Nonlinear Frequency-Dependent Synchronization in the Developing Hippocampus

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Menendez de la Prida, Liset and Juan V. Sanchez-Andres. Nonlinear frequency-dependent synchronization in the developing hippocampus. *J. Neurophysiol.* 82: 202–208, 1999. Synchronous population activity is present both in normal and pathological conditions such as epilepsy. In the immature hippocampus, synchronous bursting is an electrophysiological conspicuous event. These bursts, known as giant depolarizing potentials (GDPs), are generated by the synchronized activation of interneurons and pyramidal cells via $GABA_A$, *N*-methyl-D-aspartate, and AMPA receptors. Nevertheless the mechanism leading to this synchronization is still controversial. We have investigated the conditions under which synchronization arises in developing hippocampal networks. By means of simultaneous intracellular recordings, we show that GDPs result from local cooperation of active cells within an integration period prior to their onset. During this time interval, an increase in the number of excitatory postsynaptic potentials (EPSPs) takes place building up full synchronization between cells. These EPSPs are correlated with individual action potentials simultaneously occurring in neighboring cells. We have used EPSP frequency as an indicator of the neuronal activity underlying GDP generation. By comparing EPSP frequency with the occurrence of synchronized GDPs between CA3 and the fascia dentata (FD), we found that GDPs are fired in an all-or-none manner, which is characterized by a specific threshold of EPSP frequency from which synchronous GDPs emerge. In FD, the EPSP frequency-threshold for GDP onset is 17 Hz. GDPs are triggered similarly in CA3 by appropriate periodic stimulation of mossy fibers. The frequency threshold for CA3 GDP onset is 12 Hz. These findings clarify the local mechanism of synchronization underlying bursting in the developing hippocampus, indicating that GDPs are fired when background levels of EPSPs or action potentials have built up full synchronization by firing at specific frequencies (>12 Hz). Our results also demonstrate that spontaneous EPSPs and action potentials are important for the initiation of synchronous bursts in the developing hippocampus.

INTRODUCTION

Synchronous population discharges commonly are found in neural systems, not just as cortical oscillations associated with stimulus encoding (Farmer 1998; Gray and Singer 1989; Laurent and Davidowitz 1994) but also as spontaneous events recorded during development (Meister et al. 1991; O'Donovan et al. 1998; Yuste et al. 1995) or in epileptic seizures (Schwartzkroin and Prince 1978). In experimental models of epilepsy such as disinhibited hippocampal slices, synchronous bursts have been observed both spontaneously and when triggered by afferent stimulation (Traub and Wong 1982; Wong and Traub 1983). These bursts result from local circuit synchronization that spreads throughout the hippocampus as reported experimentally (Miles et al. 1984, 1988; Traub et al. 1995) and computationally (Traub and Dingledine 1990; Traub et al. 1993). In these studies, the role of network connectivity, synaptic conductances, and intrinsic behavior have been investigated extensively.

A similar type of activity is present in the developing hippocampus, where synchronous bursts or giant depolarizing potentials (GDPs) sustained by GABAergic transmission have been recorded (Ben-Ari et al. 1989; Garaschuk et al. 1998; Menendez de la Prida et al. 1996). $GABA_A$ receptors have an excitatory action in early postnatal life, providing the basis for hyperexcitability in immature neuronal networks (Bolea et al. 1996; Cherubini et al. 1991). Under these conditions, GDPs are recorded from the intact neonatal limbic structures (Leinekugel et al. 1998) as well as from CA3, CA1, and the fascia dentata (FD) (Garaschuk et al. 1998; Khazipov et al. 1997; Menendez de la Prida et al. 1998). GDPs are known to be generated by the synchronized release of GABA from interneurons in cooperation with glutamatergic cells (Ben-Ari et al. 1997; Khazipov et al. 1997), although the mechanism underlying synchronization still remains controversial (Garaschuk et al. 1998; Khazipov et al. 1997; Menendez de la Prida et al. 1998; Strata et al. 1997).

In this paper, we investigate the conditions under which synchronization spontaneously occurs in the immature hippocampus. Synchronization of a neuronal network is achieved when the firing of component units becomes phase locked, which is dependant on the connectivity patterns and intrinsic firing capability of the units (Colling et al. 1998; Lytton and Sejnowski 1991; Skinner et al. 1994; Stanford et al. 1998; Traub et al. 1996b). Several studies support the idea of optimal frequencies for synchronization within a neuronal network (Cobb et al. 1995; Destexhe et al. 1993; Gray et al. 1989; Stopfer et al. 1997; Whittington et al. 1995). A frequency-dependent mechanism has been proposed for the regulation of information flow from the entorhinal cortex to the hippocampus (Gloveli et al. 1997). These neuronal networks reveal nonlinear characteristics as response to extracellular stimulation ranging from 0.1 to 10 Hz (Berger et al. 1988). In the case of immature hippocampus, these possibilities have not been considered yet as a mechanism for GDP generation. We show that synchronization in the developing hippocampus arises spontaneously in a frequency-dependent manner. We have focused our attention on the period just before onset of GDPs. During this interval, which we refer to as the integration period, an increase in the number of excitatory postsynaptic

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potentials (EPSPs) is detected. By comparing EPSP frequency with the occurrence of GDPs, we demonstrate the all-or-none character of synchronous bursting, a phenomena that also can be reproduced by extracellular stimulation. The initiation of synchronous bursts by EPSPs has been reported in 4-aminopyridine (4-AP) and high-potassium media (Chamberlin et al. 1990; Ives and Jefferys 1990; Traub and Dingledine 1990). Our findings indicate that the synchronous activity of spontaneously occurring EPSPs is important not only under pathological conditions but also during postnatal development.

METHODS

Experimental preparation

Newborn New Zealand white rabbits (2–5 postnatal days) were killed by decapitation under light ether anesthesia. The whole brain was removed and chilled at 4°C in standard artificial cerebrospinal fluid. Transverse slices of hippocampus (500 μm) were prepared using a drop-blade chopper. The slices were maintained in an incubation chamber at room temperature for ≥1 h before recording, at which time one slice was transferred to a submersion-type recording chamber (Medical Systems) continuously perfused with a standard medium containing (in mM) 125 NaCl, 3 KCl, 1.2 $MgSO_4$, 1.2 NaH_2PO_4, 2 $CaCl_2$, 22 $NaHCO_3$, and 10 glucose, saturated with 95% O_2-5% CO_2, pH: 7.4. Temperature was maintained at 30–33°C (pH 7.4) with a flow speed of 1–1.5 ml/min.

Intracellular recordings

Intracellular recording electrodes were made from borosilicate glass (1.2 mm OD; Sutter Instrument) pulled with a Brown-Flaming horizontal puller (Sutter Instrument) and filled with 3 M KCl (50–100 MΩ). Simultaneous intracellular recordings were made with separated manipulators using a dual intracellular amplifier (Axoclamp II B). The intracellular penetration of CA3 and CA1 pyramidal neurons were realized in the stratum pyramidale. Recordings from FD were made at the granular layer. The criteria for a healthy record were a resting membrane potential greater than −50 mV, input resistance >20 MΩ, action potential amplitude >50 mV, and a spike train response to positive current injection. Cells in the study had a mean input resistance of 110 ± 45 MΩ in CA3 (n = 47), 48 ± 15 MΩ in CA1 (n = 9), and 64 ± 18 MΩ in FD (n = 10). In three experiments, QX314 (RBI), which suppresses sodium spikes, was added (50 mM) to the KCl pipette solution.

For intracellular injections of Neurobiotin, recording electrodes were backfilled with a 5% solution of Neurobiotin (Vector Laboratories, Burlingame, CA) in 1 M KCl and subsequently filled with 3 M KCl. Neurobiotin was injected intracellularly using depolarizing pulses (0.2–0.4 nA) at 1 Hz for 10–30 min. After the experiment, the slice was fixed overnight in 4% paraformaldehyde PBS (0.1 M, pH 7.4). After H_2O_2 (0.3%) and Triton X-100 (0.6%) pretreatment, the slice was then processed by incubation in a 1:100 dilution of ABC complex (Vector) and by a 0.03% solution of 3,3-diaminobenzidine and 0.005% H_2O_2.

Mossy fiber stimulation

Monopolar electrical stimulation was applied via tungsten electrodes at the hilus while intracellular recording at CA3 (n = 11). The stimulus duration was 100 μs. To minimize EPSP summation, the pulse amplitude was set to the value able to produce minimal EPSPs in every slice. Ten to 20 trials of periodical stimulation were tested (2–25 Hz).

Measurements and data analysis

EPSP detection complied the following criteria: only events >0.25 mV were counted as EPSPs and peaks making up clustered events were counted individually if their peak height was greater than the half-peak amplitude of the largest cluster member. To measure EPSP frequency, we established time windows of 0.5 s. GDP onset was defined at burst half-amplitude. This was the most systematic way to define GDP onset because criteria based on the depolarization underlying a GDP were difficult to apply due to EPSP accumulation. Two time windows were constructed from the GDP onset to compute EPSP frequency: 0–0.5 and 0.5–1 s. All measurements are given as means ± SD with the number of cells indicated. For statistical analysis the Student's two-tailed t-test was used (confidence level, P = 0.05).

RESULTS

Spontaneous EPSPs reflect network activity leading to GDP generation

Simultaneous intracellular recordings from n = 36 pairs of cells revealed highly synchronous GDPs within CA3 (Fig. 1*B*) and CA3-CA1 (Fig. 2*D*). The frequency of these events in CA3 and CA1 regions was 2.9 ± 1.4 GDPs/min (n = 36). GDP amplitude and duration was 21 ± 4 mV and 416 ± 209 ms, respectively (92 GDPs, n = 21 cells). The reversal potential was −30 ± 10 mV (n = 8), and they were blocked by bicuculline indicating its $GABA_A$ dependence.

Detailed examination of simultaneous recordings from pairs of CA3 cells <150 μm apart (n = 8 pairs, Fig. 1*A*) revealed a slight concurrent increase of the instantaneous firing frequency (Fig. 1*B*, see bars). Frequency changes were not intrinsic to the recorded cells because they were unrelated to the membrane potential. The synchronous increase of firing frequency lead in some cases to GDPs (Fig. 1*B*, arrow c) but failed in others (arrows a and b). These data suggest that under certain circumstances (arrows a and b) local synchronization does not fulfill the conditions for full synchronization and thus GDPs are not fired. The investigation of these conditions is the purpose of the present work.

Our recordings also indicated that the increased frequency of EPSPs before GDP onset is correlated with an increase in the number of EPSPs in the simultaneously recorded neuron (Fig. 1*C*, arrows). In this particular experiment, *cell 2* was hyperpolarized to −85 mV to show that a slight increase in the firing frequency of *cell 1* is synchronous with an EPSP recorded in *cell 2* (these 2 cells were not synaptically connected). We examined data from n = 17 CA3-CA3 recordings in which one cell was hyperpolarized to reveal EPSPs. All the EPSPs were measured and counted from hyperpolarized cells to gain in individual EPSP resolution.

GDPs typically were preceded by an increase in the number of EPSPs (Fig. 2*A*, square in *cell 2;* cells are different from those shown in Fig. 1). This interval preceding GDP had a duration between 100 and 300 ms. Concomitantly, action potentials were observed in the simultaneously impaled neurons (see action potentials in *cell 1*, Fig. 2*A*). In these neurons (P2–P5), synaptic activity was mainly GABA dependent, given that the majority of EPSPs were blocked by bicuculline (not shown). The half-duration of individual EPSPs was 38.7 ± 14.1 ms and amplitude ranged from 2.5 to 10.2 mV (n = 118 EPSPs from 5 cells). In synaptically coupled neurons (n = 3) the EPSP amplitudes, time constants, and half-durations were 6.1 ± 2.2 mV, 19.2 ± 2.1 ms, and 25.1 ± 5.2 ms, respectively, suggesting that most of the spontaneous individual synaptic

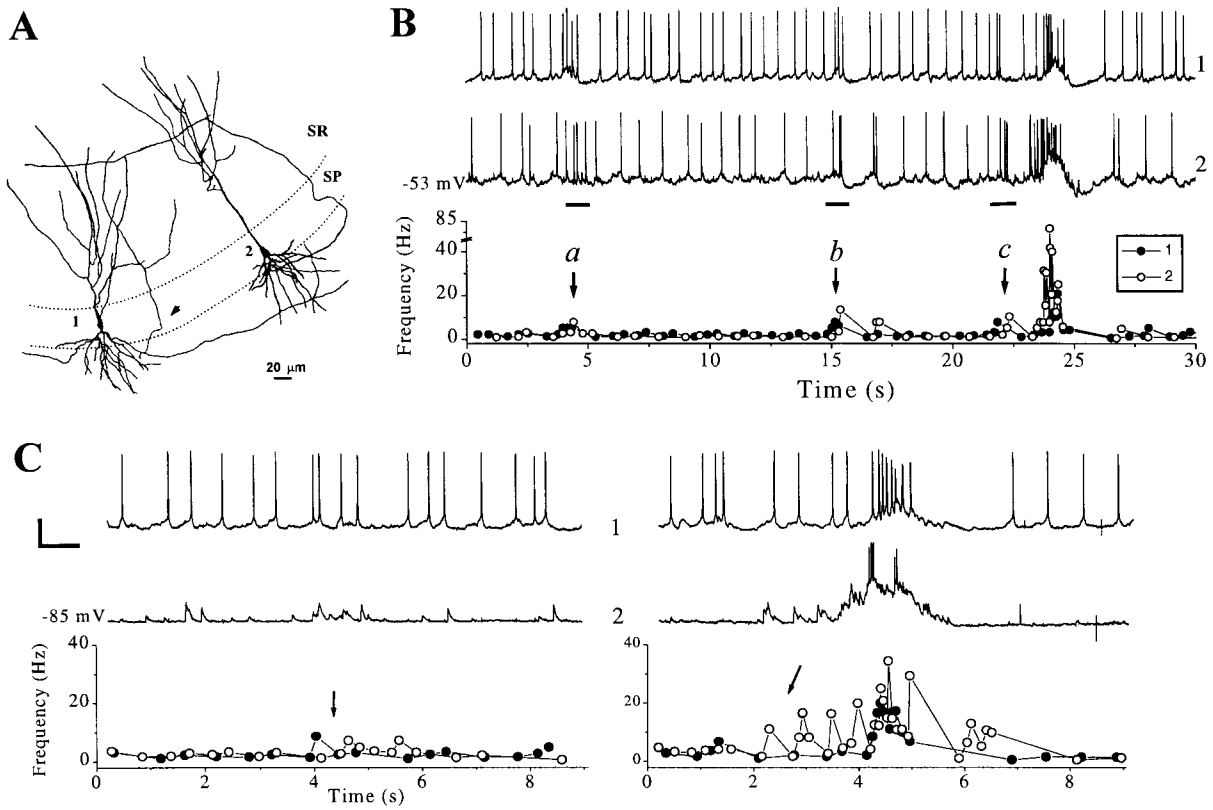

FIG. 1. Instantaneous firing frequency from simultaneous intracellular recordings from CA3 proximal cells (electrode distance <150 μm). *A*: 2 proximal pyramidal neurons were recorded simultaneously. *Cell 1* is closer to CA4 with an axon running forward to CA1 direction. Note also the axon collateral (arrowhead) entering back into CA3 stratus pyramidale (SP) and stratus radiatum (SR). On the contrary, *cell 2* has an axon that travels in the backward direction, i.e., toward CA4. *B*: spontaneous activity from *cells 1* and *2* and the instantaneous firing frequency. Slight concurrent increase of firing frequency can be observed (arrows a–c) in some cases associated with a giant depolarizing potential (GDP; arrow c). *C*: *cell 2* was hyperpolarized to show that the slight increase of firing frequency in *cell 1* is synchronous with the increase of the number of excitatory postsynaptic potentials (EPSPs) recorded in *cell 2* (arrows). These cells were not synaptically connected. Frequency in *cell 2* is referred to as the instantaneous EPSP frequency. Resting membrane potentials (RMPs), *cell 1*: −57 mV; *cell 2*: −53 mV. Calibration bars in *C*: horizontal 2 s (*B*) and 1 s (*C*); vertical 20 mV (*B* and *C*).

events may have resulted from single spikes in presynaptic neurons (Fig. 2*B*). The barrage of EPSPs thus can reflect the network activity leading to GDP generation.

Increase of EPSP frequency is related to GDP occurrence

We wondered whether the number of EPSPs preceding GDPs in a time interval between 0 and 0.5 s is significantly different from those occurring between 0.5 and 1 s. We analyzed 21 GDPs from $n = 3$ CA3 neurons using electrodes containing QX314 and 97 GDPs from $n = 8$ simultaneous intracellular recordings using KCl-filled electrodes. Reliable estimates of EPSP frequency were obtained in both cases. Results are presented in Fig. 2*C*. EPSP frequency in the 0- to 0.5-s interval was higher (18.2 ± 2.8 Hz) than in the 0.5- to 1-s interval (5.8 ± 1.6 Hz; significantly different $P = 0.8 \cdot 10^{-12}$; $t = 12.3$, $n = 118$ GDPs from 11 cells).

''Failure'' of synchronization within and between regions also provided us additional insight into the mechanisms of synchronization. Failure of synchronization refers to the situation in which a GDP was detected in an area but not in the other (Fig. 2*D*, arrow). We examined 588 GDPs from $n = 12$ simultaneously recorded cells (CA3-FD, CA3-CA3, and CA3-CA1). A low percentage of failure was detected in CA3-CA3 and CA3-CA1 pairs (2.5 and 1.8%, respectively). CA3-FD pairs showed the largest incidence of failure in FD cells (9.7%) consistent with the lower GDP frequency of this area (1.6 ± 0.9 GDPs/min; $n = 10$). Failures were always associated with an increase in the number of EPSPs (Fig. 2*D*, arrow in *cell 6*).

Frequency-dependent mechanism of synchronization

Our results allow us to state the following hypothesis: because coordinated neuronal activity underlies GDP generation, a relationship must exist between EPSP frequency and the occurrence of GDPs. In the case of failure, a GDP is not fired even though an increase in the number of EPSPs is detected. On the contrary, a different situation must be present when GDP is fired. In this case, the EPSP increase should reflect the conditions for full synchronization.

To assess this hypothesis, we analyzed $n = 10$ simultaneous recordings from CA3 and FD because FD showed the highest percentage of failures. We computed EPSP frequency under three different situations (Fig. 3*A*): when asynchronous activity was present in simultaneously impaled cells (*a*), when a GDP was recorded in CA3 and a increase in FD EPSP number failed to produce synchronization (*b*), and when a GDP in CA3 was followed by increase in EPSP number and a GDP in FD (*c*).

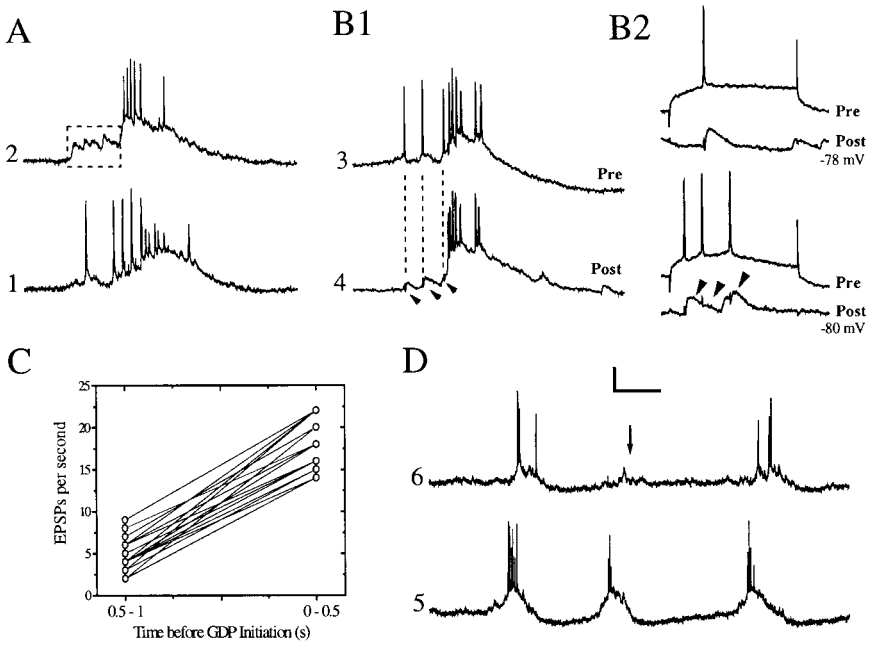

FIG. 2. Synchronous GDPs from developing hippocampal networks. *A*: synchronous GDPs from 2 different CA3 pyramidal cells impaled simultaneously (1 and 2). GDPs are typically preceded by a number of EPSPs (square in *cell 2*). Concomitantly, action potentials are present in *cell 1*. *B1*: synchronous GDPs recorded from 2 synaptically coupled CA3 cells. *Cell 3* is the presynaptic cell (Pre). Individual action potentials in *cell 3* evoke individual EPSPs in the postsynaptic *cell 4* (Post, see arrowheads). *B2*: postsynaptic EPSPs elicited by presynaptic spikes. Depolarizing pulses of 0.1 nA (*B2, top*) and 0.15 nA (*B2, bottom*) were applied to *cell 3* (Pre). *C*: EPSP frequency 0–0.5 and 0.5–1 s before GDP onset. Data from $n = 3$ cells. EPSP frequency increases 500 ms before GDPs. *D*: simultaneous intracellular recordings from CA3 (*cell 5*) and CA1 (*cell 6*). GDP failure is detected in CA1 (arrow in *cell 6*). In this case, an increase in the EPSP number is recorded in correlation with the GDP in CA3 (arrow, *cell 6*). Calibration bars in *D*: vertical 10 mV (*A, B,* and *D*); horizontal 250 ms (*A* and *B1*), 125 ms (*B2*), and 1 s (*D*). RMPs, *cell 1*: −65 mV; *cell 2*: −79 mV; *cell 3*: −69 mV; *cell 4*: −79 mV; *cell 5*: −78 mV; and *cell 6*: −69 mV.

Frequency histograms from these groups showed that cases *b* and *c* can be distinguished clearly from case *a* (Fig. 3*B*, $n = 5$). The means are: 5.8 ± 2.9 Hz, $n = 80$ time windows (*a*); 13.1 ± 2.7 Hz (*b*) and 20.3 ± 2.9 Hz (*c*), $n = 50$ time windows in both cases. Situations *b* and *c* (17.6 ± 4.7 Hz) are significantly different from situation *a* ($P = 3 \cdot 10^{-18}$; $t = 11.6$).

The relationship between EPSP frequency and GDP amplitude in *b* and *c* was examined to account for the mechanisms underlying GDP onset ($n = 10$). Because situation *b* represents the cases in which synchronization is not full and therefore GDP fails, the GDP amplitude can be taken as zero. In Fig. 3*C* the results from $n = 3$ neurons are summarized. As shown by the distribution of amplitudes GDPs arose in an all-or-none manner for every cell (represented with different symbols). The mean frequency of EPSPs for GDP triggering was 17.4 ± 2.6 Hz ($n = 10$) independent of the membrane potential. GDPs are fired when the frequency of the electrical activity responsible for EPSPs crosses a threshold of 17 Hz (Fig. 3*C*, arrow).

Nonlinear frequency-dependent properties of evoked GDPs

If the synchronization leading to GDP generation is frequency dependent, then repetitive mossy fiber stimulation should need appropriate frequencies to evoke GDPs. Figure 4*A* shows that repetitive stimulation at 1–9 Hz did not induce GDPs, irrespective of the stimulus duration. Instead GDPs were fired from 10-Hz stimulation. In pyramidal cells, a cumulative effect of successive pulses was not apparent (see 1st 3 pulses in the 10-Hz trace) or occurred far from GDP onset without triggering it (Fig. 4*B*, arrow 1). GDPs rather emerged after a sudden depolarization that was not associated with a given pulse (see arrow in the 10-Hz trace). In fact, stimulus

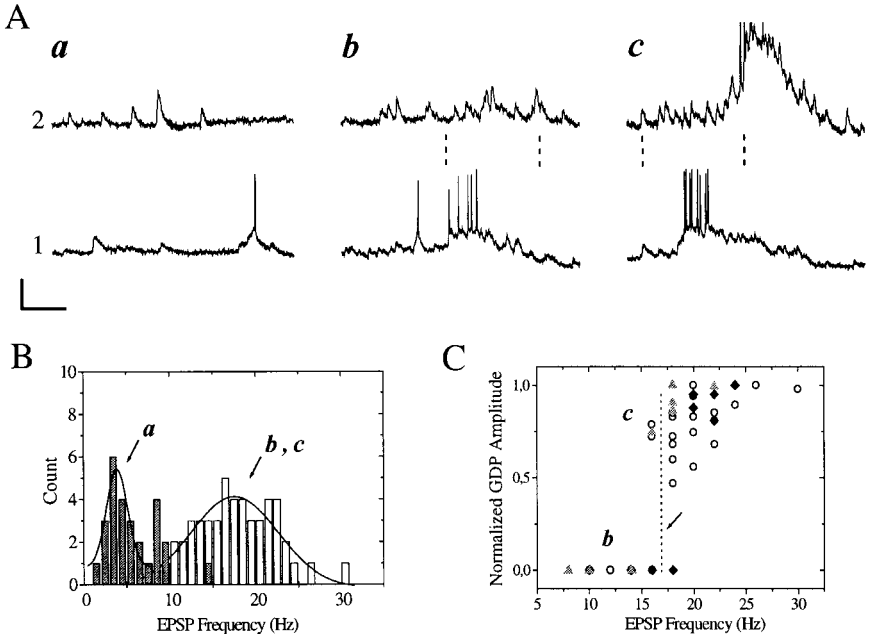

FIG. 3. Analysis of EPSP frequency within the 0- to 0.5-s integration period. *A*: 2 simultaneously recorded cells from CA3 (*cell 1*) and FD (*cell 2*). EPSP frequencies from 3 different situations are compared. *a*: both cells show uncorrelated activity. *b*: GDP is recorded in CA3 and fails in FD, where an increase in the EPSP number is recorded. *c*: GDPs are fired both in CA3 and FD. EPSP frequency from 0.5 s in *a–c* is measured (discontinuous lines in *b* and *c*). Calibration bars: vertical 20 mV (*cell 1*), 10 mV (*cell 2*) horizontal 250 ms. Spikes in *Ac* are truncated. *B*: frequency histograms show that *b* and *c* cases are statistically different from *a* ($n = 5$). *C*: normalized GDP amplitude plotted vs. EPSP frequency in *b* and *c* ($n = 3$ cells). Synchronous GDPs occurred in an all-or-none manner (arrow). RMPs, *cell 1*: −68 mV; *cell 2*: −66 mV.

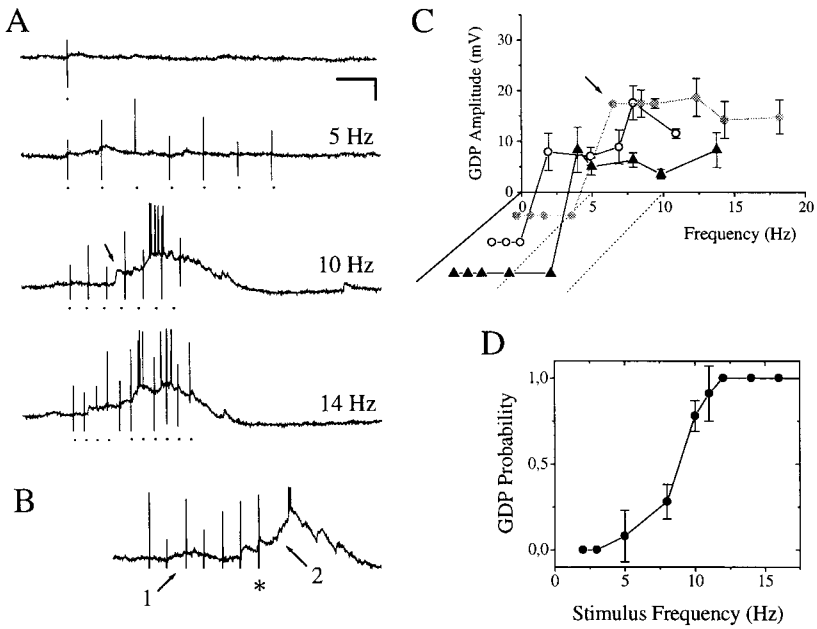

FIG. 4. Effect of repetitive mossy fiber stimulation. *A*: stimulus strength is set to evoke a minimal EPSP. Repetitive stimulation at 5 Hz does not evoke GDPs, which are triggered >10 Hz. *B*: same cell as in *A*. Twelve-Hertz stimulation is aborted (asterisk) to show that GDP triggering (arrow 2) is not a consequence of EPSP summation in pyramidal cells (arrow 1). Calibration bars in *A*: vertical 20 mV, horizontal 250 ms (*A*); vertical 10 mV, horizontal 200 ms (*B*). Spikes are truncated. *C*: evoked GDP amplitude against the stimulus frequency from 3 CA3 cells (different slices). GDPs are evoked in an all-or-none fashion. Threshold varies slightly among different slices. *D*: GDP probability plotted vs. the stimulus frequency from $n = 11$ slices. Points without error bars represent the cases with no SD (probability 0 and 1). Note that stimulation >12 Hz evokes GDPs in all the slices. RMPs, *A* and *B*: −69 mV.

interruption did not abort GDPs which were triggered after the last pulse ($n = 20$ trials, see asterisk and arrow 2 in Fig. 4*B*).

The frequency dependence of evoked GDP amplitude was similar to that described in the preceding text for spontaneous GDPs (Fig. 4*C*, 3 cells from different slices are represented). Nevertheless, GDPs in CA3 seemed to be triggered at lower frequencies (7.1 ± 2.5 Hz, $n = 11$) when compared with spontaneous GDPs in FD (~17 Hz). For a reliable determination of the population threshold for synchronization, we considered the results from different slices (Fig. 4*D*, $n = 9$). There is a range of frequency for which fluctuations in GDP probability are present. Nevertheless stimulation >12 Hz evoked GDPs in all the slices, and stimulation <5 Hz did not generate GDPs. Based on these results, the CA3 region has a frequency-threshold for GDP onset of 12 Hz.

DISCUSSION

The aim of the present work was to investigate the features of local circuit synchronization in the developing hippocampus. The results suggest that synchronous bursts or GDPs are generated by a frequency-dependent mechanism. Simultaneous recordings from pairs of proximal CA3 pyramidal cells (electrode distance <150 μm) showed a concurrent increment in the firing frequency previous to GDP onset. This increment was correlated with an increase in the number of EPSPs that reflects the spontaneous network activity. According to our results a GDP is fired when a synchronous increase in the spontaneous activity exceeds a certain frequency-threshold. This ''build-up'' of network synchronization takes place 100–300 ms before GDPs (integration period). Because GDPs involve the cooperative action of $GABA_A$, NMDA, and AMPA components (Ben-Ari et al. 1989, 1997; Bolea et al. 1999; Gaiarsa et al. 1993), the integration period represents the time in which firing increases locally due to recruitment of the appropriate neuronal populations.

A similar role of EPSPs in the initiation of synchronous bursts has been previously described in 4-AP and high-potassium media (Chamberlin et al. 1990; Ives and Jefferys 1990; Traub et al. 1995). This similarity between experimental models of epilepsy and immature hyperexcitability is particularly interesting to unify the principles of discharge generation (Schwartzkroin et al. 1995; Traub and Jefferys 1994). Both experimental and computational studies have been carried out to elucidate the components underlying network synchronization, i.e., the number of cells, network connectivity and synaptic components (Miles et al. 1984; Smith et al. 1995; Traub and Dingledine 1990; Traub et al. 1984, 1993, 1995, 1996a). However, the frequency-dependent mechanism has not been deeply investigated. Our results indicate that the activity increment responsible for EPSP accumulation does not initiate population discharges just by triggering the firing of a specific group of cells as previously proposed (Ives and Jefferys 1990; Traub and Dingledine 1990). The frequency of this firing must exceed a specific threshold to build up full synchronization.

There is evidence for the existence of specific frequencies for synchronization (Domingo et al. 1997; Farmer 1998; Murthy and Fetz 1996). In the olfactory system, information is encoded in temporal sequences in which several assemblies are recruited, regardless of the phase (Stopfer et al. 1997; Wehr and Laurent 1996). These assemblies consist of groups of neurons that fire together at 20–30 Hz, a frequency that is odor-independent (Laurent 1996). In visual perception, there are reports of synchronized responses between cortical columns at specific frequencies (40–60 Hz), irrespective of stimulus configuration (Gray et al. 1989). Different cerebral areas also interact with each other in an optimal frequency range, which in the majority of the cases has a functional content: the cortex and thalamus for example, phase lock at 7–14 Hz during spindles (Contreras et al. 1997). The cellular and network basis of the frequency-threshold synchronization might be thus investigated both in the intrinsic firing properties of neuronal groups and the connectivity patterns (Skinner et al. 1994).

Experimental and computational models of gamma-frequency oscillations have shown that there is a minimal interneuron network frequency at ~20 Hz (Traub et al. 1996a; Wang and Buzsáki 1996). In those works, the authors investigated network

frequency as a function of the time constant of $GABA_A$-mediated inhibitory postsynaptic potential (IPSP). According to their estimation a time constant of 10 ms includes five IPSPs within a period (50 ms from 20 Hz). This will cause a different hyperpolarization level in different cells preventing synchronization (Traub et al. 1996a). Nevertheless this analysis is not sufficient to predict quantitatively the minimum frequency (Traub et al. 1996a). Our data show that EPSPs from CA3 synaptically coupled pairs of pyramidal cells had time constants of ~20 ms for a minimum frequency of 12 Hz (period ~83 ms). Temporal summation occurs at time intervals shorter than the unitary EPSP time constant. This implies a minimum synchronization frequency of 50 Hz (for 20 ms), which is higher than the threshold value. Furthermore GDP triggering does not seem to result just from the summation of a given number of EPSPs in the pyramidal neurons (see Fig. 4). Whether temporal summation in the interneurons accounts for the threshold mechanism and/or whether the integration process could be taking place at dendritic locations requires further investigation. Although recent data might suggest that input summation is linear and independent of the somatic/dendritic input position in hippocampal neurons (Cash and Yuste 1998), previous studies showed that bursts can be transmitted between monosynaptically connected neurons (Miles and Wong 1987). The likely mechanism underlying burst transmission is the delayed generation of a dendritic Ca^{2+} spike in the postsynaptic cell. Therefore attention must be paid to synaptic integration at the dendrites and interneurons as plausible mechanisms underlying the frequency dependence of GDP initiation process.

Our results also show a long integration period in building up GDPs (100–300 ms), which suggests that the recruitment process involves multiple synapses. This is in accordance with previous reports showing large latency between proximal regions (~200 ms in CA3-FD recordings) (Menendez de la Prida et al. 1998). There is variability in the duration of the integration period between different cells, suggesting that the recruitment not always involve the same elements. Some of the factors that may account for this variability are the specific properties of local interconnections and/or the existence of a critical mass for full synchronization (Menendez de la Prida et al. 1998; Miles et al. 1984; Smith et al. 1995). This is specially evident in the records shown in Fig. 1*B* where the events signed by arrows b and c do not differ markedly in the firing frequency (13 and 10 Hz), but GDP fails in one of them (arrow b).

It has been proposed previously that spontaneous EPSPs may be important for brain function (Traub and Dingledine 1990). Our data show that EPSPs play a role in the initiation of synchronous bursts not only under pathological conditions, but also during postnatal development. Spontaneously occurring EPSPs provide the background level of excitation on which the activity is superimposed. This background level is the source for the generation of immature synchronous network activity (Chub and O'Donovan 1998; O'Donovan et al. 1998; Scharfman 1993; Traub and Dingledine 1990).

The capacity of developing hippocampal networks to fire synchronous bursts or GDPs has important physiological consequences by increasing intracellular calcium concentration and by promoting structural changes and trophic activity (Barbin et al. 1993; Ben-Ari et al. 1997; Leinekugel et al. 1995, 1997). Synchronization during development shapes neuronal pathways by processes depending on the electrical activity (Constantine-Patton et al. 1990; Katz and Shatz 1996; Mooney et al. 1996). It is therefore important to elucidate the conditions responsible for synchronous behavior. The developing hippocampus spontaneously fires in two modes: isolated action potentials and GDPs or bursts (Menendez de la Prida et al. 1997). Isolated action potentials encode uncorrelated activity at lower frequencies (<12 Hz), whereas GDPs gate synchronous transmission at higher frequencies (>12 Hz). The frequency-threshold mechanism described here would play the role of a switch between these two firing modes by filtering periodical inputs coming from other cortical areas and the septum (Scharfman 1991). The filtering capability would determine coordinate patterns of output activity depending on the input frequency and regulate the operative capacity of the developing hippocampus (Gloveli et al. 1997; Lisman 1997; Menendez de la Prida et al. 1997).

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