Journal of Physiology (1989), **416**, *pp.* 303–325
With 13 *text-figures*
Printed in Great Britain

GIANT SYNAPTIC POTENTIALS IN IMMATURE RAT CA3 HIPPOCAMPAL NEURONES

BY YEHEZKEL BEN-ARI, ENRICO CHERUBINI, RENATO CORRADETTI AND JEAN-LUC GAIARSA

From the Unité 29, INSERM, Hôpital de Port-Royal, 123 Boulevard de Port-Royal, 75014 Paris, France

(*Received 13 December 1988*)

SUMMARY

1. Intracellular recordings were made from rat CA3 hippocampal neurones *in vitro* during the first eighteen days of postnatal life. The cells had resting membrane potentials more negative than −51 mV, action potentials greater than 55 mV and membrane input resistances of 117 ± 12 MΩ. An unusual characteristic of these cells was the presence of spontaneous giant depolarizing potentials (GDPs) which were observed during the first eight postnatal (P) days in over 85% of neurones. They were less frequent between P9 and P12 (48%) and disappeared after P12.

2. The GDPs were synchronously generated by a population of neurones; they reversed polarity at −27 mV when recorded with KCl-containing electrodes and at −51 mV with potassium acetate- or potassium methylsulphate-filled electrodes.

3. The GDPs were blocked by bath application of bicuculline (10 μM) or picrotoxin (100–200 μM). Exogenously applied γ-aminobutyric acid (GABA; 0·2–1 mM) induced at resting membrane potential a bicuculline-sensitive membrane depolarization which reversed polarity at −25 and −51 mV when recorded with KCl- or potassium methylsulphate-filled electrodes respectively.

4. The GDPs were reduced in frequency or blocked by the *N*-methyl-D-aspartate (NMDA) receptor antagonists DL-2-amino-7-phosphonoheptanoate (AP-7; 50 μM), D(−)2-amino-5-phosphonovalerate (AP-5, 10–50 μM) and (±)3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 10–50 μM) or NMDA channel blockers phencyclidine (2 μM) and ketamine (20 μM).

5. Stimulation of the hilus during the first week of life evoked a GDP followed by a hyperpolarization. The GDPs were generated by a population of synchronized neurones and reversed polarity at −27 mV with KCl-filled electrodes and at −52 mV with potassium acetate- or potassium methylsulphate-containing electrodes.

6. Bath application of bicuculline (1–10 μM) or picrotoxin (100–200 μM) reversibly blocked the evoked GDPs in the majority of cells. The NMDA receptor antagonists AP-5 (50 μM), AP-7 (50 μM) and CPP (30 μM) usually reduced the amplitude and the duration of the evoked GDPs. In neurones in which evoked GDPs were blocked by bicuculline, a NMDA-mediated component was revealed by increasing the strength or the frequency of stimulation.

7. During the second week of postnatal life, when spontaneous GDPs were extremely rare or absent, superfusion with bicuculline (10 μM) induced, as in adult

slices, interictal discharges. These reversed polarity near 0 mV with KCl- or potassium acetate-containing electrodes and were reduced in amplitude and duration by AP-5 (50 μM).

8. During the second week of life, stimulation of the hilus evoked, as in adult CA3 cells, an excitatory postsynaptic potential (EPSP) followed by a fast and a slow inhibitory postsynaptic potential (IPSP). Exogenously applied GABA induced at resting membrane potential a hyperpolarization which reversed polarity at -66 mV with potassium methylsulphate-containing electrodes.

9. It is concluded that, in early postnatal life, hippocampal CA3 neurones display spontaneous and evoked GDPs which are mediated by GABA and presynaptically controlled by NMDA receptors.

INTRODUCTION

In adult CA3 hippocampal neurones, stimulation of the mossy fibres evokes an excitatory postsynaptic potential (EPSP), followed by a fast and a slow inhibitory postsynaptic potential (fast and slow IPSP). The fast IPSP is mediated by γ-aminobutyric acid (GABA) acting on bicuculline-sensitive $GABA_A$ receptors (Kandel, Spencer & Brinley, 1961; Ben-Ari, Krnjević, Reiffenstein & Reinhardt, 1981), whereas the slow IPSP is due to GABA acting on bicuculline-insensitive $GABA_B$ receptors (Kehl & McLennan, 1983; Alger, 1984; Newberry & Nicoll, 1985). The mossy fibre EPSP is mediated by an excitatory amino acid acting on non-*N*-methyl-D-aspartate (NMDA) receptors. In fact, it is not blocked by selective NMDA antagonists (Yamamoto, Sawada & Takada, 1983; Cotman, Flatman, Ganong & Perkins, 1986), but it is abolished by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) which preferentially acts on the quisqualate/kainate subtype of glutamate receptors (Drejer & Honoré, 1988; Neuman, Ben-Ari, Gho & Cherubini, 1988*a*). However, it is possible that, in analogy to the EPSP evoked in CA1 neurones by stimulation of the stratum radiatum, the mossy fibre EPSP includes a NMDA-mediated component blocked by Mg^{2+} near resting membrane potential. In fact, the EPSP evoked in CA1 cells by the stimulation of the stratum radiatum includes a NMDA component which can be revealed only when the powerful, voltage-dependent Mg^{2+} block of NMDA receptor-gated channels is removed (Coan & Collingridge, 1985; Dingledine, Hynes & King, 1986; Collingridge, Herron & Lester, 1988*a*, *b*; Neuman, Cherubini & Ben-Ari, 1988*c*).

In adult animals the CA3 region is well known for its pacemaker activity (Hablitz & Johnston, 1981) and its ability to generate depolarizing bursts following the application of a variety of convulsants (Neuman, Cherubini & Ben-Ari, 1988*b*). This property is due to the presence of recurrent excitatory connections between pyramidal neurones (Miles & Wong, 1986) which facilitate the initiation of a synchronized interictal-type of discharge in a population of CA3 neurones.

In immature animals, studies of the CA1 region *in vitro* have shown that the predominant form of synaptic activity is excitation and that IPSPs appear relatively later in development (Dunwiddie, 1981; Schwartzkroin, 1982; Harris & Teyler, 1983). A study of the immature rabbit hippocampus *in vitro* (Mueller, Taube &

Schwartzkroin, 1984) indicates that the depolarizing potential produced in CA1 neurones by stimulation of the stratum radiatum is partially mediated by GABA, which is depolarizing at this stage of development. In contrast, the physiological and pharmacological features of synaptic transmission in CA3 cells during development have not been investigated in detail.

In view of the high incidence of seizure disorders in the immature brain (Aicardi & Chevrie, 1970) we examined the properties of synaptic transmission in immature CA3 hippocampal neurones. A novel phenomenon was the observation of spontaneous and evoked giant depolarizing synaptic potentials during the first week of postnatal life. These giant potentials were unexpectedly found to be mediated by GABA and presynaptically controlled by NMDA receptors.

Part of this study has been reported in preliminary form (Ben-Ari, Corradetti & Gaiarsa, 1988*b*; Corradetti, Gaiarsa & Ben-Ari, 1988).

METHODS

Experiments were performed on CA3 hippocampal neurones obtained from 0- to 18-day-old Wistar rats of both sexes (P0–P18 days; 0 taken as the day of birth). Rats were anaesthetized with ether and decapitated. The brain was removed and submerged in artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl, 126; KCl, 3·5; $CaCl_2$, 2; $MgCl_2$, 1·3; NaH_2PO_4, 1·2; $NaHCO_3$, 25 and glucose, 11 (pH 7·3), gassed with 95% O_2 and 5% CO_2. Slices approximately 600 μm thick were cut using a McIlwain tissue chopper and incubated at room temperature in ACSF for at least 1 h before use. Individual slices were transferred to a submerged-type chamber and superfused with ACSF at 2·5–3·0 ml min^{-1} at 34 °C.

Extracellular field potentials were recorded with 2 M-NaCl-filled microelectrodes (resistance of 1–5 MΩ) positioned in stratum radiatum or in the vicinity of the pyramid cell layer. Intracellular recordings were made with 3 M-KCl-, 4 M-potassium acetate- or 2 M-potassium methylsulphate-filled microelectrodes (resistance of 60–150 MΩ). In four experiments, CA3 neurones were impaled with microelectrodes containing 2(triethylamino)-*N*-(2,6-dimethylphenyl) acetamide (QX-314; 50 mM, dissolved in 2 M-KCl; resistance 60–80 MΩ) in order to block fast Na^+ channels and to measure reliably the reversal of postsynaptic potentials. Current was passed through the recording electrode by means of an Axoclamp 2 amplifier. Bridge balance was checked repeatedly during the experiment and capacitative transients (with the electrode tip outside the neurone) were reduced to a minimum by negative capacity compensation. Membrane potential was estimated from the potential observed upon withdrawal of the electrode from the cell. For voltage-clamp studies, neurones were clamped using a single-electrode voltage-clamp amplifier (Axoclamp 2, Axon Instruments, Inc.). The sampling frequency was 3–4 kHz, 30% duty cycle. To ensure correct operation of the clamp, the voltage at the head stage amplifier was monitored on a separate oscilloscope. Stimulating electrodes (twisted bipolar NiCr insulated wires, 50 μm o.d. or bipolar etched tungsten electrodes) were positioned in the hilar zone. Stimulation parameters were 50–100 μs duration, 5–50 V intensity; unless otherwise stated the frequency was 0·05 Hz. Although the granule cells develop mainly postnatally, at birth a well-defined Timm-stained mossy fibre zone is already present (Gaarskjaer, 1986). At least some axons originating from the granule cells were activated by the stimulating electrode. We cannot rule out the possibility that the stimulus may have affected other neurones (e.g. GABAergic) which are present in the hilar region at an early postnatal period (F. Rosenberg, O. Robain, L. Jardin & Y. Ben-ari, unpublished observations).

Signals were digitized and displayed on a digital oscilloscope and on a computer-driven chart recorder.

Drugs were dissolved in ACSF and superfused via a three-way tap system. Steady-state conditions were achieved in 3 min. Drugs used were: bicuculline (Sigma), picrotoxin (Sigma), GABA (Sigma), isoguvacine (Sigma), DL-2-amino-7-phosphonoheptanoate (AP-7, gift of Dr P. Herrling, Sandoz), D(−)2-amino-5-phosphonovalerate (AP-5, CRB or Tocris), (±)3-(2-carboxy-

piperazin-4-yl)-propyl-1-phosphonic acid (CPP, CRB), *N*-methyl-D-aspartate (NMDA, CRB), phencyclidine (gift of Dr M. Lazdunski), ketamine (Parke–Davis), QX-314 (Astra Pharmaceutics), tetrodoxin (TTX, Sigma), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, gift of Dr T. Honoré, Ferrosan), kynurenic acid (Sigma).

RESULTS

The present study is based on long-lasting (2–6 h) intracellular recordings from 309 CA3 pyramidal neurones (P0–P18). The resting membrane potential of the cells at P0–P8 was -58 ± 7 mV ($n = 37$) and -67 ± 7 mV ($n = 50$) when recorded with KCl or potassium methylsulphate electrodes respectively. There is a significant difference between these values ($P < 0{\cdot}0005$, unpaired t test). In contrast, at P9–P18 the resting membrane potential was not significantly different when recorded with KCl electrodes (-64 ± 11 mV, $n = 10$) or potassium methylsulphate (-69 ± 10 mV, $n = 9$). When compared to adult neurones (Ben-Ari & Gho, 1988; Cherubini, Neuman, Rovira & Ben-Ari, 1988; Neuman *et al.* 1988*b*, *c*) and in agreement with other developmental studies (Mueller *et al.* 1984; Kriegstein, Suppes & Prince, 1987), immature cells had a higher input resistance (117 ± 12 MΩ; $n = 38$), longer time constant (25 ± 2 ms; $n = 14$) and action potentials greater than 55 mV with slower rising and falling phases.

Spontaneous giant depolarizing potentials (GDPs) are network driven events

During the first postnatal week the spontaneous activity of CA3 neurones consisted of large synaptic noise which occasionally triggered spike discharges. In contrast to adult neurones (Ben-Ari & Gho, 1988; Cherubini *et al.* 1988; Neuman *et al.* 1988*b*, *c*) over 85% of neurones at this developmental stage exhibited spontaneous giant depolarizing potentials (GDPs). The GDPs consisted of a large depolarization having an amplitude of 25–50 mV (when the recording electrode was filled with 3 M-KCl) and with superimposed fast action potentials (Fig. 1*D*). Smaller amplitude (15–30 mV) GDPs were found when the recording electrode was filled with 4 M-potassium acetate or 2 M-potassium methylsulphate. The GDPs lasted from 300 to 500 ms and were followed by an after-hyperpolarization. The peak of GDPs was associated with a large decrease in membrane input resistance (Fig. 1*E*). The frequency of GDPs varied from cell to cell (0·005–0·2 Hz) but was quite regular for a given neurone (Fig. 1*A* and *B*).

As shown in Fig. 1*C*, during development there was a dramatic change in the percentage of neurones showing GDPs. From P0 up to P8 most neurones (85–93%, $n = 255$) displayed GDPs whereas at P11–12 only 25% ($n = 8$) of the neurones showed these phenomena. GDPs were not observed after P12.

The following observations suggest that GDPs were network driven synaptic potentials (Johnston & Brown, 1984): (i) they were synchronous with intracellular and extracellular recordings (Fig. 1*D*); (ii) they were synchronous in pairs of CA3 neurones recorded simultaneously (not shown); (iii) their frequency was independent of membrane potential (Fig. 1*A*); (iv) they were abolished by TTX (1 μM) which blocks fast Na^+ currents and spike propagation (Fig. 1*B*); (v) they were blocked by superfusion with Co^{2+} (2 mM) or with a solution containing high concentrations of divalent cations (6 mM-Mg^{2+} and 4 mM-Ca^{2+}, known to block preferentially poly-

synaptic activity; Berry & Pentreath, 1976); and (vi) they could not be triggered by short (10–50 ms) depolarizing current pulses injected through the recording electrode.

The amplitude of GDPs increased with membrane hyperpolarization and decreased with membrane depolarization. An *actual* reversal potential was measured in twenty-

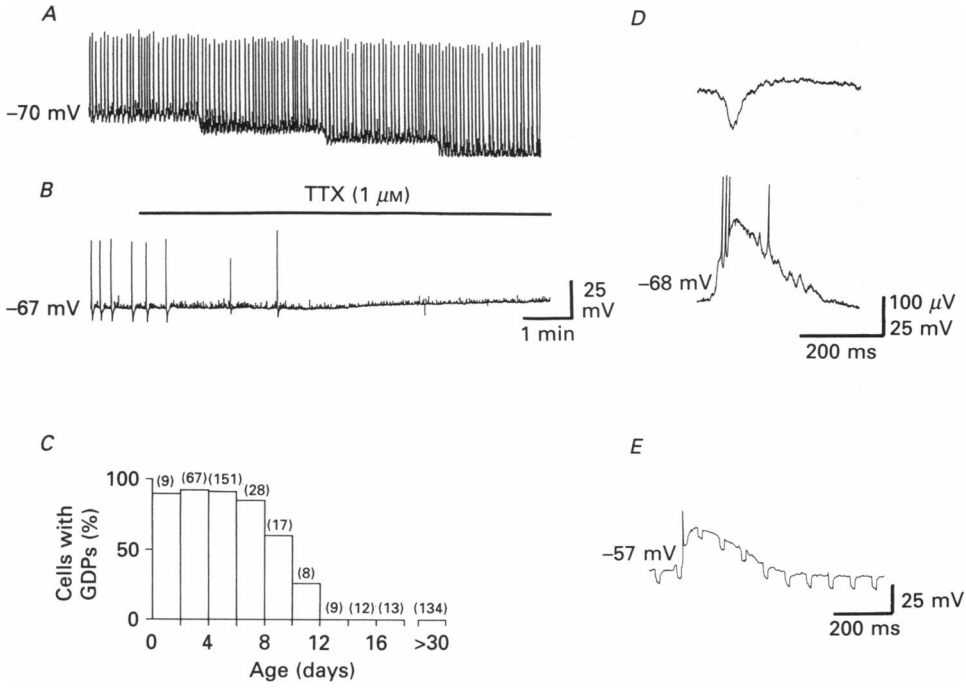

Fig. 1. Spontaneous GDPs are generated in a polysynaptic circuit. *A*, the frequency of GDPs is independent of the membrane potential. The membrane was hyperpolarized by injecting a steady current through the recording electrode. *B*, the spontaneous GDPs are blocked by TTX. *C*, histograms of the number of cells displaying spontaneous GDPs at various postnatal days. The number of neurones recorded at each period is indicated in parentheses. The histogram includes the spontaneous hyperpolarizing potentials which occur between P6 and P8. Adult neurones are from Cherubini *et al.* (1988) and Neuman *et al.* (1988*b*, *c*). *D*, concomitant extracellular (upper trace) and intracellular (lower trace) recordings of a GDP; cell different from *A*. *E*, the spontaneous GDP is associated with an input conductance increase. The downward deflections are electrotonic potentials resulting from injection of constant current pulses (−200 pA) through the recording electrode. Cells shown in *A*, *B*, *D* and *E* were recorded with KCl-containing electrodes. *A*, *D* and *E*, 6-day-old rat; *B*, 4-day-old rat.

two cells (Fig. 2). With electrodes containing potassium methylsulphate or potassium acetate the reversal potential was -51 ± 3 mV ($n = 10$), whereas with KCl-filled electrodes spontaneous GDPs reversed polarity at -27 ± 3 mV ($n = 12$). In the same neurone the reversal potential was always positive to the resting membrane potential by $15{\cdot}6 \pm 2{\cdot}5$ mV with potassium methylsulphate- or potassium acetate- and by 32 ± 3 mV with KCl-filled electrodes. The currents underlying the GDPs were measured in four neurones recorded with KCl-containing microelectrodes (Fig. 2*D*). The amplitude of the inward current was linearly related to the membrane potential:

the current was inward at resting membrane potential, became larger with membrane hyperpolarization, smaller with membrane depolarization and reversed at -22 ± 2 mV.

GDPs are mediated by GABA

The dependence of the reversal potential on the presence of chloride in the microelectrode suggested that GDPs were mediated by a Cl^- conductance. Since in

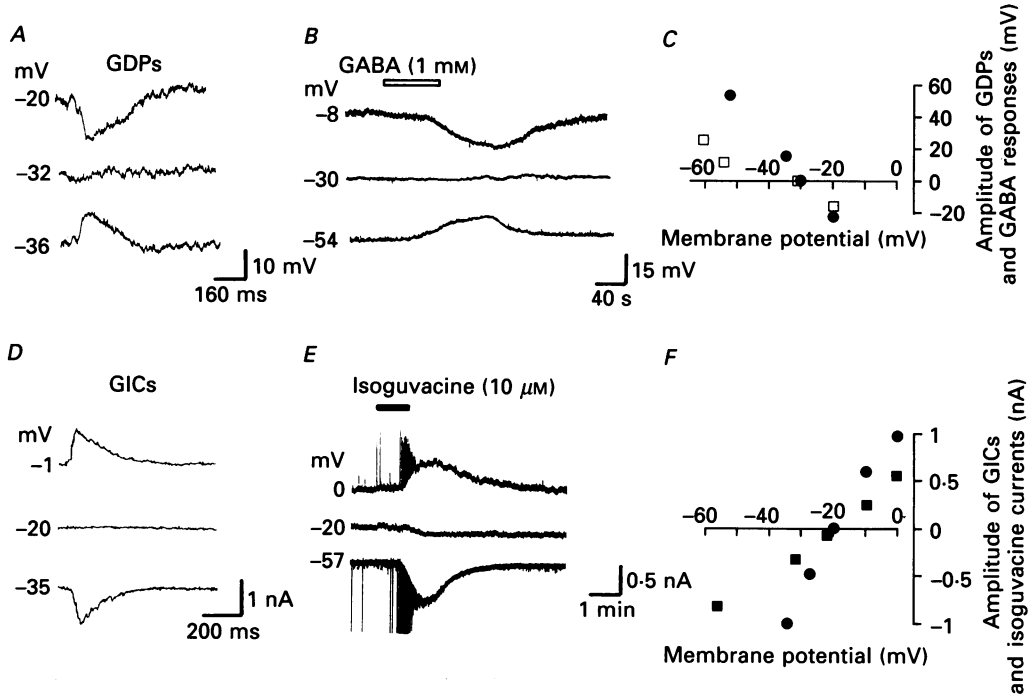

Fig. 2. The GDPs reverse polarity at the same potential as the responses to exogenously applied GABA and isoguvacine. In a 4-day-old rat spontaneous GDPs (*A*) and the response to exogenously applied GABA (*B*, open bar), in the presence of TTX change polarity at the same membrane potential. *C*, the amplitude of the responses shown in *A* (●) and *B* (□) is plotted against the membrane potential. Both responses reverse at −30 mV. *D*, 2-day-old rat: the reversal of spontaneous inward currents underlying GDPs (GICs) in a voltage-clamped neurone is compared to the reversal of the current produced by bath application of isoguvacine (*E*, filled bar). The fast upward and downward deflections are rapid spontaneous currents. Note that they reverse at the same potential as the isoguvacine current. *F*, the amplitude of the spontaneous GICs (●) and the current induced by isoguvacine (■) reverse at the same potential (−20 mV). All recordings were obtained with KCl-containing electrodes.

the hippocampus GABA is known to be an important neurotransmitter acting *via* Cl^- channels, we studied the contribution of GABA to GDPs and tested the effects of selective $GABA_A$ antagonists.

Until P5, the GABA blocker bicuculline (1–10 μM; $n = 18$) induced a small membrane hyperpolarization (4 ± 2 mV), markedly reduced the spontaneous synaptic noise and blocked GDPs within 1–3 min (Fig. 3). The effects of bicuculline, which persisted as long as the drug was applied, reversed completely after 10–30 min

wash-out. Similar results were obtained with picrotoxin (100–200 μM, $n = 4$; not shown).

In nine cells between P2 and P5 we compared the reversal potential of the response to GABA with the reversal potential of GDPs. At resting membrane potential, bath application of GABA (0·2–1 mM) depolarized the membrane and increased the

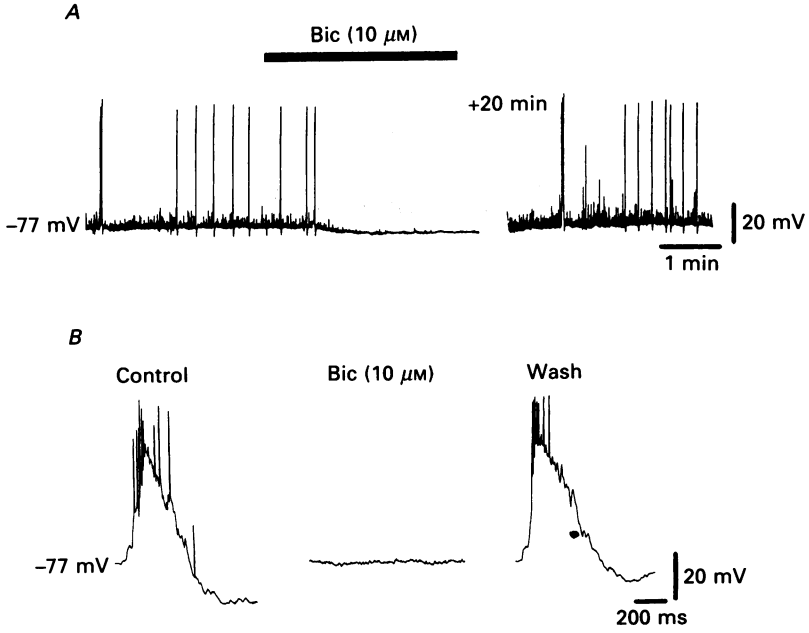

Fig. 3. Spontaneous GDPs are blocked by bicuculline. *A*, 4-day-old rat: continuous trace showing spontaneous GDPs. Bath application of bicuculline (Bic, filled bar) induced a membrane hyperpolarization, reduced the spontaneous synaptic noise and blocked GDPs. The effect of bicuculline washed out after 20 min. *B*, the three panels show faster records of the events indicated in *A*. KCl-containing electrodes.

frequency of GDPs (not shown). This effect rapidly reversed with wash-out. The responses to GABA increased with hyperpolarization and decreased with depolarization. The reversal potential of the responses to GABA, measured in nine cells, was -25 ± 3 mV ($n = 5$) with KCl electrodes and -51 ± 2 mV ($n = 4$) with potassium acetate- or potassium methylsulphate-containing microelectrodes (Fig. 2*B* and *C*; Fig. 12). These values were very close to those observed for the spontaneous synaptic potentials in the same cells (Fig. 2*A* and *C*). Furthermore, the $GABA_A$ agonist isoguvacine (10 μm) induced a depolarization at resting membrane potential in current-clamp experiments ($n = 2$) and an inward current in voltage-clamp experiments ($n = 3$). With KCl-filled electrodes the current became outward at -23 ± 3 mV, which is the same value found for the currents underlying the GDPs (Fig. 2*D–F*). Responses to both GABA and isoguvacine were blocked by bicuculline (10 μM; not shown).

GDPs are blocked by NMDA receptor antagonists

Recent studies performed on the visual cortex suggest the involvement of NMDA receptors in developmental plasticity (Kleinschmidt, Bean & Singer, 1987; Tsumoto, Hagihara, Sato & Hata, 1987). In the rat hippocampus a transient increase in the

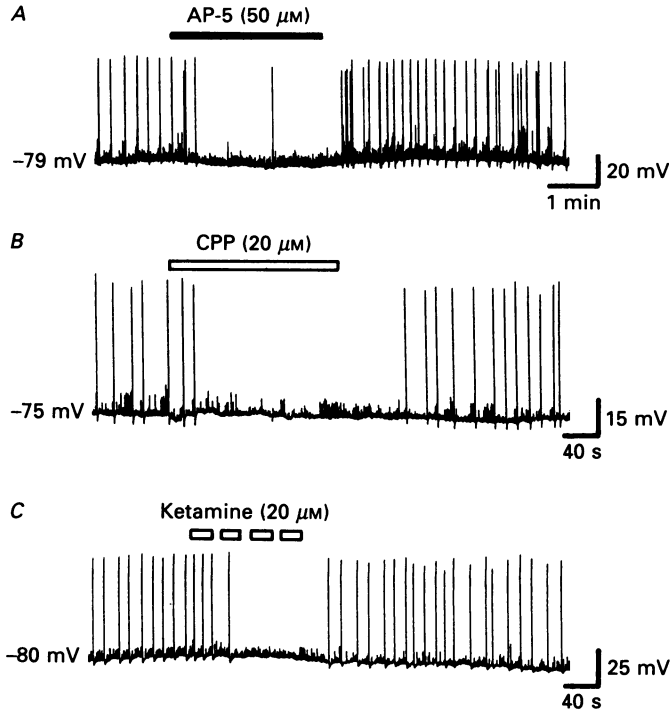

Fig. 4. Spontaneous GDPs are blocked by AP-5, CPP and ketamine. Bath application of AP-5 (*A*, filled bar), CPP (*B*, open bar) and ketamine (*C*, interrupted bar) blocked the GDPs. Note that these drugs did not change the synaptic noise. All recordings with KCl-containing electrodes. *A*, 5-day-old rat; *B*, 4-day-old rat; *C*, 6-day-old rat.

density of NMDA receptor binding sites (Tremblay, Roisin, Represa, Charriaut-Marlangue & Ben-Ari, 1988) strictly parallels the development of GDPs in early postnatal days. We therefore tested the effects of NMDA receptor antagonists and NMDA channel blockers on the spontaneous GDPs.

As shown in Fig. 4, bath application (3–5 min) of the selective NMDA receptor antagonists AP-5 (10–50 μM; $n = 38$), AP-7 (50 μM; $n = 9$) and CPP (10–50 μM; $n = 24$) reduced the frequency or blocked the GDPs in 80% of the cells without affecting the resting input resistance. A small hyperpolarization was often observed. These effects were dose dependent and washed out within a few minutes. We noted that when the antagonists were perfused for longer periods of time (> 5 min), after an initial block, the GDPs reappeared with a lower frequency than in control conditions. The NMDA channel blockers ketamine (20 μM; $n = 4$; Fig. 4*C*) or phencyclidine (1 μM; $n = 3$) also blocked GDPs. In contrast to bicuculline none of these drugs modified synaptic noise.

During the first week of life, as in adult neurones, NMDA (3–10 μM) induced a slow inward current which became outward near 0 mV. This current was selectively blocked by NMDA antagonists (Ben-Ari, Cherubini & Krnjević, 1988*a*). Furthermore, in two neurones, bicuculline (10 μM) and AP-5 (50 μM) were tested on

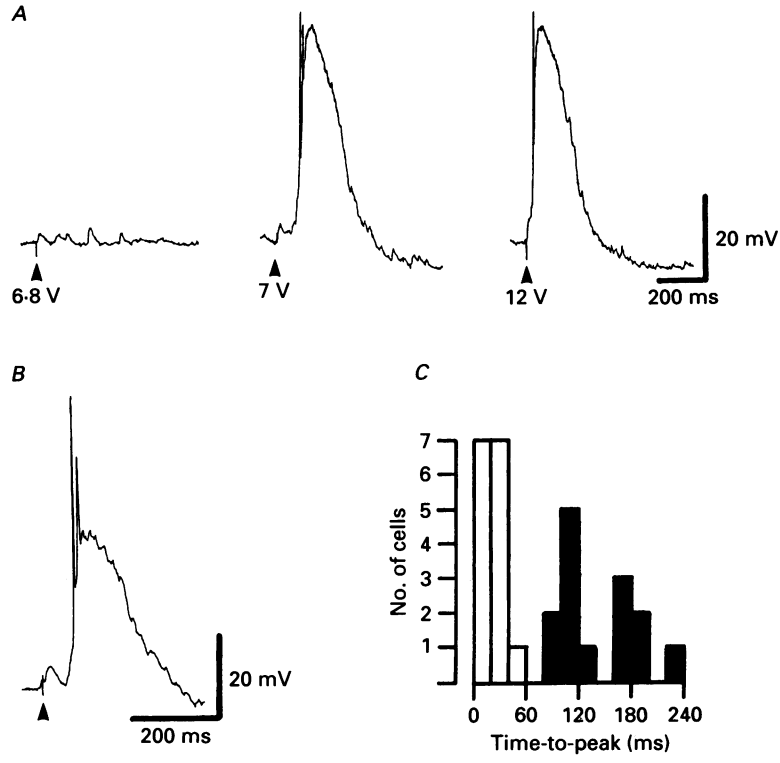

Fig. 5. Variable onset latency and all-or-none characteristics of the evoked GDPs. *A*, at resting membrane potential (−57 mV) the increase in stimulus intensity from 6·0 to 7 V or more, evoked (▲) a GDP in an all-or-none manner. Note the reduction in the latency of GDP onset produced by an increase in the stimulus strength. *B*, in a different neurone (−58 mV resting membrane potential) stimulation of the hilus evoked a small depolarizing potential followed by a GDP. *C*, the histograms show the range of latencies of the early small depolarizations (unfilled columns) and the subsequent GDPs (filled columns) in fifteen neurones. Latency was measured at the time interval between the stimulus artifact and the peak of the small depolarizations or GDPs.

both GABA- (250 μM) and NMDA- (5 μM) induced currents. As expected, bicuculline blocked the GABA response but had no effect on the NMDA current; conversely, AP-5 blocked the NMDA response without affecting the GABA current (not shown).

Hilar stimulation evokes GDPs

Electrical stimulation of the hilar region during the first days (P1–P5) of postnatal life evoked GDPs. The evoked GDPs were similar to spontaneous GDPs in terms of amplitude (25–50 mV with KCl- and 15–30 mV with potassium acetate-filled recording electrodes), duration (200–500 ms), and after-hyperpolarization. They

were associated with a decrease in membrane input resistance. Like the spontaneous GDPs, the evoked potentials were generated by a polysynaptic circuit since: (i) they were not graded and usually responded to hilar stimulation in an all-or-none manner (Fig. 5*A*); (ii) they were synchronous with extracellular and intracellular recordings;

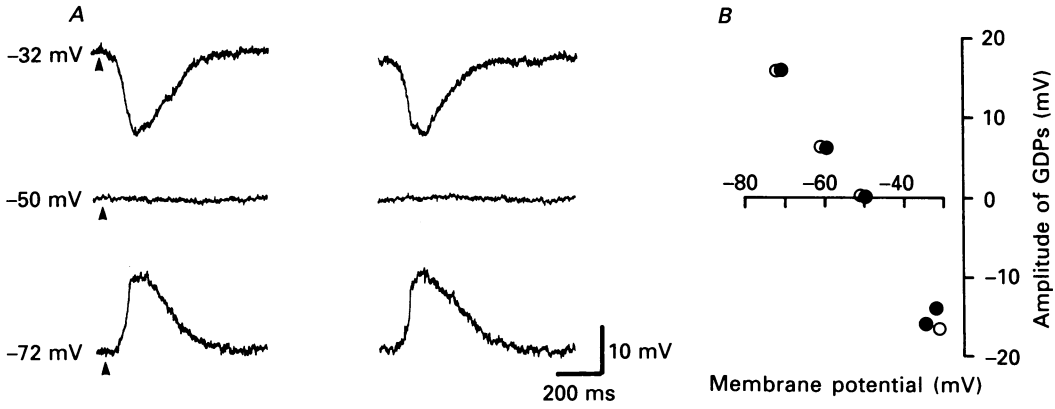

Fig. 6. Evoked and spontaneous GDPs reverse polarity at the same membrane potential. *A*, GDPs evoked by hilar stimulation (▲, left) and spontaneous GDPs (right) at three different membrane potentials in the same neurone; 6-day-old rat. *B*, the amplitude of spontaneous (●) and of evoked (○) GDPs shown in *A* are plotted against the membrane potential. Recordings made with potassium acetate-containing electrode.

(iii) they were reversibly abolished by TTX (1 μM), Co^{2+} (2 mM) or by a high divalent-cation-containing solution (4 mM-Ca^{2+} and 6 mM-Mg^{2+}). Evoked GDPs were often preceded by a small depolarizing potential (Fig. 5*B*) which frequently appeared only as a small inflexion on the initial phase of the GDP. In fifteen cells, where these two components were clearly separated, it was possible to obtain distinct time-to-peak values (taken from the stimulus artifact). The first one ranged between 10 and 60 ms, whereas the second ranged between 80 and 240 ms (Fig. 5*C*).

The reversal potential of the evoked GDPs was -27 ± 3 mV ($n = 11$) with KCl- and -52 ± 2 mV ($n = 13$) with potassium methylsulphate- or potassium acetate-containing microelectrodes; these values were very close to those obtained for the spontaneous GDPs (Fig. 6). Similar reversal values were obtained in neurones impaled with KCl–QX-314-containing microelectrodes (-33 ± 3 mV, $n = 4$; not shown). We did not succeed in reversing the small depolarizing potential preceding the GDPs.

Bicuculline blocks the evoked GDPs

Until P5, as in the case of spontaneous GDPs, bicuculline (10 μM) fully blocked the evoked GDPs in seventeen of twenty-one neurones (81%; Fig. 7*A*), suggesting that the evoked potentials were mediated by GABA acting on $GABA_A$ receptors. Bicuculline also blocked the small depolarizing potential which sometimes preceded the GDPs (Fig. 7*A*), whereas neither AP-5 (50 μM) nor CNQX (4 μM) blocked it. The action of bicuculline was rapid in onset (3–5 min) and completely reversed 20–30 min after wash-out. The blocking effect of bicuculline was not modified by shifting the membrane potential to more hyperpolarized levels. Similar results were obtained with picrotoxin (100–200 μM, $n = 3$; not shown).

In four neurones bicuculline (10 μM) did not completely block the evoked potentials but only reduced their amplitude.

Contribution of NMDA receptors to the evoked GDPs

Bath application of AP-5, AP-7 or CPP, at concentrations (30–50 μM) which fully block the effect of exogenously applied NMDA (5 μM, $n = 9$; see Ben-Ari *et al.* 1988*a*;

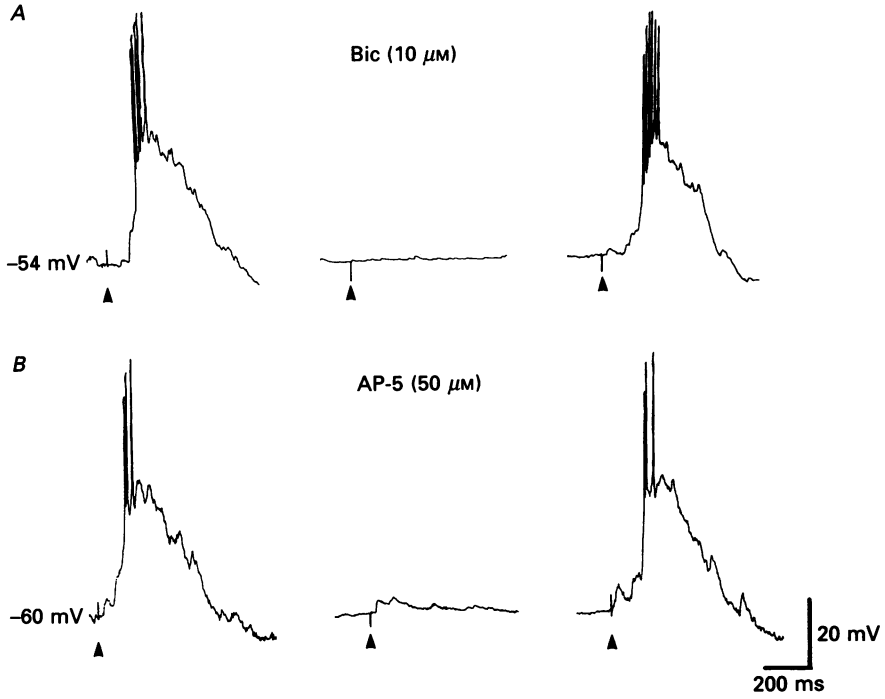

Fig. 7. Evoked GDPs are blocked by bicuculline and reduced by AP-5. *A*, GDPs evoked by hilar stimulation (▲) were blocked by bicuculline. *B*, in the same cell, superfusion of AP-5 greatly reduced the amplitude and duration of the GDPs in a reversible manner. KCl-containing electrode, 5-day-old rat.

King, Cherubini & Ben-Ari, 1989), reduced the amplitude and the duration of the evoked GDPs in twenty-three of twenty-seven neurones (85%; Fig. 7*B*). The effects of NMDA receptor antagonists were rapid in onset (3–5 min) and washed out within 5–10 min. Furthermore, in the four neurones in which bicuculline failed to block the evoked GDPs, NMDA receptor antagonists blocked the bicuculline-insensitive component. This suggests a contribution of NMDA-mediated events in the generation of evoked GDPs.

The possible role of NMDA receptors in the evoked GDPs was further examined following changes in the strength or in the frequency of stimulation of those neurones in which the evoked GDPs were fully blocked by bicuculline. After block of GDPs by bicuculline, increasing the strength of stimulation revealed a depolarizing potential followed by a hyperpolarization. Both the bicuculline-insensitive component and the following hyperpolarization were completely abolished by the NMDA receptor antagonist AP-5 (50 μM, $n = 4$; Fig. 8*A*). In the cell shown in Fig. 8*A* (recorded with a KCl-filled electrode) the reversal potential of the evoked postsynaptic response was

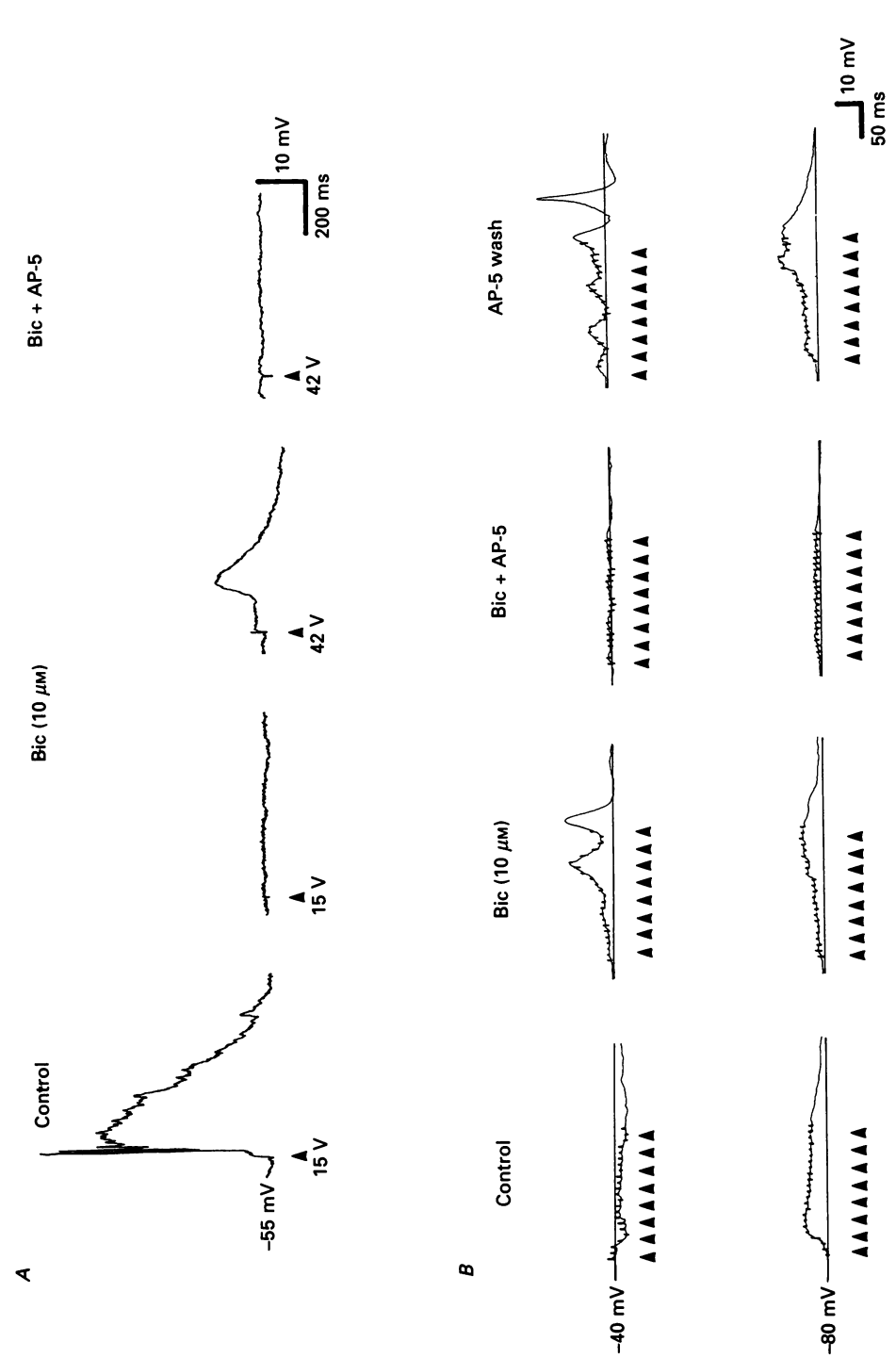

Fig. 8. Increase in strength or frequency of stimulation reveals an NMDA-mediated component of the evoked GDPs in the presence of bicuculline. *A*, evoked GDPs (▲) were blocked by bicuculline. The increase of stimulation strength revealed a depolarization followed by a hyperpolarization. Both responses were blocked by AP-5 (50 μM). KCl-containing electrode, 3-day-old rat. *B*, high-frequency trains of 13 V, 50 μs pulses at 100 Hz for 200 ms (multiple triangles) at −80 mV evoked a depolarizing response. At −40 mV the same train evoked a hyperpolarizing response. Bicuculline slightly depressed the initial part of the depolarizing potential at −80 mV and revealed a large, depolarizing, oscillating response at −40 mV. Both of these responses were blocked, in a reversible manner, by AP-5 (50 μM). Potassium methylsulphate-containing electrode, 3-day-old rat.

−18 mV before bicuculline application and shifted to 3 mV for the high threshold response obtained in the presence of bicuculline.

An AP-5-sensitive component of the evoked postsynaptic potential could also be revealed in the presence of bicuculline by a brief high-frequency train of stimuli. As shown in Fig. 8*B*, the high-frequency train (at −80 mV) induced a depolarizing potential which became hyperpolarizing at −40 mV. Bicuculline reduced the amplitude of the initial part of the depolarization at −80 mV, but revealed a depolarizing component at −40 mV. This component was blocked by AP-5 (50 μM).

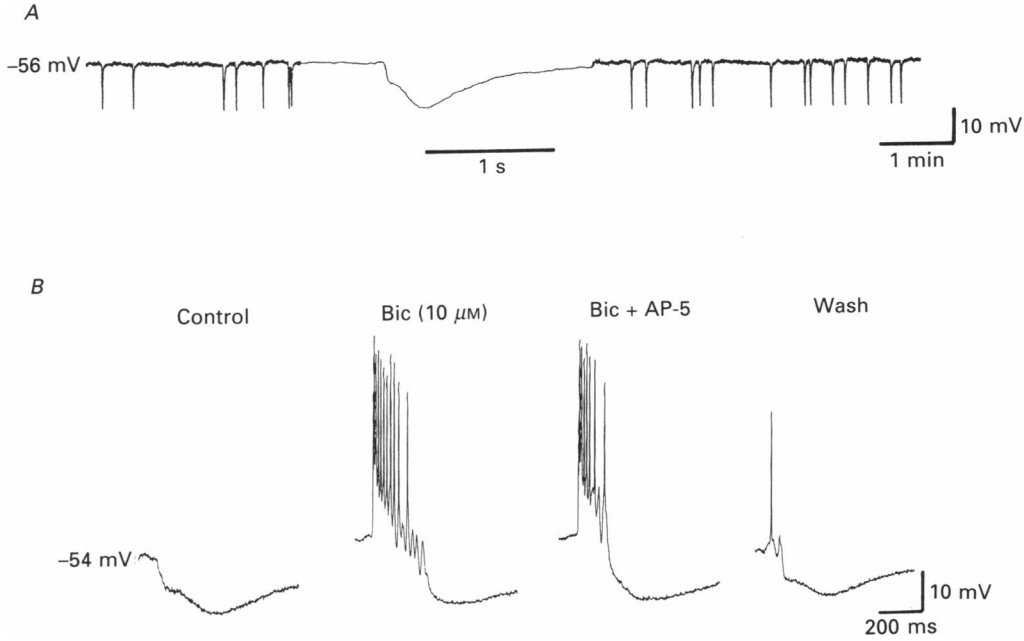

Fig. 9. Spontaneous, recurrent hyperpolarizing potentials recorded between P6 and P8. *A*, spontaneous hyperpolarizing potentials occurred at 0·05–0·13 Hz. As shown on an expanded time scale (middle) the hyperpolarizing potentials had a fast and a slow component. Six-day-old rat. *B*, in another neurone spontaneous hyperpolarizations were blocked by bicuculline (Bic) which induced spontaneous synchronized interictal discharges. These were reduced in amplitude and duration by AP-5 (50 μM). Seven-day-old rat. Both neurones were recorded with potassium acetate-containing electrodes.

These results imply that the depolarizing potential revealed during bicuculline superfusion was due to the activation of NMDA receptors.

Changes in spontaneous and evoked GDPs at the end of and after the first week of life

Between P5 and P12 there was a transitional period characterized by the progressive disappearance of the spontaneous GDPs (Fig. 1*C*) and by the occasional appearance of spontaneous large hyperpolarizing potentials in cells lacking GDPs. Whenever present, the GDPs showed the same properties of those recorded until P5.

The transitional properties of this developmental stage also influenced the effects of bicuculline on GDPs. In fact, in ten of twenty-seven neurones, bicuculline (10 μM) fully blocked GDPs and reduced synaptic noise as long as the antagonist was applied

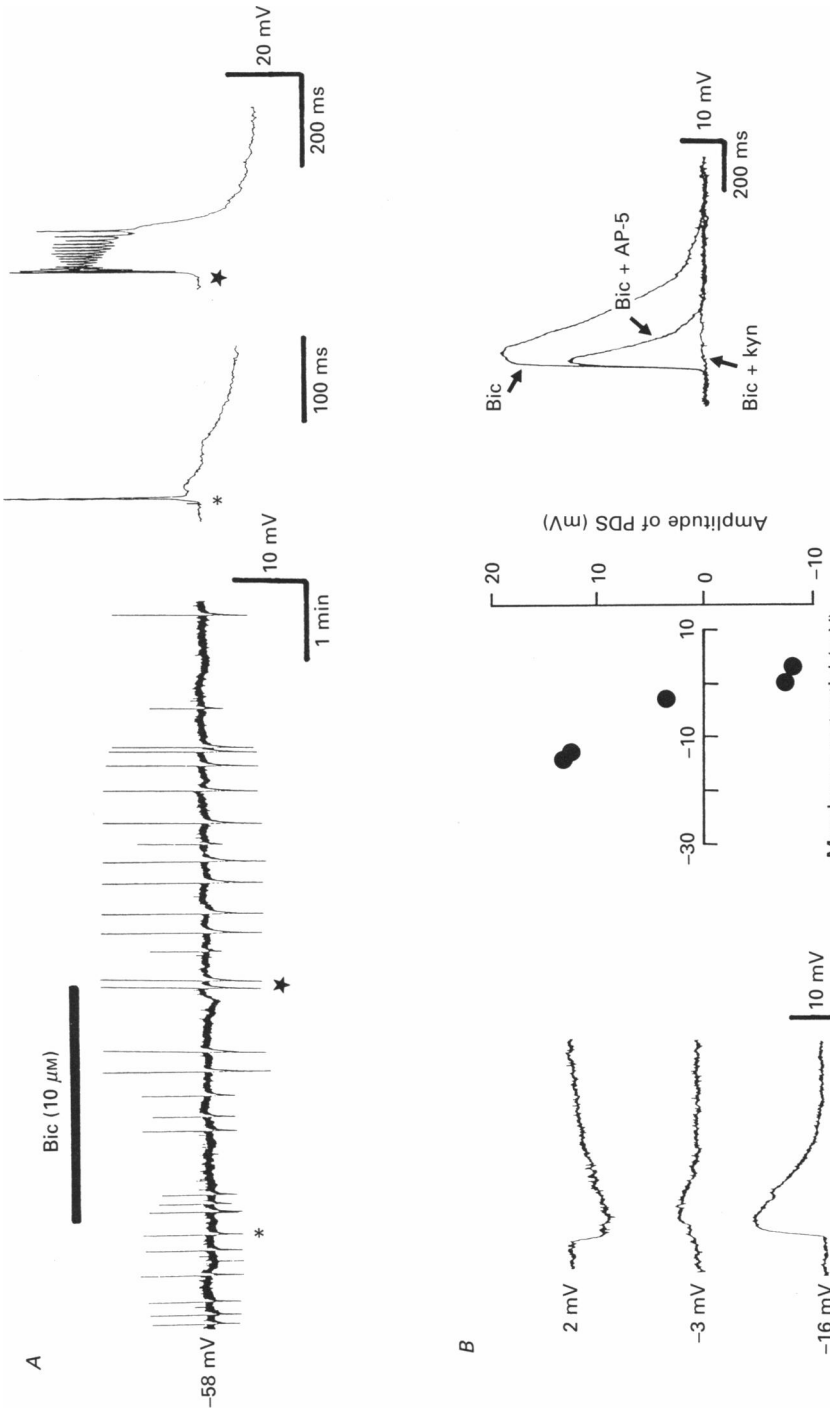

Fig. 10. Bicuculline induces spontaneous synchronized interictal discharges during the second week of life. *A*, the left trace shows that, in a 13-day-old rat, spontaneous spikes were present at resting membrane potential. Superfusion of bicuculline (Bic; filled bar) induced the appearance of interictal discharges. Expanded traces of a single spike (*) and of an interictal discharge (★) are shown on the right. Note the different time scale between the expanded traces. Recordings made with a KCl-containing electrode. *B*, recordings from an 8-day-old rat neurone impaled with an electrode containing KCl and QX-314. In control conditions no spontaneous GDPs were present. Superfusion with bicuculline (10 μM) induced the appearance of interictal discharges. Left panel shows the interictal discharges induced by bicuculline at three different membrane potentials. In the graph (middle) the amplitude of the interictal discharges is plotted against the membrane potential. The right panel shows that the bicuculline (Bic)-induced interictal discharges were greatly reduced by AP-5 (50 μM) and completely blocked by kynurenic acid (kyn, 1 mM).

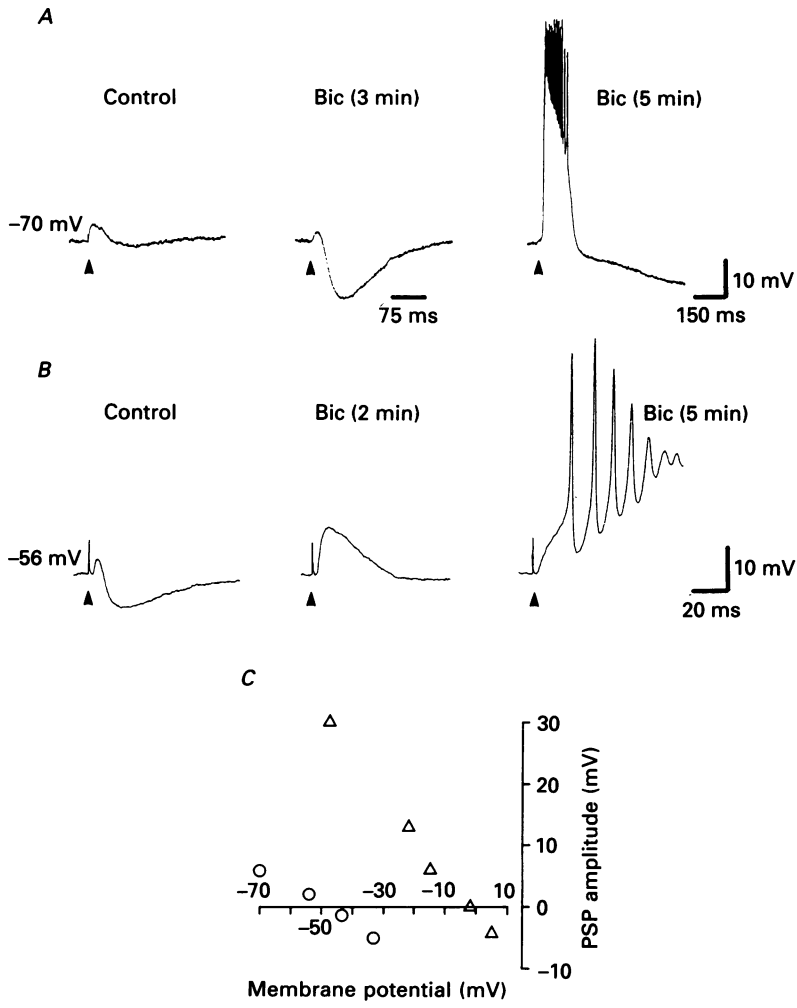

Fig. 11. Bicuculline induces evoked synchronized discharges during and after the second week of life. *A*, in an 11-day-old animal stimulation of the hilus (▲) evoked a depolarizing response followed by a small hyperpolarization. Bicuculline (Bic) initially (< 5 min) reduced the depolarizing response and increased the hyperpolarization, then after 5 min of application, induced the appearance of evoked bursts. *B*, in a 16-day-old rat stimulation of the hilus evoked an EPSP followed by an IPSP. Bicuculline (Bic) abolished the hyperpolarization and promoted the appearance of bursts. *A* and *B* were recorded with potassium methylsulphate-containing electrodes. *C*, the amplitude of the evoked depolarizing potential before bicuculline (○) and the amplitude of the synchronized discharge during bicuculline (△) for the cell shown in *A* is plotted against the membrane potential. Note the shift in reversal potential after application of bicuculline.

(> 15 min). In contrast, in the remaining seventeen neurones, bicuculline initially blocked the GDPs but, after 3–5 min, induced the appearance of synchronized interictal discharges. These differed from the GDPs since: (i) they had a faster rising phase and (ii) they reversed polarity at 0 mV with KCl- or potassium acetate-containing electrodes.

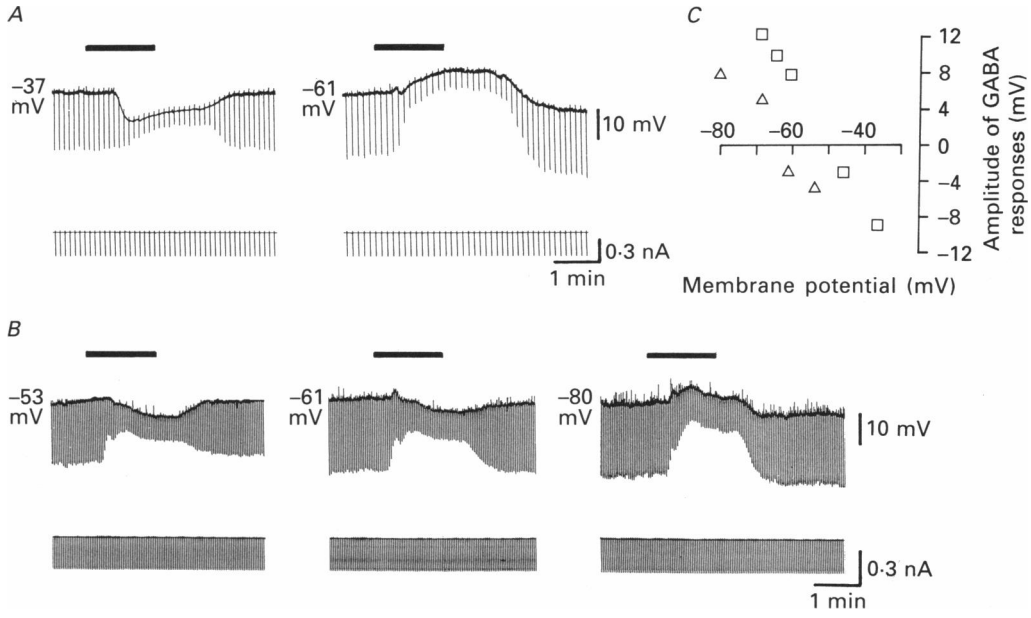

Fig. 12. The response to exogenously applied GABA is depolarizing at resting membrane potential during the first postnatal days and becomes hyperpolarizing after the second week of life. *A*, in a 4-day-old rat neurone application of GABA (0·5 mM; filled bars) induced at −61 mV a membrane depolarization and at −37 mV a membrane hyperpolarization. Resting membrane potential, −60 mV. *B*, in a 17-day-old rat neurone application of GABA (2 mM; filled bars) induced at resting membrane potential (−61 mV) a hyperpolarization which become larger at −53 mV. GABA induced a depolarization at −80 mV. Downward deflections in *A* and *B* are electrotonic potentials resulting from application of constant hyperpolarizing current pulses (lower traces) of 400 ms duration. Note the different concentrations of GABA in *A* and *B*. Both recordings with potassium methylsulphate-containing electrodes in the presence of extracellular TTX (1 μM). *C*, the responses of the cells shown in *A* (□) and *B* (△) to GABA application are plotted against the membrane potential.

Between P6 and P8, spontaneous large hyperpolarizing potentials were found in six of sixteen neurones (37%) recorded with potassium acetate- or potassium methylsulphate-containing electrodes (Fig. 9). These hyperpolarizations occurred with the same frequency of GDPs (0·05–0·13 Hz) and were generated by a polysynaptic circuit since their frequency was independent of membrane potential and they were blocked by TTX. The spontaneous hyperpolarizing potentials were biphasic, comprising a fast and a slow component. The first component reversed polarity at -62 ± 2 mV (always negative to the resting membrane potential by -6 ± 2 mV). The second component reversed polarity at -65 ± 3 mV. In two cells the spontaneous hyperpolarizations reversed polarity at the same value of reversal potential of the responses to GABA (−61 mV in one cell and −64 mV in the other).

Bicuculline (10 μM) blocked both components of the spontaneous hyperpolarizing potentials and induced interictal discharges which were reduced in amplitude and duration by AP-5 (50 μM, Fig. 9*B*). Moreover, sustained application of bicuculline (10 μM, $n = 5$) to slices in which GDPs or spontaneous hyperpolarizations were absent, induced the appearance of interictal discharges (Fig. 10*A*). The latter were similar to those observed in adult neurones, reversed in polarity near 0 mV and were blocked by CNQX (4 μM) or kynurenic acid (1 mM, Fig. 10*B*). In contrast to adults, the interictal discharges observed in this period were greatly reduced in amplitude and duration by NMDA receptor antagonists (Fig. 10*B*).

As for the spontaneous GDPs, there was a transitional period between P5 and P12, during which stimulation of the hilus still evoked GDPs followed by a hyperpolarization. However, in only twelve of twenty-three neurones, bicuculline (10 μM) persistently blocked the evoked GDPs. In the remaining eleven cells (in analogy with the case of spontaneous GDPs) after an initial block bicuculline induced the appearance of stimulus-evoked bursts. Like the spontaneous interictal discharges induced by bicuculline, they had a faster rising phase, they reversed at 0 mV and they were blocked by CNQX or kynurenic acid. After P11, stimulation of the hilus evoked, instead of GDPs, a depolarizing response, followed by a hyperpolarization (Fig. 11*A*). Bath application of bicuculline (10 μM) initially reduced the amplitude and duration of the depolarizing response, then, with a variable latency (3–5 min), it induced the appearance of bursts similar to those observed in adult neurones (Wong & Traub, 1983). As shown in Fig. 11*C* the control reversal potential of the depolarizing response (−47 mV) shifted in the presence of bicuculline to a more positive value (−2 mV). Interestingly, during the first minutes of bicuculline application, before the development of evoked bursts, the hyperpolarization which followed the bicuculline-insensitive depolarizing potential became larger (Fig. 11*A*). It is possible that, at this stage, the depolarizing GABA-mediated component 'masked' a large hyperpolarizing component reflecting the effect of GABA on $GABA_B$ receptors.

From P12 onwards, in parallel with the disappearance of spontaneous GDPs (Fig. 1*C*), stimulation of the hilus evoked an EPSP followed by an IPSP similar to those found in mature neurones (Cotman *et al.* 1986; Neuman *et al.* 1988*a*; Fig. 11*B*). Superfusion of bicuculline (10 μM, $n = 13$) blocked the fast inhibitory potential and produced, as in adult cells, spontaneous and evoked synchronized discharges (Fig. 11*B*). The bursts, which reversed in polarity near 0 mV, were blocked by CNQX (4 μM, $n = 5$) or kynurenic acid (1 mM, $n = 8$). They were only slightly reduced by AP-5 (50 μM, $n = 6$).

In keeping with these results, exogenous application of GABA during and after the second week of life induced at resting membrane potential hyperpolarizing or biphasic depolarizing–hyperpolarizing bicuculline-sensitive responses, which reversed in polarity at -66 ± 2 mV ($n = 3$; Fig. 12).

DISCUSSION

The main finding of this study is the novel observation of spontaneous and evoked GDPs in CA3 hippocampal neurones during the first week of life. GDPs are

suggested to be mediated by GABA and to involve NMDA receptors in their generation.

Evidence for GABA mediation of spontaneous and evoked GDPs

Two lines of evidence support the notion that GDPs are mediated by GABA. (i) Both GDPs and GABA (or isoguvacine) reversed at the same membrane potential, as expected if the exogenous agonist activates the same conductance(s) as the GDPs. Since use of KCl-filled electrodes simultaneously shifted the reversal potential from −51 mV (obtained with potassium acetate- or potassium methylsulphate-containing electrodes) to −27 mV, it seems that the activation of a Cl^- conductance was the mechanism underlying both the GDPs and the response to exogenous GABA. In the same experimental conditions, regardless of the type of electrode employed, the reversal of the responses to NMDA was −2 mV (Ben-Ari *et al.* 1988*a*). (ii) The spontaneous GDPs were blocked by the GABA antagonists bicuculline and picrotoxin. This effect of the antagonists was associated with a membrane hyperpolarization (from the second week of life bicuculline usually depolarized the membrane) suggesting that a tonic release of GABA contributed to maintain the cell membrane at a more depolarized level. This is also confirmed by the observation that there is a significant difference in the resting membrane potential of the cells recorded with KCl and potassium methylsulphate-containing microelectrodes only during the first week of life.

Although the present study indicates that a Cl^- permeability increase was likely to be responsible for the action of GABA, it is not fully clear why such an effect of GABA should be depolarizing and thus capable of eliciting GDPs. In adult hippocampal cells, somatic or dendritic application of GABA produces a hyperpolarization and a depolarization respectively (Andersen, Dingledine, Gjerstad, Langmoen & Mosfeldt Laursen, 1980; Alger & Nicoll, 1982). It is presently not clear whether in adult neurones the hyperpolarizing and depolarizing responses to GABA are only due to the activation of Cl^- channels. Assuming that only Cl^- is involved, it is possible that in immature cells the depolarizing responses to GABA, like those observed following dendritic application of GABA to adult neurones, were due to a modified Cl^- gradient resulting from the unidirectional operation of the Cl^- membrane pumps (Misgeld, Deisz, Dodt & Lux, 1986). Regardless of the ionic mechanisms underlying their generation, GDPs were excitatory events, since when recorded with potassium methylsulphate-containing electrodes, they reached threshold for spike generation in spite of a fall in input resistance.

At the end of the first week of life the responses to GABA shifted from the depolarizing to the hyperpolarizing direction in concomitance with a progressive reduction of spontaneous GDPs and with the appearance of spontaneous hyperpolarizing potentials. The spontaneous hyperpolarizing potentials were likely to be inverted GDPs since: (i) like the GDPs these were generated in a polysynaptic network at the same frequency and (ii) like the GDPs they reversed at the same potential as GABA and they were blocked by bicuculline. During this transitional period bicuclline generated for the first time the interictal synchronized discharges typically found in adult CA3 neurones (Wong & Traub, 1983). This interictal type of discharge is due to the removal of inhibition which reveals quiescent recurrent excitatory synapses interconnecting the pyramidal neurones.

NMDA receptors are involved in spontaneous and evoked GDPs

The second important finding of this study was the modulation of spontaneous GDPs by NMDA receptors and the presence (at resting membrane potential) of a NMDA component in the GDPs evoked by hilus stimulation.

Both NMDA receptor antagonists and NMDA channel blockers reduced the frequency of spontaneous GDPs or abolished them. This phenomenon was presumably caused indirectly via antagonism of NMDA receptor activity driving the discharge of GABA neurones. It is unlikely that NMDA receptors directly mediated the generation of GDPs at the level of pyramidal cell membrane since NMDA-mediated responses have a reversal potential different from the one of GDPs (Ben-Ari *et al.* 1988*a*). It should be noted that NMDA receptors did not seem to control the tonic release of GABA, since NMDA antagonists, in contrast to bicuculline or picrotoxin, did not reduce GABA-mediated synaptic noise.

Evoked GDPs included a NMDA component since: (i) in the presence of bicuculline an AP-5-sensitive, voltage-dependent response was revealed and (ii) this synaptic response reversed near 0 mV as that induced by bath application of NMDA to immature neurones (Ben-Ari *et al.* 1988*a*). The overall contribution of the NMDA component to the excitatory synaptic transmission of immature CA3 cells appears to be relatively small, in keeping with the delayed maturation of the excitatory connections (Amaral & Dent, 1981). Preliminary observations (R. Corradetti, J. L. Gaiarsa, Y. Ben-Ari and E. Cherubini, unpublished observations) suggest that non-NMDA receptors may also be involved in modulating GDPs, since the rather selective quisqualate/kainate receptor antagonist CNQX (Drejer & Honoré, 1988; Neuman *et al.* 1988*a*) and the broad spectrum excitatory amino acid antagonist kynurenate blocked spontaneous GDPs without reducing synaptic noise.

Mechanisms of generation of GDPs and possible physiological significance

In contrast to the delayed maturation of the excitatory connections (Amaral & Dent, 1981), the GABAergic system has a relatively early development. In the hippocampus, numerous GABA and glutamic acid decarboxylase immunoreactive interneurones are already present at birth in the vicinity of pyramidal cells (F. Rosenberg, O. Robain, L. Jardin & Y. Ben-Ari, unpublished observations). At this early developmental stage, however, the synapses are primarily asymmetric and have several immature features (McLaughlin, Wood, Saito, Roberts & Wu, 1975; Blue & Parnavelas, 1983; Bahr & Wolff, 1985). Axosomatic contacts appear only during the second and third postnatal week. A non-synaptic GABA release may precede GABAergic synaptogenesis (Balcar, Damm & Wolff, 1986; Hicks, Ruwe & Veale, 1986) possibly originating from growth cones (Gordon-Weeks, Lockerbie & Pearce, 1984).

On the basis of our observations and the above-mentioned anatomical data, we propose a simple model to explain the generation of spontaneous GDPs. As shown in Fig. 13 and analogous to other central neurones (cf. the well-documented case of cells in the lamprey spinal cord, Grillner, Wallen, Dale, Brodin, Buchanan & Hill, 1987), GABAergic interneurones are thought to undergo oscillatory activity. The cycle might be initiated by a depolarization of GABA-containing cells via activation of their NMDA receptor–channel complex by glutamate released by adjacent

pyramidal cells. The NMDA receptor activation will promote a Ca^{2+} influx (MacDermott, Mayer, Westbrook, Smith & Barker, 1986). This increase in cytosolic Ca^{2+} in turn might activate a Ca^{2+}-dependent K^+ conductance, which would hyperpolarize the GABAergic cell. The oscillating GABAergic interneurone would release GABA onto pyramidal neurones and participate in a positive feedback loop

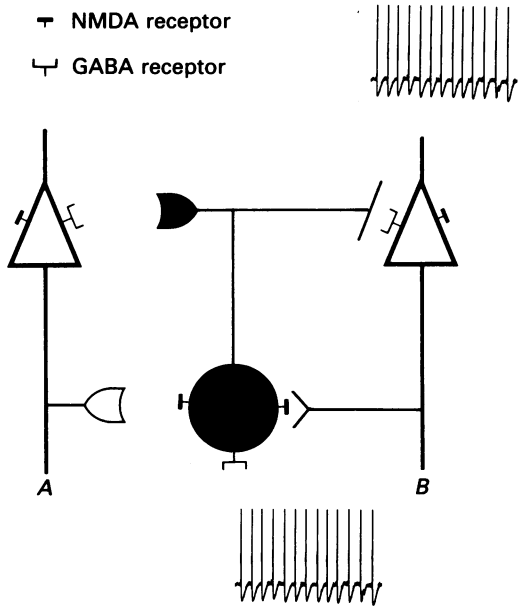

Fig. 13. Model of oscillatory behaviour of immature CA3 neurones. The schematic drawing shows a GABAergic interneurone (filled circle) which oscillates, as depicted by the lower continuous trace, in response to activation of NMDA receptors. The interneurone is driven by an excitatory amino acid released by axon collaterals of pyramidal cells (open triangles). The GABAergic neurone releases GABA and activates GABA receptors which mediate depolarizations able to produce oscillations in the pyramidal neurones as shown by the upper intracellular recordings. Assuming such a pattern of activity in a small cluster of cells (for simplicity termed an 'oscillatory unit') the activation of such 'units' may elicit synchronous discharge of a population of neurones if excitation is spread via recurrent collaterals of pyramidal cells or via multiple innervation of many pyramidal cells by a single interneurone. Further contribution to synchronized discharges may derive from reciprocal connections between GABAergic interneurones. *A* and *B* propose two distinct anatomical arrangements: in *A*, the pyramidal cell releases from free endings (open calix) the neurotransmitter acting on the NMDA receptors located on the interneurone; similarly the interneurone releases GABA from free endings (filled calix). In *B*, typical synaptic contacts are envisaged to connect a pyramidal cell and an interneurone. Anatomical arrangements intermediate between *A* and *B* may be assumed. Other receptors which may participate in this oscillating behaviour have been omitted from this simplified model.

through which the synchronous discharge of a population of pyramidal cells might be produced. Should the recurrent 'excitatory' connection among pyramidal neurones be functional at this stage, it would then be a suitable mechanism to facilitate synchronization. The cyclic release of GABA could either occur at axodendritic synapses or from non-synaptic free endings such as those described in

the immature cortex (McLaughlin *et al.* 1975; Blue & Parnavelas, 1983; Bahr & Wolff, 1985). Regardless of the precise mechanism underlying the generation of GDPs, their presence in such a restricted period of postnatal life suggests that these recurrent events are an important physiological feature of immature CA3 neurones and may represent a significant signal in cell growth and differentiation.

The authors wish to thank Dr A. Nistri for his helpful comments on the manuscript, and Mrs G. Idriss and S. Guidasci for technical and photographic assistance. The financial support from DRET is acknowledged. R.C. was recipient of the EMBO fellowship ALTF 203-1987.

REFERENCES

AICARDI, J. & CHEVRIE, J. J. (1970). Convulsive status epilepticus in infants and children. A study of 239 cases. *Epilepsia* **11**, 189–197.

ALGER, B. E. (1984). Characteristics of a slow hyperpolarizing synaptic potential in rat hippocampal pyramidal cells *in vitro*. *Journal of Neurophysiology* **52**, 892–910.

ALGER, B. E. & NICOLL, R. A. (1982). Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied *in vitro*. *Journal of Physiology* **328**, 125–141.

AMARAL, D. G. & DENT, J. A. (1981). Development of the mossy fibres of the dentate gyrus: I. A light and electron microscopic study of the mossy fibres and their expansions. *Journal of Comparative Neurology* **195**, 51–86.

ANDERSEN, P., DINGLEDINE, R., GJERSTAD, L., LANGMOEN, I. A. & MOSFELDT LAURSEN, A. (1980). Two different responses of hippocampal pyramidal cells to application of gamma-aminobutyric acid. *Journal of Physiology* **305**, 279–296.

BAHR, S. & WOLFF, J. R. (1985). Postnatal development of axosomatic synapses in the rat visual cortex: morphogenesis and quantitative evaluation. *Journal of Comparative Neurology* **233**, 405–420.

BALCAR, V. J., DAMM, S. & WOLFE, J. R. (1986). Ontogeny of K^+-stimulated release of ^3H GABA in rat cerebral cortex studied by a simple technique *in vitro*. *Neurochemistry International* **89**, 573–580.

BEN-ARI, Y., CHERUBINI, E. & KRNJEVIĆ, K. (1988*a*). Changes in voltage dependence of NMDA currents during development. *Neuroscience Letters* **94**, 88–92.

BEN-ARI, Y., CORRADETTI, R., & GAIARSA, J. L. (1988*b*). Spontaneous and evoked giant EPSPs in neurones from immature rat hippocampal slices involve the activation of *N*-methyl-D-aspartate (NMDA) receptors. *Journal of Physiology* **406**, 7*P*.

BEN-ARI, Y. & GHO, M. (1988). Long-lasting modification of the synaptic properties of rat CA3 hippocampal neurones induced by kainic acid. *Journal of Physiology* **404**, 365–384.

BEN-ARI, Y., KRNJEVIĆ, K., REIFFENSTEIN, R. J. & REINHARDT, W. (1981). Inhibitory conductance changes and action of γ-aminobutyrate in rat hippocampus. *Neuroscience* **6**, 2445–2463.

BERRY, M. S. & PENTHREATH, V. W. (1976). Criteria for distinguishing between monosynaptic and polysynaptic transmission. *Brain Research* **105**, 1–20.

BLUE, M. & PARNAVELAS, J. G. (1983). The formation and maturation of synapses in the visual cortex of the rat. II. Quantitative analysis. *Journal of Neurocytology* **12**, 697–712.

CHERUBINI, E., NEUMAN, R., ROVIRA, C. & BEN-ARI, Y. (1988). Epileptogenic properties of the mast cell degranulating peptide in CA3 hippocampal neurones. *Brain Research* **445**, 91–100.

COAN, E. J. & COLLINGRIDGE, G. L. (1985). Magnesium ions block an *N*-methyl-D-aspartate receptor mediated component of synaptic transmission in rat hippocampus. *Neuroscience Letters* **53**, 21–26.

COLLINGRIDGE, G. L., HERRON, C. E. & LESTER, R. A. J. (1988*a*). Synaptic activation of *N*-methyl-D-aspartate receptors in the Schaffer collateral–commisural pathway of rat hippocampus. *Journal of Physiology* **399**, 283–300.

COLLINGRIDGE, G. L., HERRON, C. E. & LESTER, R. A. J. (1988*b*). Frequency-dependent-*N*-methyl-D-aspartate receptor-mediated synaptic transmission in rat hippocampus. *Journal of Physiology* **399**, 301–12.

CORRADETTI, R., GAIARSA, J. L. & BEN-ARI, Y. (1988). D-Amino-phosphonovaleric acid-sensitive

spontaneous giant EPSPs in immature rat hippocampal neurones. *European Journal of Pharmacology* **154**, 221–222.

COTMAN, C. W., FLATMAN, J. A., GANONG, A. H. & PERKINS, M. N. (1986). Effects of excitatory amino acid antagonists on evoked and spontaneous excitatory potentials in guinea-pig hippocampus. *Journal of Physiology* **378**, 403–415.

DINGELDINE, R., HYNES, M. A. & KING, G. L. (1986). Involvement of *N*-methyl-D-aspartate receptors in epileptiform bursting in the rat hippocampal slice. *Journal of Physiology* **380**, 175–189.

DREJER, J. & HONORÉ, T. (1988). New quinoxalinediones show potent antagonism of quisqualate responses in cultured mouse cortical neurons. *Neuroscience Letters* **87**, 104–108.

DUNWIDDIE, T. V. (1981). Age-related differences in the *in vitro* rat hippocampus: development of inhibition and the effects of hypoxia. *Developmental Neuroscience* **4**, 165–175.

GAARSKJAER, F. B. (1986). The organization and development of the hippocampal mossy fibre system. *Brain Research Reviews* **11**, 335–357.

GORDON-WEEKS, P. R., LOCKERBIE, R. O. & PEARCE, B. R. (1984). Uptake and release of ^{3}H GABA by growth cones isolated from neonatal rat brain. *Neuroscience Letters* **52**, 205–212.

GRILLNER, S., WALLEN, P., DALE, N., BRODIN, L., BUCHANAN, J. & HILL, R. (1987). Transmitters, membrane properties and network circuitry in the control of locomotion in lamprey. *Trends in Neuroscience* **10**, 34–41.

HABLITZ, J. J. & JOHNSTON, D. (1981). Endogenous nature of spontaneous bursting in hippocampal pyramidal neurons. *Cellular and Molecular Neurobiology* **1**, 325–334.

HARRIS, K. M. & TEYLER, T. J. (1983). Evidence for a late development of inhibition in area CA1 of the rat hippocampus. *Brain Research* **268**, 339–343.

HICKS, T. P., RUWE, W. D. & VEALE, W. L. (1986). Release of gamma-aminobutyric acid from the visual cortex of young kittens. *Developmental Brain Research* **24**, 299–304.

JOHNSTON, D. & BROWN, T. H. (1984). Mechanisms of neuronal burst generation. In *Electrophysiology of Epilepsy*, ed. SCHWARTZKROIN, P. A. & WHEAL, H. V., pp. 227–301. New York: Academic Press.

KANDEL, E. R., SPENCER, W. A. & BRINLEY, F. J. (1961). Electrophysiology of hippocampal neurones. I. Sequential invasion and synaptic organization. *Journal of Neurophysiology* **24**, 225–242.

KEHL, S. J. & MCLENNAN, H. (1983). Evidence for a bicuculline insensitive long lasting inhibition in the CA3 region of the hippocampal slice. *Brain Research* **279**, 278–281.

KING, A. E., CHERUBINI, E. & BEN-ARI, Y. (1989). *N*-Methyl-D-aspartate induces recurrent synchronized burst activity in immature hippocampal CA3 neurones *in vitro*. *Developmental Brain Research* **46**, 1–8.

KLEINSCHMIDT, A., BEAN, M. & SINGER, W. (1987). Blockade of 'NMDA' receptors disrupts experience dependent plasticity of kitten striate cortex. *Science* **238**, 355–358.

KRIEGESTEIN, A. R., SUPPES, T. & PRINCE, D. A. (1987). Cellular and synaptic physiology and epileptogenesis of developing rat neocortical neurons *in vitro*. *Developmental Brain Research* **34**, 161–171.

MACDERMOTT, A. B., MAYER, M. L., WESTBROOK, G. L., SMITH, S. J. & BARKER, J. L. (1986). NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature* **321**, 519–522.

MCLAUGHLIN, B. J., WOOD, J. G., SAITO, K., ROBERTS, E. & WU, J. Y. (1975). The fine structural localization of glutamic acid decarboxylase in developing axonal processes and pre-synaptic terminals of rodent cerebellum. *Brain Research* **85**, 355–371.

MILES, R. & WONG, R. K. S. (1986). Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus. *Journal of Physiology* **373**, 397–418.

MISGELD, U., DEISZ, R. A., DODT, H. U. & LUX, H. D. (1986). The role of chloride transport in postsynaptic inhibition of hippocampal neurons. *Science* **232**, 1413–1415.

MUELLER, A. A., TAUBE, J. T. & SCHWARTZKROIN, P. A. (1984). Development of hyperpolarizing inhibitory postsynaptic potentials and hyperpolarizing response to γ-aminobutyric acid in rabbit hippocampus studied *in vitro*. *Journal of Neuroscience* **4**, 860–867.

NEUMAN, R. S., BEN-ARI, Y., GHO, M. & CHERUBINI, E. (1988*a*). Blockade of excitatory synaptic transmission by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the hippocampus *in vitro*. *Neuroscience Letters* **92**, 64–68.

NEUMAN, R., CHERUBINI, E. & BEN-ARI, Y. (1988*b*). Epileptiform bursts elicited in CA3 hippocampal neurons by a variety of convulsants are not blocked by *N*-methyl-D-aspartate antagonists. *Brain Research* **459**, 267–274.

NEUMAN, R., CHERUBINI, E. & BEN-ARI, Y. (1988*c*). Endogenous and network bursts induced by *N*-methyl-D-aspartate and Mg^{2+}-free medium in the CA3 region of the hippocampal slice. *Neuroscience* **28**, 393–399.

NEWBERRY, N. R. & NICOLL, R. A. (1985). A bicuculline-resistant inhibitory post-synaptic potential in rat hippocampal pyramidal cells *in vitro*. *Journal of Physiology* **348**, 239–254.

SCHWARTZKROIN, P. A. (1982). Development of rabbit hippocampus physiology. *Developmental Brain Research* **2**, 469–486.

TREMBLAY, E., ROISIN, M. P., REPRESA, A., CHARRIAUT-MARLANGUE, C. & BEN-ARI, Y. (1988). Transient increased density of NMDA binding sites in the developing rat hippocampus. *Brain Research* **461**, 393–396.

TSUMOTO, T., HAGIHARA, K., SATO, H., & HATA, Y. (1987). NMDA receptors in the visual cortex of young kittens are more effective than those of adult cats. *Nature* **327**, 513–514.

WONG, R. K. S. & TRAUB, R. D. (1983). Synchronized burst discharge in disinhibited hippocampal slice. *Journal of Neurophysiology* **49**, 442–458.

YAMAMOTO, C., SAWADA, S. & TARADA, S. (1983). Suppressing action of 2-amino-4-phosphonobutyric acid on mossy fiber-induced excitation in the guinea pig hippocampus. *Experimental Brain Research* **51**, 128–134.