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Origin of the synchronized network activity in the rabbit developing hippocampus

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Abstract

Rhythmic spontaneous bursting is a fundamental hallmark of the immature hippocampal activity recorded *in vitro*. These bursts or giant depolarizing potentials (GDPs) are GABA- and glutamatergic-driven events. The mechanisms of GDPs generation are still controversial, since although a hilar origin has been suggested, GDPs were also recorded from isolated CA3 area. Here, we have investigated the origin of GDPs in hippocampal slices from newborn rabbits. Simultaneous intracellular recordings were performed in CA3, CA1 and the fascia dentata. We found a high degree of correlation between the spontaneous GDPs present in CA3 and CA1 regions. Cross-correlation analysis demonstrated that CA3 firing precedes CA1 by about 192 ms, although a significant population of discharges was recorded first in CA1 (20%). Granule cells (GCs) in the fascia dentata also showed GDPs. The frequency of these events (1.46 $\pm$ 1.25 GDPs/min, $n = 7$) is significantly lower when compared with that from CA3 (3.13 $\pm$ 1.43 GDPs/min, $n = 10$) or CA1 (2.94 $\pm$ 1.36 GDPs/min, $n = 17$). Dual recordings from CA3 and fascia dentata cells showed synchronous bursts in both regions with no prevalent preceding area. By recording from isolated areas we found that CA1, CA3 and the fascia dentata can produce GDPs, suggesting that they emerge as a property of local circuits present throughout the hippocampus.

Introduction

The presence of synchronized spontaneous bursting in the developing central nervous system has received considerable support in the last years. Since the pioneer work by Galli & Maffei (1988), showing the existence of spontaneous electrical activity in prenatal retinal ganglion cells, bursts of activity have been reported in the retina (Meister *et al*., 1991), spinal cord (Gu and Spitzer, 1995) and cerebral cortex (Yuste *et al*., 1995). Highly correlated bursting activity has also been detected in hippocampal slices from newborn rats and rabbits (Ben-Ari *et al*., 1989; Menendez de la Prida *et al*., 1996), strengthening the idea that hyperexcitability is a feature of specific periods during postnatal development. However, the mechanisms which generate and synchronize bursting activity differ among the systems. In the case of immature hippocampus, the synchronized bursts (called giant depolarizing potentials or GDPs) are network-driven events involving excitatory action, via glutamate receptors, and GABAergic interneurons (Ben-Ari *et al*., 1989; Gaiarsa *et al*., 1991; McLean *et al*., 1995).

In a recent paper, Strata *et al*. (1997) discussed the possible role of hilar interneurons in pacing the GDPs. In their view, the hyperpolarization-activated current, I_h, contributes to phase lock the activity of the hilar interneurons and thus to the correlated release of GABA. According to their results, the dissection of the hilus eliminates GDPs in CA3 and CA1, although the bursting was still recorded in the isolated hilus. Nevertheless, another recent report shows that the isolation of CA3 does not eliminate GDPs, suggesting that all the elements required for their generation are present within this area (Khazipov *et al*., 1997). The pacemaker role of I_h in the activity of hippocampal interneurons is not a strict feature of the hilar field. In fact, it has been shown that I_h is clearly involved in the spontaneous repetitive firing of CA1 stratus oriens-alveus interneurons (Maccaferri & McBain, 1996). On the other hand, the existence of local synchronized inhibitory circuits via GABAergic mechanisms has been described throughout the hippocampus (Michelson & Wong, 1994) suggesting that the mechanisms responsible for generating GDPs are not restricted to the hilus, but are in fact present in every region of the hippocampus.

In this paper, we investigated the capacity of each region of the hippocampus to generate synchronized GDPs in immature rabbit hippocampal slices (postnatal days P2–P5). Simultaneous intracellular recordings from CA3, CA1 and fascia dentata in intact slices confirmed the presence of highly correlated GDPs. Analysis of these records revealed a heterogeneous source for synchronized GDPs, which were also present in isolated slices from these three regions. We conclude that the elements required for generation of GDPs are present in every area of the hippocampus. Here, we discuss these findings in view of the connectivity patterns within the hippocampus.

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Methods

Slicing procedures

The methods used in these experiments were similar to those previously described (Sanchez-Andres & Alkon, 1991). New Zealand white rabbits (2–5 postnatal days) were killed by decapitation under slight ether anaesthesia. The whole brain was removed and chilled to 4 °C in standard artificial cerebrospinal fluid (ACSF, composition in mM: 125 NaCl, 3 KCl, 1.2 $MgSO_4$, 1.2 NaH_2PO_4, 2 $CaCl_2$, 22 $NaHCO_3$, 10 glucose, saturated with 95% O_2–5% CO_2, pH 7.4). The hippocampus was dissected from a ventral approach and its dorsal part cut into 500-μm transverse slices with a drop-blade chopper. The slices were maintained in an incubation chamber at room temperature for at least 1 h before recording. The experiments were performed in two configurations: (i) intact slices and (ii) isolated slices from CA3, CA1 and fascia dentata obtained from the intact slices (see Fig. 4B). For recording, individual slices were transferred to a submerged-type recording chamber (Medical System), continuously perfused with ACSF at a temperature of 32–34 °C (pH 7.4) and a flow of 1–1.5 mL/min. In the case of experiments using isolated regions, once the intact slice was placed in the recording chamber, a minislice was obtained by microdissection with a razor blade. Its size was measured with a calibrated eyepiece. Isolated slices from CA3 ($n = 9$), CA1 ($n = 10$) and fascia dentata ($n = 5$) were obtained. Bicuculline was from Sigma. Steady concentrations in the bath were reached after 2 min perfusion.

Intracellular recording

Intracellular recording electrodes were made from capillary tubes with intraluminal glass fibres (borosilicate, OD 1.2 mm, ID 0.69 mm; Sutter Instrument Co.) pulled with a Brown-Flaming horizontal puller (Sutter Instrument Co.), and filled with 3 M KCl (electrode resistances: 50–100 MEΩ). Simultaneous intracellular recordings were made with separated manipulators using a dual intracellular amplifier (Axoclamp II B). The intracellular penetrations of CA3 and CA1 pyramidal neurons were made in the stratum pyramidale. GCs were impaled at the granular layer. The criteria for a healthy record were a resting membrane potential greater than –50 mV, input resistance larger than 20 MΩ, action potential amplitude greater than 50 mV and a spike train response to positive current injection (Schwartzkroin, 1982). Cells in the study had a mean input resistance of 97 ± 33 MΩ in CA3 ($n = 21$), 45 ± 15 MΩ in CA1 ($n = 27$) and 58 ± 18 MΩ in GCs ($n = 12$). In some experiments, the neurons were injected with Neurobiotin (Vector Laboratories, Burlingame, CA) for late identification. To achieve this, the tips of the recording electrodes were filled with Neurobiotin 5% dissolved in 1 M KCl and backfilled with 3 M KCl. Neurobiotin was injected intracellularly using depolarizing pulses (0.2–0.4 nA) at 1 Hz for 10–30 min. In these experiments, the electrical properties were analysed (response to depolarizing and hyperpolarizing pulses). 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 a 0.03% solution of 3,3-diaminobenzidine and 0.005% H_2O_2.

Data analysis

Cross-correlation analyses were performed by computing the auto- and cross-correlation functions to determine the degree of synchrony and to measure the time lags between the neuronal discharges. The auto- and cross-correlation functions of the membrane potential fluctuations were computed using the FFT by a well known programmable routine (Press *et al*., 1992). In the abscissa of these correlograms, function values to the right of zero indicate the cases in which the reference cell fires first. All the measures are given as mean ± SD, the number of cells are indicated in every case. Statistical significance analysis using student's two-tailed *t*-test was applied (confidence level, $P = 0.05$). Graphic design was performed with Origin (v.3.73, Microcal Software).

TABLE 1. Frequency of the spontaneous giant depolarizing potentials (GDPs) in intact and isolated hippocampal slices

	CA3	CA1	FD
Control (intact slices)	3.13 ± 1.43*† $n = 10$	2.94 ± 1.36 ‡ $n = 17$	1.46 ± 1.25 †‡ $n = 7$
Isolated slices	5.63 ± 2.93* $n = 11$	2.73 ± 1.40 $n = 10$	1.04 ± 0.47 $n = 5$

Fascia dentata (FD). Values are given as GDPs/min. GDPs frequency in CA3 and CA1 in control is not significantly different: $t = 0.33$, $P = 0.74$.
*Isolated CA3 versus control; significantly different: $t = 2.44$, $P = 0.024$.
†CA3 versus FD in control conditions; significantly different: $t = 2.48$, $P = 0.023$.
‡ CA1 versus FD in control conditions; significantly different: $t = 2.47$, $P = 0.021$.

Results

As previously reported, spontaneous GDPs are present in the CA1 rabbit hippocampal slices from the first two postnatal weeks (Menendez de la Prida *et al*., 1996). These events are similar to those described in the rat (Ben-Ari *et al*., 1989). They consisted of a large depolarization (21 ± 4 mV) lasting from 190 to 750 ms and crowned by 2–6 spikes (92 GDPs, $n = 21$ cells). Their reversal potential was –30 ± 10 mV ($n = 8$) and they were blocked by bicuculline (10 μM, data not shown) as previously reported (Ben-Ari *et al*., 1989; Zhang *et al*., 1991; Menendez de la Prida *et al*., 1996). To investigate the origin of these events, we performed simultaneous intracellular recordings from CA3, CA1 and the fascia dentata.

Synchronized GDPs occur in CA3 and CA1 neurons from intact slices

Twelve simultaneous intracellular recordings from CA3 and CA1 pyramidal neurons in intact hippocampal slices were performed: CA3–CA3 ($n = 5$) and CA3–CA1 ($n = 7$). The GDPs recorded in CA3 have the same properties as those cited above. Dual recordings from CA3 and CA1 cells revealed a high degree of correlation between GDPs, in accordance with previous findings (Khazipov *et al*., 1997; Strata *et al*., 1997). The frequency of these events was similar to that computed from single recordings in either CA1 or CA3 at the same postnatal ages, i.e. 2.94 ± 1.36 GDPs/min ($n = 17$) and 3.13 ± 1.43 GDPs/min ($n = 10$), respectively (see Table 1). Figure 1(A) illustrates the electrical activity from two pyramidal neurons impaled simultaneously in the CA3 stratus-pyramidale (cell 1 and cell 2). As can be seen, the synchronous activity between these neurons was restricted to the GDPs. After 40 min of stable recording, cell 2 was discarded and another pyramidal neuron in the CA1 area was impaled (cell 3, Fig. 1B). Again, the synchrony between the firing of the two cells was restricted to the GDPs.

Cross-correlation analysis of the membrane potential fluctuations was carried out to evaluate the time delay in GDP onset. The auto-correlation function from every cell showed a clear maximum at zero and multiple secondary peaks, confirming the rhythmic nature of the

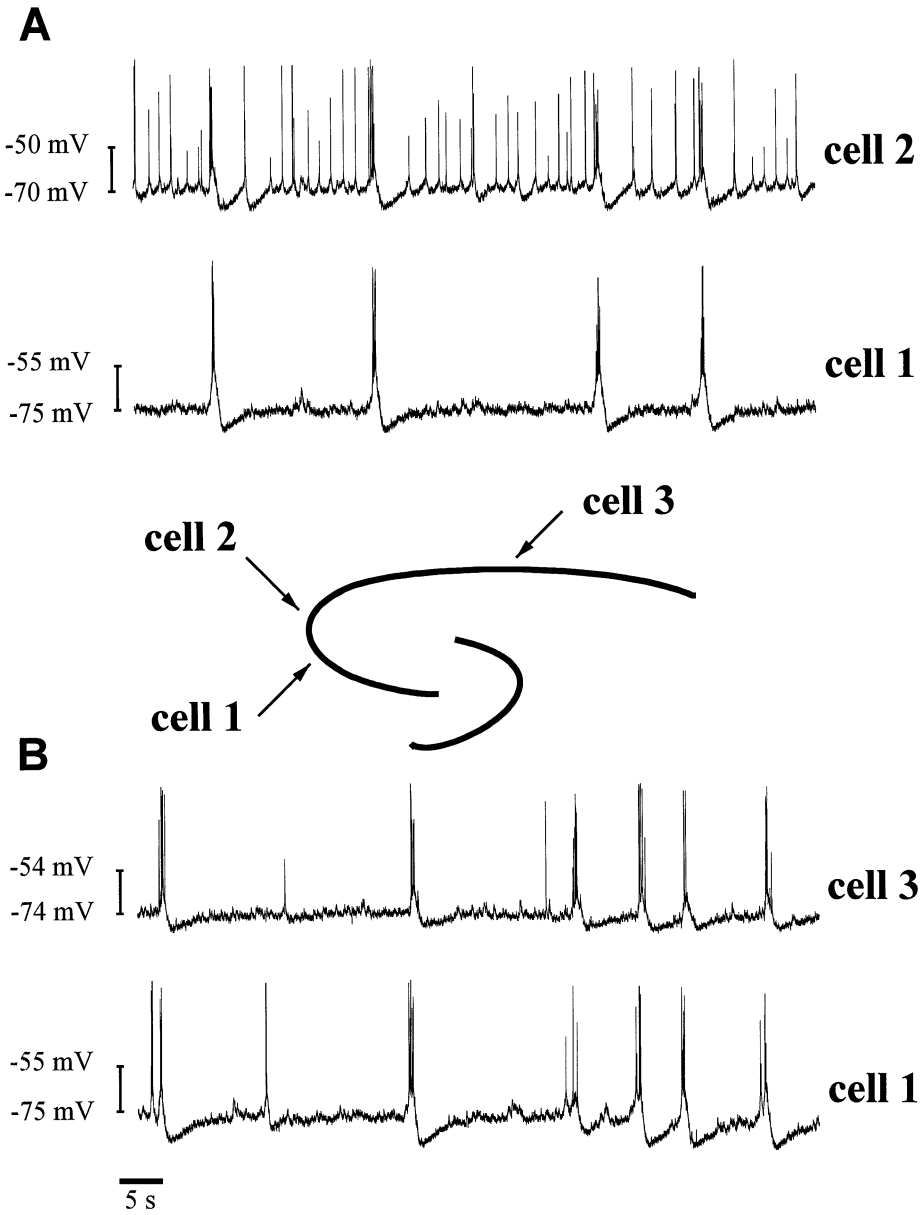

FIG. 1. Synchronized giant depolarizing potentials (GDPs) recorded simultaneously from CA3–CA3 and CA3–CA1 pairs (postnatal day 4, P4). Two CA3 pyramidal cells were impaled simultaneously (cell 1 and cell 2) as represented in the central diagram. After 40 min of stable recording, cell 2 was discarded and a third neuron (cell 3) was impaled at the CA1 pyramidal layer. (A) Intracellular activity from cells 1 and 2 show highly synchronized GDPs at a frequency of about 3.08 GDPs/min. (B) The activity from cells 1 and 3 also shows a high degree of synchrony. Input resistance from these cells was 65 MΩ (cell 1), 60 MΩ (cell 2) and 40 MΩ (cell 3).

individual firing patterns (not shown). The cross-correlation function from cells 1 and 2 of Fig. 1 showed a wide central peak reflecting the presence of strong synchronous events (Fig. 2A). The slight asymmetry of this peak suggests that the underlying depolarization of the cell 1 discharges preceded those of cell 2 (see inset in Fig. 2A). An example of these highly correlated discharges is shown in Figure 2(B).

The cross-correlogram from cells 1 and 3 (Fig. 2C) displayed a peak clearly shifted to the right, with a time lag of about 179 ms (cell 1 is the reference neuron). This allows us to conclude that in general, cell 1 (CA3) fired first followed by cell 3 (CA1) with a delay of 179 ms (Fig. 2D, left). We observed a mean delay of 192 ± 52 ms between the onset of GDPs in the CA3 and CA1 regions (data from four cross-correlograms). However, when larger periods were analysed (epochs longer than 15 min, in all the slices), CA1 neurons preceded CA3 in 20% of the synchronized discharges (Fig. 2D, right). In these cases, the mean delay was about 215 ± 87 ms (from 15 synchronized GDPs, $n = 3$ pairs).

GDPs are present in GCs in synchrony with CA3 region

Single intracellular recordings from GCs in the fascia dentata showed that GDPs were also present in this region ($n = 5$), although their frequency was significantly lower than in the CA3 and CA1 areas (1.46 ± 1.25 GDPs/min, $n = 7$; see Table 1). These cells had a mean membrane potential of –67 ± 6 mV, input resistance of 54 ± 19 MΩ and action potential amplitude of about 58 ± 7 mV ($n = 7$). Typically,

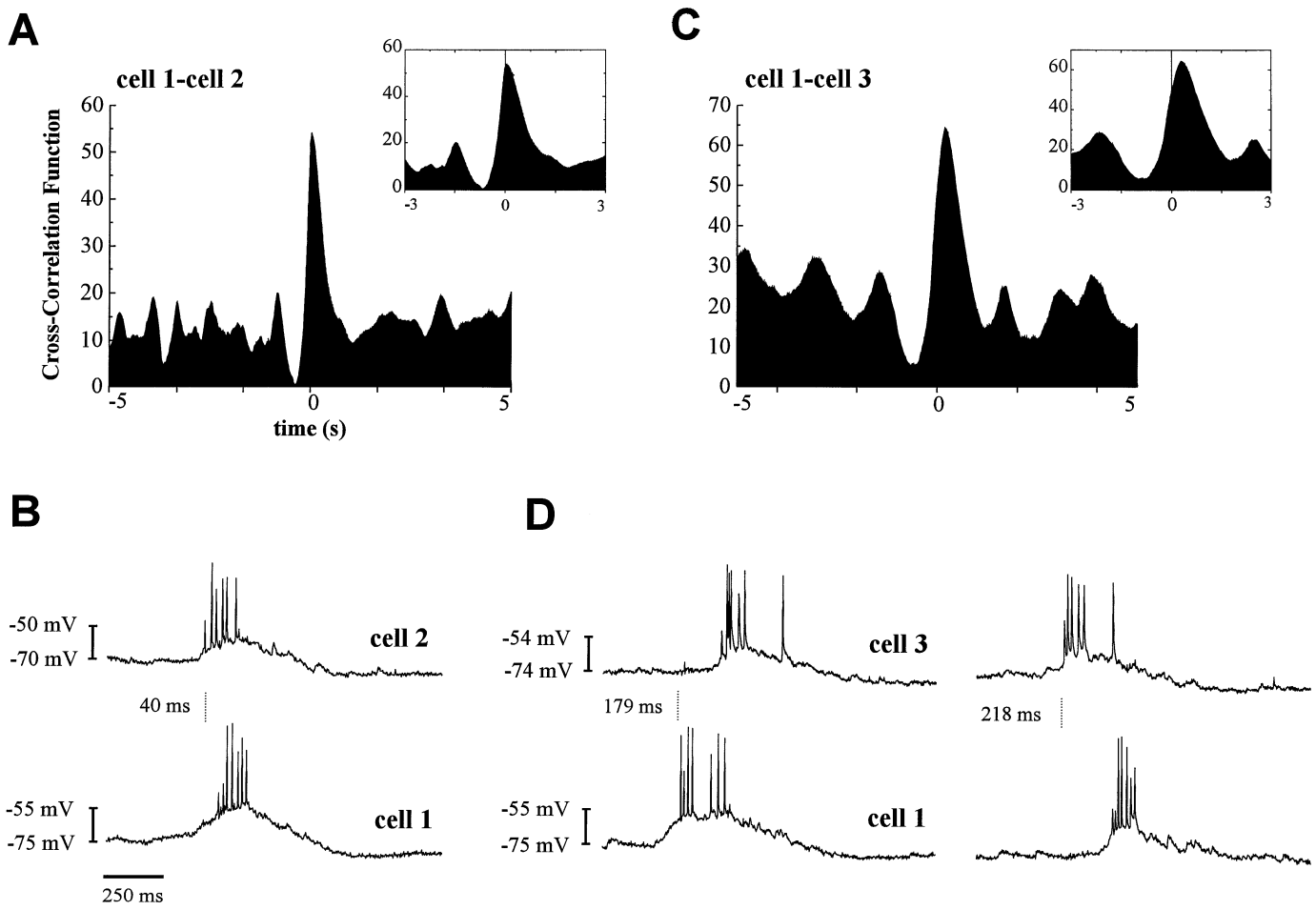

FIG. 2. Analysis of the onset of GDPs in CA3 and CA1 cells shown in Figure 1. (A) Cross-correlation function from cell 1 and 2 clearly shows a central peak and several secondary peaks confirming the presence of highly correlated events. Cell 1 is the reference neuron. (B) Analysis of the simultaneous recordings from cells 1 and 2 reveals that GDPs are synchronous in both neurons. The dotted line marks the position of the first spike to show the time lag between the events. In this case, cell 2 precedes to cell 1 in 40 ms. (C) Cross-correlation function from cells 1 and 3 shows a central peak clearly shifted 179 ms to the right (see inset), indicating that in general cell 3 (CA1) was triggered 179 ms after cell 1 (CA3). Cell 1 is the reference neuron. (D) Analysis of simultaneous electrical activity from cells 1 and 3 shows that in general CA3 neuron precedes CA1 in firing (left), although in some cases CA1 may initiate the burst (right).

they showed low frequency spontaneous spiking activity and abundant synaptic noise.

Dual recordings from CA3 pyramidal neurons and GCs ($n = 8$) showed that every time a GDP was registered in the fascia dentata, a similar burst appeared in CA3 (Fig. 3A). However, not all the GDPs observed in CA3 were accompanied by the GCs bursting, although in these cases an increase in the number of depolarizing postsynaptic potentials was frequently recorded (not shown). We found no region preceding the other in firing, i.e. in the 61% of the synchronized discharges, the GDP in CA3 preceded GCs firing. The typical cross-correlation function from GC–CA3 records showed a central peak (Fig. 3B). The mean latency between these two events was 272 ± 105 ms (from $n = 25$ GDPs) in the cases when CA3 fired first (see Fig. 3C) and 210 ± 100 ms (from $n = 21$ GDPs) when the discharge was initiated in the fascia dentata.

In one case, an interneuron (int) was impaled at the border of the granule layer, as confirmed by morphological reconstruction (Fig. 3D). This cell did not show the typical GDPs consisting of large depolarizations crowned by fast spikes. Instead, bursts of action potentials (3.3 ± 1.8 spikes, data from eight bursts) were recorded in synchrony with GDPs in CA3 (Fig. 3D, right). Similar results have been reported previously in a cell-attached configuration, confirming the implication of a synchronized action by GABAergic interneurons in the generation of GDPs (Khazipov *et al.*, 1997).

GDPs are present in isolated CA3, CA1 and fascia dentata areas

The above data show that the entire hippocampus bursts synchronously during early postnatal development, but do not allow us to conclude that a given area plays the role of pacemaker, as far as bursting is not initiated systematically in any of them. The existence of a region working as a pacemaker would imply that other areas when isolated would lack bursting capability. To check this hypothesis, we performed a series of experiments recording the spontaneous activity from neurons in isolated areas of the hippocampus. Twenty-three recordings were performed: nine from isolated CA3, 10 from CA1 and five from GCs in the fascia dentata.

GDPs were present in every isolated region, as can be seen in Table 1 and Figure 4. Cells from isolated CA3 showed the higher frequency of spontaneous GDPs when compared with the other isolated regions, and with the CA3 and CA1 frequency in control (intact slices). The GDPs recorded in isolated areas had the same

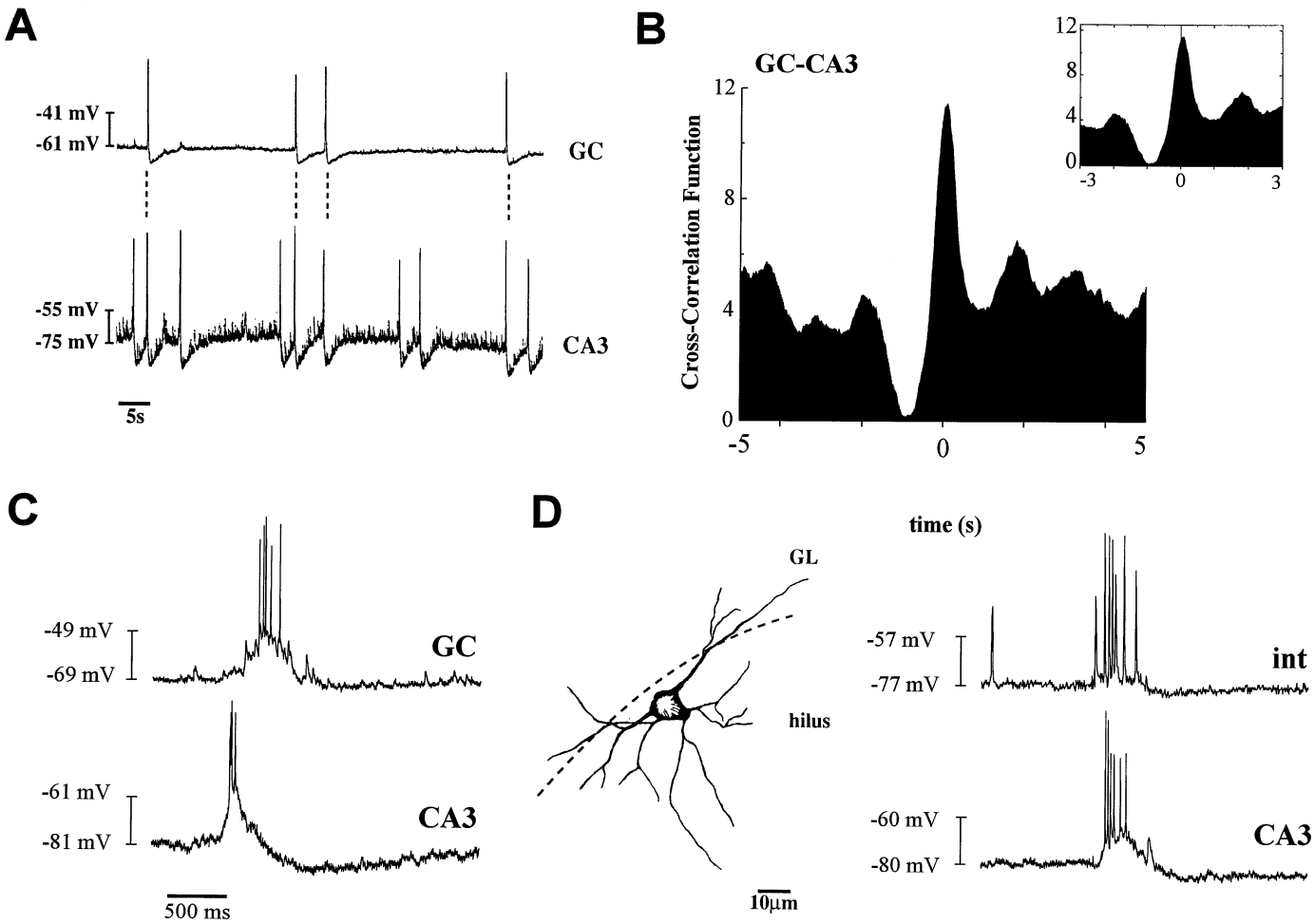

FIG. 3. Simultaneous recordings in CA3 and the fascia dentata. (A) Dual intracellular recordings from GC and CA3 pyramidal neuron (P4) show that GDPs occur at different frequencies (10 GDPs are present in CA3 cell while only four in GC). Input resistance: 40 MΩ (GC) and 120 MΩ (CA3). (B) The cross-correlation function clearly shows a central peak. Here, GC is taken as the reference neuron. (C) In 61% of cases, GDPs are fired first in CA3. Input resistance: 50 MΩ (GC) and 100 MΩ (CA3). (D) In one case, an interneuron (int) was impaled at the border of the granule layer as concluded from its morphological reconstruction. Camara Lucida drawing is shown on the left. This interneuron shows bursts of action potentials in correlation with GDPs recorded in a CA3 neuron. Input resistance is 40 MΩ from the fascia dentata interneuron and 60 MΩ from the CA3 cell. The granule layer (GL) and hilus are indicated.

electrical properties as those recorded in the control (Fig. 4A), i.e. duration, amplitude and reversal potential (31 ± 12 mV, $n = 4$), and they were blocked by bicuculline (10 µM, data not shown). Simultaneous intracellular recordings in isolated CA3 ($n = 2$) corroborated the high degree of synchrony of these events (data not shown).

To investigate whether the emergence of GDPs depends on the number of neurons present in the specific neuronal network (Smith *et al.*, 1995), we analysed the dependence of their frequency against the size of the CA3-isolated minislices. The minislice was obtained by means of two cuts perpendicular to the CA3 body layer. The size is therefore a measure of the length of the stratus pyramidale. We found a large variability in the frequency of GDPs from minislices of different sizes. The values are not significantly different from slices greater than 1500 µm (mean: 1800 µm; $n = 9$), when compared with 600 µm ($n = 4$) and 300 µm ($n = 5$) minislices (Fig. 4C). This suggests that GDPs are generated by local circuits which are still present in the smaller sized slices (Miles, 1990; Fortunato *et al.*, 1996).

Discussion

The results of the present work can be summarized as follows: (i) GDPs are present in GCs of the fascia dentata, and pyramidal cells of the CA3 and CA1 areas of immature hippocampus. (ii) GDPs are highly correlated among the different regions of intact hippocampal slices. This correlation allows us to state that GDPs appear synchronously, and strongly suggests that the hippocampus bursts as a whole during early postnatal development. Such synchrony could be the consequence of a given area acting as pacemaker. The answer to this possibility can be checked by analysing the delays in GDP onset, since the hypothetical pacemaker region would burst earlier. (iii) Cross-correlation analysis applied to dual records from CA3–CA1 and GC–CA3 cells verifies the presence of delays between different hippocampal areas in the onset of GDPs and a prevalent firing direction from CA3 to CA1. However, there is a significant population of discharges fired first in CA1 (20%). In the case of GC–CA3 records, there is no regular sequence of GDPs production. All these data suggest that one region triggers the synchronous bursting,

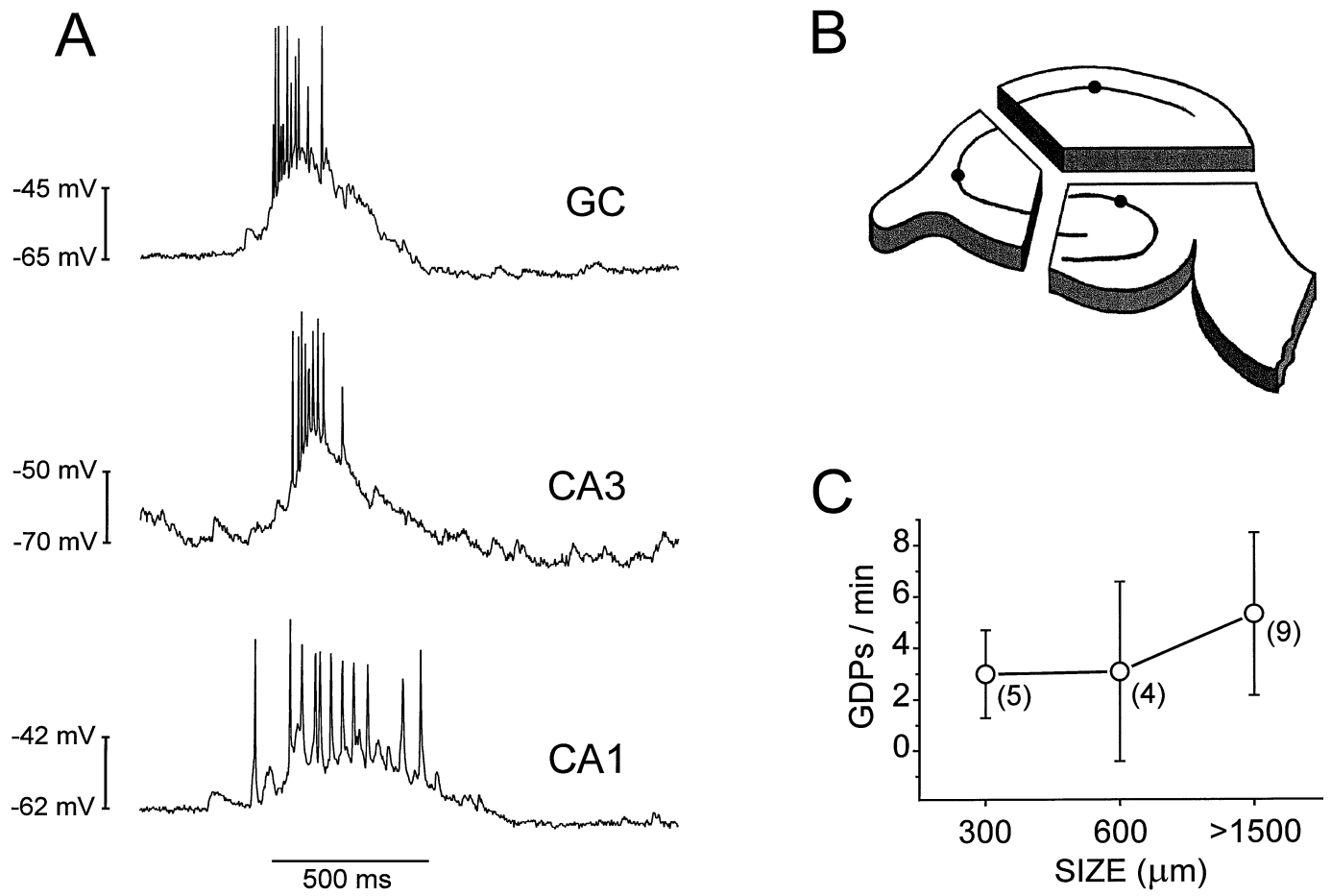

FIG. 4. GDPs recorded in isolated slices from CA3, CA1 and fascia dentata areas. (A) GDPs similar to those recorded in the control are also present in isolated subfields of CA3, CA1 and the fascia dentata. (B) Schematic representation of the minislices from isolated regions. Dots mark where cells were impaled. (C) Relationship between the GDP frequency and the CA3 minislice size. A large variability was detected. Smaller minislices (300 μm) also showed spontaneous GDPs at a frequency not significantly different from those recorded under control conditions (intact slices). Input resistance is 80 MΩ from GC (P3); 120 MΩ from CA3 cell (P2) and 40 MΩ from CA1 cell (P4).

but not as a fixed pacemaker as there is not a systematic preceding region. An intrinsic capability of different regions to initiate GDPs requires that every area could be able to generate them in isolation. (iv) GDPs are in fact present in isolated minislices from CA3, CA1 and the fascia dentata, as well as in CA3 minislices of about 300 μm in size. Interestingly, the frequency of GDPs in GCs and CA1 neurons is not significantly different when recorded from isolated or intact slices, while the frequency from isolated CA3 is significantly higher than in the control. (v) Our results allow us to estimate the speed at which GDPs spread, i.e. from CA3 to CA1. Assuming an average distance between recorded cells of about 500 μm and a delay of about 200 ms, then GDPs spread at a speed of 0.025 m/s.

To date, the mechanisms of GDPs generation are under discussion: a hilar origin has been proposed (Strata *et al*., 1997), while these events were recorded from isolated CA3 area (Ben-Ari *et al*., 1989; Khazipov *et al*., 1997). Our data suggest that each hippocampal area is able to trigger the onset of GDPs. A substantial amount of evidence supports the $GABA_A$ and glutamatergic nature of these events (Ben-Ari *et al*., 1989; Strata *et al*., 1995). The synchronous activation of GABAergic interneurons is determined by their polysynaptic cooperative action with the pyramidal cells involving excitatory GABA and glutamatergic connections (Corradetti *et al*., 1988; McLean *et al*., 1995; Khazipov *et al*., 1997). These elements are present in all hippocampal subfields which, as confirmed by the present work, are able to generate GDPs. Nevertheless, the greater frequency recorded in isolated CA3, when compared with isolated CA1 and fascia dentata, suggests the existence of differences among these areas.

The CA3 area is specially known as a highly recurrently network (Li *et al*., 1997), even at early stages of development in comparison to CA1 and the fascia dentata (Seress *et al*., 1989). Electrophysiological and morphological evidence shows that CA3 pyramidal cells and interneurons are extensively connected (Buhl *et al*., 1994; Cobb *et al*., 1995). More than 1000 CA3 pyramidal cells may converge onto one inhibitory neuron providing optimal mechanisms to control the electrical activity within the network (Miles, 1990; Gulyas *et al*., 1993). The higher frequency of spontaneous GDPs recorded from isolated CA3 may reflect such a capability in contrast with CA1 and the fascia dentata. Nevertheless, despite the absence of recurrent pathways, the CA1 region shows specific local synaptic contacts between pyramidal cells and interneurons from the oriens/alveus (o/a) and the lacunosum/moleculare (l/m) layers (Lacaille *et al*., 1987; Lacaille & Schwartzkroin, 1988; Sik *et al*., 1995). In fact, it has been shown that single GABAergic interneurons can synchronize the firing of large neuronal populations in the CA1 area (Cobb *et al*., 1995). Recordings in cell-attached configuration from o/a and l/m

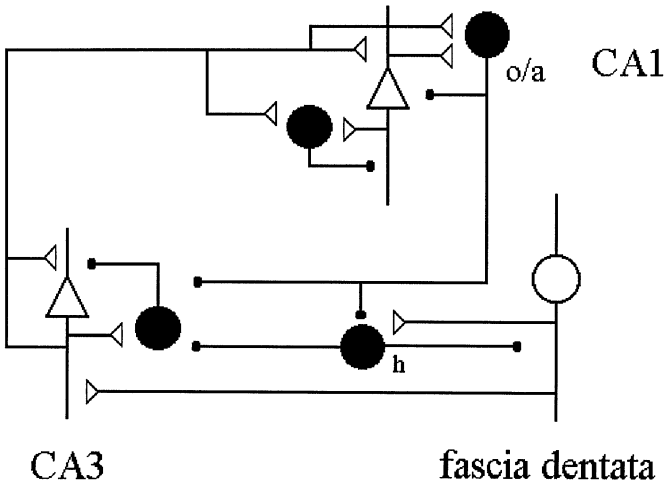

FIG. 5. A schematic diagram of the circuits hypothetically implicated in GDPs generation. Each hippocampal region (fascia dentata, CA3 and CA1) contains the basic GABA and glutamatergic mechanisms required to generate GDPs, as supported by the results in isolated slices. In intact slices, the typical connectivity direction is from fascia dentata to CA1. This explains that in general GDPs are first fired in the CA3 region, since the fascia dentata shows lower frequency values (see Table 1). In certain cases, however, GDPs are initiated in CA1. We propose that they are propagated to the fascia dentata and CA3 through the GABAergic feedback loop involving interneurons from the oriens/alveus layer. Pyramidal excitatory cells and synaptic contacts are represented as open triangles. GABAergic interneurons and synapses are represented as black circles. The granule cells are represented as open circles. (o/a), oriens/alveus, (h) hilar interneurons.

interneurons in intact slices confirm that they fire bursts of action potentials in synchrony with GDPs recorded from pyramidal cells (Khazipov *et al*., 1997). This suggests that the isolated CA1 area contains elements required to sustain GDP oscillation. Our results demonstrated that CA1 is also able to generate GDPs with a frequency not significantly different from that recorded in intact slices.

Although GDPs are more frequently observed first in CA3, the 20% of cases in which CA1 pyramidal cells preceded CA3 in firing seems surprising considering the classical view of hippocampal excitatory connections. Recently, Sik and colleagues have provided evidence for an inhibitory feedback pathway from CA1 to CA3 and hilar regions (Sik *et al*., 1994). This feedback pathway was found to be due to axon collaterals from a class of GABA immunoreactive o/a interneurons from the CA1 region, that spread widely into the stratum radiatum of the CA3 and hilar areas. The flow of information in the hippocampus is fundamentally unidirectional from the fascia dentata to CA1. This explains that in general, when the three regions are coupled (intact slices), the GDP firing is initiated at CA3 and in minor cases at the fascia dentata (as this isolated region shows the lower frequency values). We propose that the GABAergic feedback is mediating the transmission of synchronized output from local circuits in CA1 to both CA3 pyramidal neurons and GCs (Fig. 5). By means of this circuit, the GDPs autonomously generated at CA1 can be backward propagated contributing to the synchronized firing. In Figure 5, we summarize the present discussion presenting a simplified model of the GDPs generation.

The capability of immature hippocampal networks to fire GDPs in a synchronized fashion seems to have important physiological consequences. Experimental evidence supports the view that $GABA_A$ receptor activation causes an increase in intracellular calcium concentration in interneurons and pyramidal cells from immature hippocampal slices (Leinekugel *et al*., 1995). The role of calcium in coding different aspects of neuronal development has been widely documented (Kater *et al*., 1988; Gu & Spitzer, 1995). On the other hand, GABA and glutamate mechanisms are found to also be involved in structural changes and certain trophic activities (Lipton & Kater, 1989; Barbin *et al*., 1993). The presence of the $GABA_A$-and glutamate-dependent spontaneous GDPs in the immature hippocampal circuit must therefore have an important effect on the normal hippocampal development.

From a computational point of view, bursting appears to have a critical role in neuronal information processing (see Lisman, 1997 for review). We have recently demonstrated that these bursts (or GDPs) emerging from undeveloped hippocampal networks, are the events that carry the information, while single spikes are noise (Menendez de la Prida *et al*., 1997; Stollenwerk *et al*., 1997). Although there is evidence supporting a neurophysiological role for neural noise (Arieli *et al*., 1996; Ferster, 1996), in this case the capability of the developing hippocampus to sustain highly correlated discharges is a more optimal mechanism for computational purposes. The role of synchronized spontaneous activity in configuring the adult connectivity pattern has been reported (Katz & Shatz, 1996; Mooney *et al*., 1996). Here we have shown that GDPs are synchronously recorded in cells from the fascia dentata, CA3 and CA1. Not all of these neurons are connected, but those which are synaptically coupled will also be firing in a synchronous fashion. Such a synchrony between pre- and postsynaptic neurons could result in the strengthening of their synaptic contacts by means of Hebbian mechanisms. This possible role of GDPs in the consolidation of connectivity patterns during hippocampal development seems to be an extremely suggestive field for further investigations.

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Abbreviations

FD	fascia dentata
GCs	granule cells
GDPs	giant depolarizing potentials
GL	granule layer
h	hilus
int	interneuron
LM	Lacunosum Moleculare
o/a	oriens/alveus

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