in mammalian CSF ($2.7–2.9$ mM) and nearly twofold higher $[\text{Ca}^{2+}]$ would also favor epileptiform activity (Langmoen and Andersen 1981). Furthermore, the localized shocks used to evoke epileptiform discharges in the present study probably do not resemble natural forms of activity seen in vivo.

Nevertheless, the present report of epileptiform activity without drug treatments or prior induction raises the concern about slice condition, especially in view of the high vulnerability of hippocampal interneurons (Lowenstein et al. 1992; Oliva et al. 2002; Scharfman and Schwartzkroin 1990; Sloviter 1987). This is a difficult point to address, but we note that slices used in the present study show highly characteristic subthreshold responses spreading over considerable distances along well-established hippocampal pathways. Our slices also express LTP quite well (Chang and Jackson 2006). The epileptiform discharges reported here follow a highly stereotypical and reproducible pattern, which suggests that if interneuron loss plays a role, then the spatial distribution of this loss can consistently support the spatiotemporal pattern of the epileptiform discharges observed here. Finally, we note that the increase in evoked synaptic responses after the addition of a GABA$_A$-receptor antagonist indicates that our slices contain some healthy interneurons. Using measurements of response enhancement during GABA$_A$-receptor blockade should serve as a useful benchmark for the evaluation of inhibitory function in slices. This could provide the basis of a strategy for systematic investigation of the relation between epileptiform activity and interneuron function in slices.

**Propagation of epileptiform activity in the hippocampal slice**

The propagation of epileptiform activity within the CA3 region was slower than the slowest known velocities of action potentials in fine unmyelinated vertebrate axons. By contrast, the propagation of normal responses in the CA1 region, which depends on axonal action potential conduction in Schaffer collaterals, was
The fact that epileptiform activity spreads with a slower velocity suggests the involvement of a multisynaptic excitatory network of connections (Demir et al. 1998; Hoffman and Haberly 1993; Miles et al. 1988; Traub et al. 1987).

Interestingly, the propagation of epileptiform activity within the CA1 region was also slower than action potential propagation, but comparable to (although slightly lower than) the velocity of epileptiform activity in the CA3 region. Thus the mechanism underlying the spread of epileptiform discharges in the CA1 region may be similar. It is known that associational excitatory connections between pyramidal neurons exist in the CA1 region, but the density is lower than that in the CA3 region (Alger and Teyler 1977; Christian and Dudek 1988; Deuchars and Thomson 1966; Kishin et al. 1995). These associational excitatory connections may be too sparse to initiate epileptiform activity in the CA1 region, but might be sufficient to support seizure propagation when a large group of CA1 pyramidal neurons are strongly activated by inputs from CA3 pyramidal neurons. Furthermore, the discharge under way in the CA3 region will produce ongoing activation of synaptic inputs through the entire CA1 region, and this barrage could facilitate the spread of a discharge. These inputs could provide some depolarization to relieve Mg2+ block of N-methyl-D-aspartate receptors, which mediate a portion of both Schaffer collateral and recurrent excitation of CA1 pyramidal neurons (Deuchars and Thomson 1966; Kishin et al. 1995). However, because recurrent excitation in the CA1 region is generally thought to be weak, one must also consider the possibility of significant contributions from gap junctions and ephaptic interactions in the spread of epileptiform activity through the CA1 region (Dudek et al. 1986).

After epileptiform activity initiated at a focus within the CA3 region, a response emerged abruptly in the CA1 region at the CA2 border. Only later did the epileptiform activity invade the CA2 region from the CA3 region (Fig. 3). The emergence of epileptiform activity in the CA1 region by such a saltatory process in advance of spread through the intervening CA3 and CA2 regions implies a more rapid conduction by action potentials in Schaffer collaterals. These action potentials were not visible in our voltage imaging records, but this is not surprising. Signals from axonal volleys are visible in these experiments only when their synchrony is within about 1 ms.

The stereotypical spatiotemporal pattern of epileptiform activity

The initiation and propagation of epileptiform activity in hippocampal slices follows a stereotypical pattern. Discharges usually initiated in the CA3 region and emerged abruptly in the CA1 region bordering the CA2 region. The abrupt emergence of activity in the CA1 region resembles a form of propagation reported by Sekino et al. (1997). After emerging in the CA1 region at the CA2 border, discharges propagated through the CA1 region to the subiculum. This propagation through the CA1 region occurred simultaneously with propagation from the site of onset in the CA3 region toward the CA2 region.

Blocking GABA_A receptors and applying TS or theta bursts all reduced the latency of onset in the CA3 region, as well as the latency of subsequent emergence in the CA1 region, but did not change the amplitude or alter the spatiotemporal pattern of the ensuing discharge. These experiments suggest that the underlying circuitry that generates this pattern is very robust and not very sensitive to a variety of treatments that alter other aspects of the hippocampal network. It would appear that the excitatory synaptic connections of the hippocampal slice rather than inhibitory connections determine the spatiotemporal characteristics of epileptiform discharges in hippocampal slices, because this same pattern was observed after blockade of GABA_A receptors. GABA_A-receptor blockade, TS, and theta bursts all reduced the latency of discharge onset, which could reflect either strengthening of excitatory synapses or weakening of inhibitory synapses. Each of these changes in synaptic function could accelerate the buildup of reverberating activity between CA3 pyramidal cells.

Unique properties of the CA1–CA2 border

Anatomical tracing has shown that neurons in the proximal CA3 region (near the dentate gyrus) tend to send Schaffer collaterals to the distal portion of the CA1 region (near the subiculum), whereas neurons in the distal portion of the CA3 region tend to innervate neurons in the proximal part of the CA1 region (Ishizuka et al. 1990). It is difficult to see how this arrangement could contribute to any of our results, but this is not surprising because this same study found that slices contain relatively few axons projecting from the CA3 region to distal parts of the CA1 region. In fact, consideration of axon preservation during slice preparation would suggest that the proximal CA3 region would have a higher density of Schaffer collateral inputs. This is one possible explanation for the emergence of the epileptic response in the CA1 region at the CA2 border. However, epileptic responses were also larger there than elsewhere in the CA1 region. It has been reported that isolated CA1 regions containing this portion can generate sharp wave-ripple bursts spontaneously or in response to electrical stimulation (Maier et al. 2003; Nimmrich et al. 2005). Our slices also showed stronger LTP at the same site in the CA1 region near the CA2 border. Taken together, these observations suggest that the neural circuitry and/or the excitability are not uniform in the CA1 region. The CA1 neurons close to the CA2 region behave differently and this could account for both the initiation of epileptiform responses in the CA1 region and the greater magnitude of LTP.

We noted a reverse situation in the CA2 region, where epileptiform discharges showed a long latency and low amplitude. Likewise, LTP was also weak, and there was a striking contrast with the strong LTP in the adjacent part of the CA1 region. Thus just as the CA1 region at the border shows enhanced excitability, so the CA2 region shows diminished excitability. This finding is intriguing because the CA2 region is known to be more resistant to epilepsy-induced principal cell loss compared with the CA3 and CA1 regions (Sloviter et al. 1991; Williamson and Spencer 1994).

The distinct differences in excitability between the CA2 region and the nearby CA1 region draw attention to numerous anatomical studies that mapped molecular expression in the hippocampus. The distinctiveness of molecular expression in the CA2 region has been invoked previously to explain interesting spatiotemporal patterns of electrical signals (Sekino et al. 1997). The CA2 region shows higher levels of a number of proteins, including fibroblast growth factor (Matsuyama et al. 1992), neurotrophin-3 (Wetmore and Olson 1993), a2+ binding proteins (Leranth and Ribak 1991), and extracellular matrix
constituents (Celio 1993). Gene mapping studies further showed that the CA2 region is unique in the expression of several genes (Lein et al. 2005, 2007). These differences in gene expression could underlie our observations of reduced excitability in the CA2 region. A particularly intriguing insight from the gene mapping studies is that according to the gene expression maps the CA2 region is a little thicker than according to morphological criteria. However, the ambiguous zone inferred from the discrepancy between these two mapping methods appears to fall on the CA3 border of the CA2 region rather than on the CA1 border. Nevertheless, the possibility remains that interactions between neurons in the CA1 and CA2 regions enhance the excitability of the former and diminish the excitability of the latter.

Gene expression maps also may indicate that the CA1 region is not uniform. One can discern gradients in expression in many of the figures shown in Lein et al. (2004). The maps of serine protease 19 and neuronal guanine nucleotide exchange factor show variations within the CA1 region, although these gradients received no comment in the text. The relation between these molecular maps and the functional variations illuminated in the present study by voltage imaging represents an important challenge for future study.

Another interesting possibility for the heightened excitability in the CA1 border region relates to the fields of inhibition of CA1 interneurons. If pyramidal cells located more centrally in the CA1 region receive inhibition from interneurons spread out over equal distances on both sides within a slice, then CA1 pyramidal cells near the border will receive fewer inhibitory synapses from other CA1 interneurons. Moreover, if CA2 interneurons cross into the CA1 region less frequently, then the nearby CA1 pyramidal cells would receive fewer inhibitory synaptic inputs. This would make the heightened excitability of the CA1 border region an “edge effect,” rather than a consequence of unique cellular properties of the neurons at that location.

CONCLUSIONS

In summary, our findings revealed a stereotypical initiation and propagation pattern of epitilepticiform activity in the hippocampal slice. This same pattern of epilepticiform activity was seen with different stimulation sites throughout the hippocampus and dentate gyrus. TS, theta bursts, or blocking GABA_A receptors did not change this stereotypical pattern but decreased the onset latency, suggesting the intrinsic excitatory connections in the hippocampus predominate in the determination of the initiation site and propagation pattern of epilepticiform activity. Finally, the portion of the CA1 region close to the CA2 region presented both stronger epilepticiform responses and larger theta burst–induced LTP, suggesting the neural circuitry and/or intrinsic excitability of neurons at the CA1–CA2 border are unique. This work has brought to light functional variations within hippocampal subfields, highlighting locations that may play a special roll in seizures and in relaying information through the synaptic circuitry of the hippocampus.

ACKNOWLEDGMENTS

We thank Drs. Daniel Johnston, Helen Scharfman, and Paul Rutecki for critical comments on this manuscript.

REFERENCES


