Initiation and Propagation of Neuronal Coactivation in the Developing Hippocampus

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1Department of Physiology and Biophysics, Georgetown University, Washington, DC; 2Institute of Neurosciences, University Miguel Hernandez, Alicante, Spain; and 3Department of Fisiologia, University of La Laguna, Tenerife, Spain

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Bolea, Sonia, Juan V. Sanchez-Andres, Xiaoying Huang, and Jian-young Wu. Initiation and propagation of neuronal coactivation in the developing hippocampus. J Neurophysiol 95: 552–561, 2006. First published September 21, 2005; doi:10.1152/jn.00321.2005. Correlated neuronal activity is ubiquitous in developing nervous systems, where it may introduce spatiotemporal coherence and contribute to the organization of functional circuits. In this report, we used voltage-sensitive dyes and optical imaging to examine the spatiotemporal pattern of a spontaneous network activity, giant depolarizing potentials (GDPs), in rat hippocampal slices during the first postnatal week. The propagation pattern of the GDP is closely correlated to the anatomical organization of the network. In the hilus, where mossy cells and interneurons are not organized in layers, GDPs propagate at the same velocity in all directions. In CA3 and CA1, the activation is synchronous along the axis of the pyramidal cells’ dendritic tree. The velocity of wave propagation is significantly different in three hippocampal subfields: it is slowest in the hilus, faster in CA3, and fastest in CA1. The velocity of horizontal propagation (along the axis of the pyramidal layer) has a large variation from trial to trial, suggesting that the horizontal velocity is determined to some extent by dynamic network factors. Imaging revealed that each GDP event is initiated from a small focus. The location of the initiation focus differs from event to event. All together, our data suggest that GDP is a propagating excitation wave, initiated from a small site, and propagating to the whole hippocampus. The spatiotemporal patterns of the wave in CA3 and CA1 areas show better synchrony along the pyramidal cell dendritic trees and progressive activation along the axis of the pyramidal cell layer.

INTRODUCTION

Spontaneous neuronal activity during development has the potential to shape many aspects of neuronal wiring throughout the brain (Goodman and Shatz 1993; Katz and Shatz 1996). Such activity has been found in many parts of the CNS, including retina (Maffei and Galli-Resta 1990; Meister et al. 1991; Wong 1999), hippocampus (Ben-Ari et al. 1989; Menendez de la Prada et al. 1998), neocortex (Garaschuk et al. 2000; Lippe 1994; McCormick 2001; Yuste et al. 1992), thalamus (Andersen et al. 1996), spinal cord (O’Donovan et al. 1994; Spitzer and Gu 1997), and embryonic CNS (Momose-Sato et al. 2003).

In rodent hippocampus, synchronous neuronal discharges known as giant depolarizing potentials (GDPs) appear during the first postnatal week (Ben-Ari et al. 1989; Khalilov et al. 1997; Leinekugel et al. 1998; Menendez de la Prada et al. 1996). GDPs have been well characterized by intracellular recordings, as depolarizations of 20–50 mV for 300–500 ms, with superimposed action potentials and an occurrence rate of 3–12 GDPs/min. The depolarization of the neurons is thought to be generated by the synchronized activation of interneurons and pyramidal cells through GABA_A, AMPA, and N-methyl-D-aspartate (NMDA) receptors (Ben-Ari et al. 1989; Bolea et al. 1999; Gaiarsa et al. 1991; Khazipov et al. 1997). GDPs can be blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H,2,3-benzodiazepine (GYKI 53655) (Bolea et al. 1999; Gaiarsa et al. 1991), suggesting that AMPA-mediated depolarization is essential to GDP events. NMDA receptors may also contribute to the GDP as observed calcium influx through NMDA- and voltage-dependent calcium channels (Garaschuk et al. 1998; Leinekugel et al. 1995, 1997). However, n-2-amino-5-phosphono- pentoic acid (APV) only partially blocks the occurrence of GDPs (Bolea et al. 1999), suggesting that NMDA receptors and subsequent calcium influx are not essential.

Spontaneous GDPs may occur in isolated CA3, CA1, or the hilus (Menendez de la Prada et al. 1998), suggesting that they emerge as a property of local circuits in all hippocampal structures. Recently, GDPs have also been recorded in vivo in rat pups, where they occur during immobility periods, sleep, and feeding (Leinekugel et al. 2002) at similar frequency as in acute slices (6–20 GDPs/min).

GDPs may play a role in the control of functional synapse number (Lauri et al. 2003), as well as “unsilencing” silent connections and strengthening those with low probability of transmitter release (Gasparini et al. 2000). Increased GABA_A receptor activation seems to accelerate the switch of GABAergic responses from excitatory to inhibitory (Ganguly et al. 2001). Neurons rarely fire between GDP bursts at this age, leaving GDPs as the main source of correlated neuronal activity. Understanding the spatiotemporal pattern of GDPs is
crucial to understanding when and where the neurons will fire together and how such synchronized population activation refine the neuronal connectivity in this developing stage.

The spatiotemporal pattern of the GDP has not been examined by imaging methods. There may be two possible spatiotemporal regimens. If the GDP initiates from a point pacemaker and propagates through local connections, the activity would progressively invade the tissue and appear as a propagating wave. If the propagation follows the trisynaptic circuit between dentate gyrus (DG), CA1, and CA3, the excitation would appear almost simultaneously (with about 1 synaptic delay) in the CA3 and CA1. Obviously the two possible regimens would provide different spatial synchrony in the developing hippocampus. In this report, we use voltage-sensitive dye (VSD) imaging to visualize the initiation and propagation of GDPs in hippocampal slices from neonatal rats. Our data show that the propagation of GDPs follows the first regimen: GDP initiated from a small focus and progressively propagated as a wave front. Such spatiotemporal pattern may contribute to refining of hippocampal circuits by providing a localized spatial synchrony.

**Methods**

**Slice preparation**

Hippocampal slices were obtained from P2–P6 Sprague-Dawley rats of both sexes. The animals were killed following National Institutes of Health guidelines, deeply anesthetized with halothane, and quickly decapitated. The whole brain was immersed in cold ACSF in a submerged chamber. During the experiment, the brain tissue slicer (Stoelting) and allowed to recover at room temperature for 1 h before optical imaging. The stained preparation was perfused with dye-free ACSF at room temperature for 1 h before optical imaging. During the experiment, the tissue was continuously perfused with ACSF in a submerged chamber.

**VSD imaging**

Optical imaging was performed with a 124-element photodiode array (Centronics, Newbury Park, CA) at a frame rate of 1,000 frames/s. The image of the preparation was projected on the array, such that each detector received light from a 42 × 42-, 84 × 84-, or 168 × 168-μm² area of the slice, depending on the objective used (×40, ×20, or ×10, respectively). The photocurrent of dye-related absorption signals (705 nm) from each photodetector was individually amplified through a two-stage amplifier. The first stage performed a current to voltage conversion with a feedback resistor of 50 MΩ. The signals were amplified and filtered by a second stage amplifier before digitizing. This second stage provided a voltage gain of 200, high-pass filtering with a 100-ms time constant, and a four-pole Bessel analog low-pass filter with a 300-Hz corner frequency. The commercial version of the recording apparatus (with 464 detectors) is available as NeuroPDA from Redshir Imaging: www.redshir imaging.com. The recording apparatus allows low dark noise (10⁻⁶ of the illumination intensity), large effective dynamic range (>19 bits), and fast sampling rate (1,000 frames/s). With RH479 and absorption measurement, the optical signal size (dI/I) for the GDP was about 5 × 10⁻⁴–2 × 10⁻³. This value was calculated by dividing the measured signal by the resting light intensity. In trace displays (Figs. 1 and 2, B and C), dI/I was used to show the amplitude of the optical signal. In pseudocolor displays (Fig. 2D and all contour displays), we did not calculate dI/I; instead, the measured signal on each detector was normalized to its own maximum amplitude. The resting photocurrent on the diode was ~100 nA. At this illumination intensity the signal-to-noise ratio was ~10, so signal averaging was not necessary. Optical recording trials were 2 s long for the evoked GDPs and 8–40 s for spontaneous GDPs. On each slice, we recorded 4–20 trials (with a total exposure of <160 s). Phototoxicity was not a concern with this level of exposure time and intensity (Jin et al. 2002). For additional details about the apparatus and the recording methods, see Jin et al. 2002; Kosmidis et al. 2004; Wu and Cohen 1993.

The preparation was placed in a submerged chamber under an upright microscope (Zeiss, Axio scope, fixed stage). During optical recordings, the preparation was illuminated by a 100-W tungsten halogen lamp using a 705/30-nm interference filter. To reduce the noise caused by mechanical vibration, the microscope was placed on a special isolation stage (Minus K stage; www.minus k.com) that significantly reduces the noise above 5 Hz. To reduce the vibration noise below 5 Hz, the perfusion was paused during optical recording trials and a coverslip was used when a dry lens (×10) was used.

**Electrical recording**

Tungsten (epoxy-coated) microelectrodes with tip resistance of ~75 kΩ (FHC, Bowdoinham, ME) were used for recording local field potentials simultaneously with the optical recordings. The electrodes were carefully inserted into cortical tissue so that the tissue surrounding it produced normal VSD signals. The field potential signals were amplified 1,000 times and band-pass filtered between 0.1 and 400 Hz by a Brownlee Precision 440 amplifier. During imaging experiments, the field potential was digitized simultaneously with VSD signals. The same tungsten microelectrode was also used for stimulation to trigger GDP events. Stimuli consisted of rectangular pulses of 10–30 V and 100 μs in duration.

**Data analysis**

The optical data were analyzed using the program NeuroPlex (Redshirt Imaging, Fairfield, CT). NeuroPlex displays the data in the form of a time course of the optical signal as a function of time. This value was calculated by dividing the measured signal by the resting light intensity. In trace displays (Figs. 1 and 2, B and C), dI/I was used to show the amplitude of the optical signal. In pseudocolor displays (Fig. 2D and all contour displays), we did not calculate dI/I; instead, the measured signal on each detector was normalized to its own maximum amplitude. The resting photocurrent on the diode was ~100 nA. At this illumination intensity the signal-to-noise ratio was ~10, so signal averaging was not necessary. Optical recording trials were 2 s long for the evoked GDPs and 8–40 s for spontaneous GDPs. On each slice, we recorded 4–20 trials (with a total exposure of <160 s). Phototoxicity was not a concern with this level of exposure time and intensity (Jin et al. 2002). For additional details about the apparatus and the recording methods, see Jin et al. 2002; Kosmidis et al. 2004; Wu and Cohen 1993.

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form of traces for numerical analysis (Fig. 2B) or pseudocolor images for visualizing the spatiotemporal pattern (Fig. 2D). The digitized data were further filtered digitally by NeuroPlex [low-pass, 300 Hz (Butterworth) and high-pass, 1.2 Hz (RC filter)] before displaying. The pseudocolor display was made using variable scaling, where the data from each diode are first normalized to their own maximum and then each normalized data point is assigned a color according to a linear color scale (0/100% deep blue and 1/100% red). This kind of scaling best preserves the timing information of the signals. The display was either in pixel (Fig. 2D, top row) or contour mode (Fig. 2D, bottom row). For analysis of the propagation activity, contour mode was chosen. Contour mode low-pass filters the raw data spatially, so that areas of equal signal amplitude are assigned the same color, and the amplitude difference between neighboring diodes is interpolated. To determine the initiation point of the discharges, pixel mode can better show which detectors depolarize first.

To calculate the propagation velocity between two locations, we first measured the time for the GDP to reach 50% of the peak amplitude at the two locations (Fig. 3C); the propagation velocity is calculated as the distance between the two locations divided by the difference of the two times.

**RESULTS**

**VSD signals of GDP**

In our experimental arrangement, the VSD signal is wavelength-dependent light absorption. The light absorption increased at 705 nm and decreased at 650 nm in association with the membrane depolarization of the cortical neuronal population. This wavelength-dependent signal is referred to as optical signal or VSD signal (Cohen et al. 1974). This VSD signal occurred simultaneously with the local field potential signals of GDPs (Fig. 1), and could be blocked by GABA$_A$ and glutamate antagonists (data not shown). Therefore we presume that the VSD signals reflect the population neuronal depolarization of the GDPs. Local field potential recordings from nonstained and nonimaged slices showed similar amplitude, duration, and initiation frequency, indicating that the dye staining and the light exposure during imaging did not have noticeable effects. The optical signal has a smooth waveform, whereas local field potential signal has more high-frequency components (Fig. 1). This might be because the optical signal is the sum of the membrane depolarization of a population (in an area of 42 $\times$ 42 $\mu$m$^2$), whereas the local field potential electrode collects both spikes and local current because of depolarization and repolarization.

**Propagation of GDP waves**

The propagation of the optical signals during an evoked GDP in the CA3 area is shown in Fig. 2. The GDP was evoked by an electrical stimulation in the hilus (outside the field of

![Fig. 2. Raw signals and contour map. A: recording arrangement. Area CA3 was imaged with a $\times$40 objective. Stimulation electrode was placed in the hilus (stim). B: sample of the optical recordings obtained at each detector during an evoked GDP. C: selected optical recording traces from 3 detectors (location indicated by circles in B) showing different time-courses, indicating that the signal is propagating across the field of view. Signals were filtered between 1.2 and 100 Hz. Calibration: $5 \times 10^{-4}$ d$I$/d$t$. D: pseudocolor maps of the optical signals. Amplitude of the optical signal is converted to a color according to a 16-color linear scale (top right). Top row: in each image, the color on every detector shows amplitude of the optical signal at the poststimulation time (marked at bottom of images). Detectors located out of the slice, framed with dotted lines in B, have been discarded from the maps. Bottom row: colors on the detectors are interpolated to obtain isoamplitude lines (black lines) to show propagation. E: progression of an isoamplitude line, drawn at 1-ms intervals of poststimulation time. Dashed lines outline the CA3 pyramidal cell layer.](http://jn.physiology.org/doi/abs/10.1152/jn.01876.2005)
All of the optical detectors recorded optical signals of the evoked GDP, except those measuring light from an area at the border or outside the slice (Fig. 2B). Isoamplitude lines show propagation of an evoked GDP (lines are drawn at 1-ms interval). Note that in the hilus, the isoamplitude lines have a convoluted pattern, which is not directionally selective; lines become parallel to the dendritic tree of pyramidal cells when entering the CA3 area. Optical signals from detectors labeled in A show that the wave gradually accelerated when propagating along the major axis in CA3. The activity propagated from the top of the recording area (closer to the stimulation electrode in Fig. 3B) and toward CA3 (see location of stimulation electrode in Fig. 3A). The isoamplitude contour lines show a different pattern in the hilus compared with that in CA3 (Fig. 3B). In the hilus, the propagation was slower (lines are closer) and more convoluted. The propagation velocity became higher in CA3, and the wave front became less convoluted, running perpendicular to the pyramidal layer. These results suggest that the propagation pattern of GDPs is influenced by cellular architecture of the hippocampus. In Fig. 3C, optical signals from three groups of optical detectors (blue, red, and green squares in Fig. 3A and B) were normalized to their maximum and plotted. The green squares were located along the axis of an imaginary pyramidal neuron in CA3 and had the best level of synchronization; the activation at these locations reached 50% of the maximum almost simultaneously (Fig. 3C). The blue squares were located in the hilus area and showed the least synchrony; the time to reach 50% of activation differed by ~12 ms. The red squares were at the hilus–CA3 border and had an intermediate level of synchrony.

These results suggest that the shape of the wave front is highly correlated to the cellular organization in hilus and CA3. The propagating pattern in CA1 is similar to that of CA3 but with different velocity.

**Propagation velocity**

Observations in Figs. 2E and 3B suggest that the propagation of GDPs becomes faster along the path in hilus and CA3. To test if this is true across the whole propagation path, we used a low power objective (×10) to image hilus, CA3, and CA1 simultaneously (Fig. 4A). In 28 events from two different slices, the GDPs propagated across CA1 in the same pattern as that in CA3, with isoamplitude lines perpendicular to the pyramidal layer (Fig. 4A, contour lines). However, as a general tendency, the velocity in CA1 was higher than that in CA3. In the front edge contour map, it is clear that the distance between isoamplitude lines increases in CA1, suggesting an increase in

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**Different propagation patterns in hilus and CA3**

To further analyze the relationship between anatomic structure and GDP propagation, we examined the propagation patterns in different regions of the developing hippocampus. In Fig. 3, we show an example of the propagation patterns of GDPs evoked in the hilus. The activity propagated from the left, the initiation point in the hilus, to the right, toward CA3 (see location of stimulation electrode in Fig. 3A). The isoamplitude contour lines show a different pattern in the hilus compared with that in CA3 (Fig. 3B). In the hilus, the propagation was slower (lines are closer) and more convoluted. The propagation velocity became higher in CA3, and the wave front became less convoluted, running perpendicular to the pyramidal layer. These results suggest that the propagation pattern of GDPs is influenced by cellular architecture of the hippocampus. In Fig. 3C, optical signals from three groups of optical detectors (blue, red, and green squares in Fig. 3A and B) were normalized to their maximum and plotted. The green squares were located along the axis of an imaginary pyramidal neuron in CA3 and had the best level of synchronization; the activation at these locations reached 50% of the maximum almost simultaneously (Fig. 3C). The blue squares were located in the hilus area and showed the least synchrony; the time to reach 50% of activation differed by ~12 ms. The red squares were at the hilus–CA3 border and had an intermediate level of synchrony.

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the propagation velocity. A row of detectors along the pyramidal cell layer in CA3 and CA1 was selected for calculating the propagation velocity in the example presented. In Fig. 4, eight detectors were selected. The optical signals were normalized to the maximal amplitude on each detector, and the time for the signal to reach its 50% of the maximal amplitude was marked respectively on each detector. Times for signals to reach 50% of the peak amplitude (broken line) are marked for each detector and used in C for the calculation of propagation velocity. C: propagation velocity along the pyramidal cell line, calculated as the time difference of the wave arrival on each detector divided the distance between the two detectors. Higher velocity is in point 5 (between detectors 5 and 8), which anatomically corresponds to the transition area between CA3 and CA1 (see detector location in A).

**Fig. 4.** Propagation of a GDP in different areas. A: schematic diagram of the position of the detector array (× 10 objective) and the stimulation electrode over the tissue. Eight detectors are marked (1–8), and signals on them are shown in B. Inset: contour map showing the propagation of a GDP evoked in the hilus that propagated from over CA3 and CA1 areas. Isoamplitude lines are drawn at 2-ms intervals. B: plot of signals (1–8) from the 8 detectors marked in A. Signals are normalized to peak amplitude of the GDP event respectively on each detector. Times for signals to reach 50% of the peak amplitude (broken line) are marked for each detector and used in C for the calculation of propagation velocity. C: propagation velocity along the pyramidal cell line, calculated as the time difference of the wave arrival on each detector divided the distance between the two detectors. Higher velocity is in point 5 (between detectors 5 and 8), which anatomically corresponds to the transition area between CA3 and CA1 (see detector location in A).

**Dynamical variations of propagation velocity**

In the previous sections, we have examined the variation of the GDP propagation related to anatomical organization in three hippocampal areas. Here we examine variations of the propagation velocity influenced by dynamic factors, i.e., variations of the velocity from trial to trial in a given area of the same slice.

For this purpose, we imaged 17 evoked GDPs in the same slice and examined the propagation in CA3 (Fig. 5). The GDP episodes were evoked by an extracellular electrode placed in the hilus, outside the field of view (Fig. 5A). In these GDP episodes evoked by stimuli of identical intensity and interstimulus interval, we found that some episodes were significantly faster or slower than the average. The contour maps of two example trials, Fast and Slow, are shown in Fig. 5B to demonstrate overall variability in the propagation velocity.

In a more detailed analysis, we selected 11 detectors along the pyramidal layer (Fig. 5A, black boxes). The propagation velocity between the 10 pairs of detectors was calculated. Figure 5C shows the results of all 17 evoked GDPs. The top panel of Fig. 5C shows the propagation profile of all trials in color maps according to a linear pseudocolor scale (red, 60 mm/s; blue, 0 mm/s). The number on the right of each color map is the average velocity of each event over the entire propagation path. As shown in Fig. 5C, consecutive imaging trials from the same tissue show some fast trials, with an average velocity of ~30 mm/s (e.g., trials 2 and 3), which is about twice as fast as the slow trials (trials 6, 16, and 17). The average velocity appeared to be independent of the initial velocity at the beginning of the propagation path (e.g., trial 13 has a faster velocity than trial 3 at the beginning of the path, but a slower averaged speed overall), suggesting that the variation is not caused by the initiation of GDP.

Another type of trial-to-trial variation is the local velocity. Along the propagation path, waves in some regions are faster than in the other places (e.g., the red areas surrounding the broken line in trials 1–3, 5, and 15). The velocity varies largely (10–60 mm/s). The fast regions are not fast in all trials, because slower velocity is seen in trials 6, 10, 14, and 16. This strongly suggests that, in a given anatomical structure, dynamic factor(s) can significantly change the velocity.

The averaged propagation profile of all trials is shown in the bottom panel of Fig. 5C. This averaged profile shows an overall tendency of gradual increase in propagation velocity in the CA3 area, consistent with the data shown in Figs. 2E, 3C, and 4C. However, the propagation profile of individual events may differ from this general tendency. In trials 6 and 16, the velocity was faster in the first portion of CA3 and then became slower. Even the locations with a faster velocity on average (Fig. 5C, arrows) can sometimes show a much slower velocity. Overall, the large trial-to-trial variations in propagation velocity suggest that dynamic factors contribute significantly over the spreading of the GDP. Large trial-to-trial variations in propagation velocity were observed in all slices from animals of P2–P6. In each slice, we measured ~10 GDP events evoked...
in hilus (total of 114 trials from 10 slices). The average velocity over the entire CA3 area was 21.8 ± 5.6 mm/s. However, at different local areas, the velocity varied from 2.8 to 60 mm/s, consistent with the example shown in Fig. 5.

Initiation foci of GDPs

It has been controversial whether spontaneous GDPs originate only in the hilus (Strata et al. 1997) or also can be initiated in other hippocampal areas (Bolea et al. 1999; Menendez de la Prida et al. 1998). VSD imaging is fast enough to locate the initiation foci of spontaneous and evoked GDPs. We have observed multiple initiation foci in each slice distributed in both hilus and CA3 areas (Fig. 6). Figure 6A shows the locations of the initiation foci for seven spontaneous GDPs (black boxes). Three of them are in the CA3 pyramidal layer and the other four are in the hilus. This suggests that the rhythm of GDPs may be produced by multiple pacemakers distributed both in the hilus and the CA3. We therefore set out to learn whether evoked GDPs were initiated from the same foci that generated spontaneous GDPs. We imaged five evoked GDP events in the same slice. Each initiation focus is labeled in Fig. 6A with gray boxes. Surprisingly, the evoked GDP did not start at the tip of the electrode, and identical stimuli did not evoke GDPs from the same focus (interstimulus interval > 3 min). These foci were distributed as far as ~150 μm from each other, and some were far away from the electrode tip (Fig. 6A, gray boxes). In addition, the initiation foci of spontaneous GDPs did not overlap with those of evoked ones (Fig. 6A, gray and black boxes). Figure 6, B and C, shows the front edge contour maps (1-ms isoamplitude lines) of two spontaneous and three evoked GDPs from the same slice. In all cases, the GDPs started from small foci (●) and propagated toward CA3 and CA1 (right) and the hilus (left). Note that the propagation velocity became lower when the waves enter the hilus (~1–5 mm/s). The isoamplitude lines on the top left of all the contour maps in Fig. 6, B and C, are more densely arranged than those in other areas, indicating slower velocity. In another slice, we imaged the initiation of 24 spontaneous GDPs with a larger field of view (×20 objective; Fig. 6D). Again the initiation foci for spontaneous GDPs were marked as black boxes and initiation foci for evoked GDPs as gray. In this slice, all foci were in the CA3 area. There were two preferred locations within CA3: one close to the hilus (16/24 GDPs) and the other close to CA1 (8/24). The distance between the two preferred areas was ~350 μm, and the initiation foci of consecutive GDPs jumped from one to the other. In this slice, we did not see spontaneous initiation foci in the hilus. However, an electrode placed in the hilus was able to reliably evoke GDPs (gray box in Fig. 6D). When the stimulation electrode was placed in CA1, microshocks were also able to reliably evoke GDPs at the tip of the electrode, suggesting that CA1 has a potential to generate GDP events from local recurrent interactions (203 trials in 12 slices).

In conclusion, GDP was initiated from a small focus and progressively propagated in the tissue with a large variation in velocity at different areas.
Dendritic trees (Figs. 2 lines) became parallel to the orientation of the pyramidal cell in the CA3 area, the progression of the wave front (isoamplitude lines) aligned toward the center of the slice. When the wave reaches densely grouped in the pyramidal layer and the dendritic trees of pyramidal neurons was highly organized, with the somas pattern (Figs. 3 and 4). In CA3 and CA1 areas, the distribution of waves without distortion of current density distribution (source/sink pairs) in cortical tissue (Bao and Wu 2003; Jin et al. 2002). This technique has been used to visualize epileptiform and oscillatory activities in hippocampal and neocortical slices, and propagating waves are a common spatiotemporal characteristic in these activities (Albowitz and Kuhnt 1995; Bao and Wu 2003; Demir et al. 1998, 2000; Huang et al. 2004; Miyakawa et al. 2003; Tsau et al. 1998; Wu et al. 1999b, 2001). In this study, we found that GDP activity in developing hippocampus appears as propagating waves and that the propagation patterns are strongly correlated to anatomical structure in the CA3 and CA1 areas. Large trial-to-trial variations in the propagation velocity were found, suggesting dynamic control of GDP spatiotemporal distribution. We also show that GDPs are initiated from a small focus and that, in each slice, different GDP events may be initiated from different locations.

**DISCUSSION**

Optical recording with VSDs measures membrane depolarization during neuronal population activity, which allows accurate visualization of initiation sites and propagating patterns of waves without distortion of current density distribution (source/sink pairs) in cortical tissue. We found a correlation between GDP propagation pattern and the anatomical organization in different regions of hippocampus. In the hilus, where mossy cells and interneurons are not organized in layers, the propagation of GDP waves had similar velocity in all directions, and the isoamplitude lines in the contour map were arranged in a relatively concentric pattern (Figs. 3 and 4). In CA3 and CA1 areas, the distribution of pyramidal neurons was highly organized, with the somas densely grouped in the pyramidal layer and the dendritic trees aligned toward the center of the slice. When the wave reaches the CA3 area, the progression of the wave front (isoamplitude lines) became parallel to the orientation of the pyramidal cell dendritic trees (Figs. 2E, 3B, and 4A). Neurons located within the same isoamplitude line depolarized with greater synchrony. Other reports showed that, not only do pyramidal cells fire synchronous bursts, interneurons also show this property (Menendez de la Prada and Sanchez-Andres 2000), and the firing is associated with the increase in \( [Ca^{2+}]_i \) (Garaschuk et al. 1998; Leinekugel et al. 1995). Taken together, this indicates that, in the CA3 and CA1 areas, the wave of excitation implicates regions of maximal activity associated with the pyramidal neurons, both temporally and spatially. This is similar to the developing neocortex, where the activation of muscarinic acetylcholine receptors results in waves of correlated activity that propagate parallel to the pial surface and perpendicular to the cortical lamina (Peinado 2000).

Our data also show that GDP propagation is correlated to anatomical structures but is not restricted by the classical trisynaptic hippocampal circuit. The propagation of GDPs follows the major axis of the hippocampus from dentate gyrus to CA3 then to CA1, which is compatible with the classical trisynaptic circuit (Andersen et al. 1971). However, if GDPs propagated through the trisynaptic circuits from hilus to CA1, we would expect the signal arriving at CA1 cells monosynaptically through the Schaffer collaterals before running polysynaptically. We would not see progressive wave fronts running step by step along the major axis of hippocampus. We also found that GDPs evoked in CA1 or CA3 propagated “backward” to the hilus. This backward propagation cannot be explained in terms of the trisynaptic circuit. Thus the observations in this report argue against an essential contribution of the long excitatory pathways but are in agreement with the limited functionality of the glutamatergic synapses at this early stage of development (Durand et al. 1996). However, we noticed that the long fibers in the trisynaptic circuit were more easily damaged during slicing of the brain, so our results remain to be verified in vivo. Our arguments are supported by other studies. Simultaneous electrical recordings showed that GDPs could appear first in CA1 and later in CA3 pyramidal cells (Menen-
dez de la Prida et al. 1998). Amaral and Witter (1989), and Amaral (1993) have questioned the validity of the trisynaptic circuit in the newborn. Canepari et al. (2000) used calcium imaging to examine the GDP propagation during the first postnatal week. They found that, in the presence of kynurenic acid, which blocks the glutamate component, the GDP can still propagate toward the CA1, probably through GABA-mediated local networks (Canepari et al. 2000).

Sequential activation along the axis of pyramidal laminar may contribute to the consolidation of developing circuits by providing reduced synchrony over distance. Recently, it has been reported that the pairing of GDPs with mossy fiber (MF) stimulation induced a persistent increase in synaptic strength at MF-CA3 synapses. The potentiation was maximum when pre- and postsynaptic signals were coincident and decreased when the interval between GDPs and MF stimulation increased (Kasyanov et al. 2004). In light of this observation, we would speculate that the propagation wave of GDP would contribute to the construction of local synaptic domains.

**Dynamic variations in propagation velocity**

In the same slice, the propagation velocity and the pattern of the contour map varied from trial to trial. These variations were large and suggest that dynamic factors contribute significantly to the control of propagation.

We observed two types of dynamic variations in the GDP propagation: one was the variation in averaged velocity over the entire CA3 (Fig. 5C, fast and slow trials). Another was the trial-to-trial variation of local velocity (Fig. 5C), which showed a large difference. Our analysis also suggests that the differences in averaged velocity may be primarily determined by the occurrence of fast/slow regions. For example, fast trials in Fig. 5C (trials 2 and 3) had more fast regions than slow trials (trials 6 and 14). Examining the variations of individual events also suggests that the general tendency of acceleration in CA3 is caused by the increasing of occurrence rate of fast regions along the propagation path from CA3 to CA1.

**Initiation of GDP**

We observed that GDP started from a small point focus instead of an area of coactivation (Fig. 6). Such “point initiation” was also seen in the initiation of epileptiform events observed in disinhibited neocortex (Ma et al. 2004; Tsu et al. 1998, 1999), whereas “area initiation” (or “plateau activity” Demir et al. 2000) has been observed in rat piriform cortical slices. The synaptic mechanism for GDP initiation is different from that of epileptiform activity (Khalilov et al. 1999). In disinhibited cortex, the initiation is likely to be mediated by a recurrent excitation network with glutamate synapses (McCormick and Contreras 2001). In the developing hippocampus, both glutamatergic and GABAergic networks contribute to the recurrent excitations (Bolea et al. 1999; Chen et al. 1996; Cherubini et al. 1991). Thus the initiation of GDPs is likely to be mediated by both networks.

The hippocampal region for GDP initiation has been controversial. Earlier studies pointed to an exclusive localization in the hilus (Strata et al. 1997). Later simultaneous intracellular recordings from CA3 and CA1 pyramidal cells suggested CA3 and CA1 also can initiate GDP (Menendez de la Prida and Sanchez-Andres 2000; Menendez de la Prida et al. 1998). In addition, isolated tissue with CA3 and CA1 fields can also generate spontaneous GDPs (Bolea et al. 1999; Garaschuk et al. 1998; Menendez de la Prida et al. 1998). In this study, we confirmed that spontaneous GDPs can originate both in the hilus and in CA3 (Fig. 6). The initiation site is not fixed but varies from event to event. Even within the hilus or CA3, it changes from one burst to the next. While we did not see spontaneous GDPs originating in the CA1 area, our optical recording trials sampled a very small fraction of time, and we would definitely miss infrequent initiations in CA1. Electrical stimulation to CA1 can initiate GDP events starting in the CA1 area (data not shown), suggesting CA1 at least has the potential to initiate GDPs.

In conclusion, GDPs initiate from a small focus and propagate to other areas as waves, similar to many cortical population neuronal events. In the developing hippocampus, both GABAergic and glutamate networks are excitatory, and either of them may mediate the initiation/propagation of GDP. Questions remain following this report. Are different propagation patterns in hilus and CA3/CA1 mediated by different neuronal networks? Further experiments are needed to understand the roles of these two networks.

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