Synchronization of inhibitory neurones in the guinea-pig hippocampus *in vitro*

Hillary B. Michelson and Robert K. S. Wong

Department of Pharmacology, State University of New York Health Science Center, Brooklyn, New York, NY 11203, USA

1. Intracellular recordings were obtained from pyramidal, granule and hilar cells in transverse slices of guinea-pig hippocampus to examine synaptic interactions between GABAergic neurones.

2. In the presence of the convulsant compound 4-aminopyridine (4-AP), after fast excitatory amino acid (EAA) neurotransmission was blocked pharmacologically, large amplitude inhibitory postsynaptic potentials (IPSPs) occurred rhythmically (every 4–8 s) and synchronously in all principal cell populations (triphasic synchronized IPSPs). In the presence of the $GABA_A$ receptor blocker picrotoxin (PTX), a large amplitude IPSP continued to occur spontaneously in all principal cells simultaneously (monophasic synchronized IPSP).

3. Burst firing occurred simultaneously in a group of hilar neurones (synchronized bursting neurones) coincident with triphasic synchronized IPSPs in principal cells. After PTX was added, the bursts and the underlying depolarizing synaptic potentials were completely suppressed in some of the synchronized bursting neurones (type I hilar neurones), while others (type II hilar neurones) continued to fire in bursts coincident with monophasic synchronized IPSPs in principal cells. Intense hyperpolarization blocked burst firing and revealed underlying attenuated spikes of less than 10 mV, but did not uncover any underlying depolarizing synaptic potentials.

4. In type II hilar neurones, during sufficient hyperpolarization, spontaneous activity consisted of attenuated spikes. With depolarization, the small spikes began to trigger full size action potentials. These data suggest the presence of electrotonically remote spike initiation sites.

5. The morphology of synchronized bursting neurones was revealed by intracellular injection of the fluorescent dye Lucifer Yellow. Attempts to inject dye into one type II hilar neurone often resulted in the labelling of two to four cells (dye coupling). Dye coupling was not observed in type I hilar neurones.

6. These findings indicate that excitatory interactions synchronizing the firing of GABAergic neurones can occur in the absence of fast EAA neurotransmission. GABAergic neurones can become synchronized via their recurrent collaterals through the depolarizing action of synaptically activated $GABA_A$ receptors. In addition, a subpopulation of GABAergic neurones can become synchronized by a mechanism probably involving electrotonic coupling.

Until recently, experiments addressing cellular and synaptic properties of cortical neurones have mainly focused on the excitatory pyramidal cells (Andersen, Blackstad & Lømo, 1966; Andersen, Bliss & Skrede, 1971; Schwartzkroin, 1975; Prince, 1978; Miles & Wong, 1986). Such studies were facilitated by the use of the *in vitro* slice preparation, which allowed for the study of individual neuronal properties within an intact circuit. Similar information regarding inhibitory GABAergic neurones has been difficult to obtain, since activation of inhibition in isolation from excitatory synaptic transmission can only be obtained under very restrictive conditions such as direct paired recording and antidromic stimulation (Miles & Wong, 1984, 1987; Lacaille & Schwartzkroin, 1988). The advent of selective excitatory amino acid (EAA) receptor antagonists (Honoré *et al.* 1988) greatly facilitated the study of inhibitory processes in isolation.

GABAergic neurones, constituting about 20% of all neurones in the mammalian cortex, are widely distributed in all areas of the cortex (Gabbott & Somogyi, 1986;

Hendry, Schwark, Jones & Yan, 1987). Immunocytochemical studies show that every type of cortical neurone receives GABAergic input (Houser, Vaughn, Hendry, Jones & Peters, 1984), suggesting that GABAergic neurones play a significant role in the signalling process in the cortex. Indeed, *in vitro* experiments show that $GABA_A$ receptor blockade leads to epileptiform burst discharges in the cortex (Schwartzkroin & Prince, 1977). These data suggest that an understanding of the signal integration process in the cortex requires a detailed knowledge of the properties of inhibitory neurones and the circuit formed by these neurones. Using EAA receptor blockers, we have begun to examine the property of networks formed by GABAergic neurones. We demonstrated that the firing of inhibitory neurones could become synchronized in the absence of glutamate-mediated excitation (Aram, Michelson & Wong, 1990). Furthermore, by recording directly from inhibitory neurones, we have demonstrated that interneurones are recurrently connected and that the firing of inhibitory cells could become synchronized by synaptic excitation mediated by $GABA_A$ receptors (Michelson & Wong, 1991).

We have continued to examine the connectivity of inhibitory neurones. The present results show that, while GABA-mediated synaptic excitation is the primary process pacing synchronized GABAergic neuronal discharges in the hippocampus, another mechanism, independent of chemical synaptic transmission, is present to synchronize the activity of these neurones. Portions of the study have been presented previously (Michelson & Wong, 1992).

METHODS

The methods used in these experiments were similar to those described in previous reports (Miles & Wong, 1984; Michelson & Wong, 1991). Transverse slices of hippocampus, 400 μm in thickness, were prepared with a vibratome from guinea-pigs weighing 200–300 g, which were anaesthetized with 2-bromo-2-chloro-1,1,1-trifluoroethane and rapidly decapitated. In some experiments, slices were microdissected into isolated minislices of the CA1 subfield, the CA3 subfield, or the area dentata. Slices were then transferred onto the nylon mesh of a gas–fluid interface recording chamber (Fine Science Tools, Foster City, CA, USA) and maintained at 37 °C, at a pH of 7·4, exposed to a warmed, humidified atmosphere of 5 % CO_2 in O_2. The lower surfaces of the slices were in contact with a perfusion solution of normal composition (mM): NaCl, 124; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1·6; $NaHCO_3$, 26; and D-glucose, 10. 4-AP (75 μM), 3-3(2-carboxypiperazine-4-yl)propyl-1-phosphonate (CPP; 10 μM), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM), picrotoxin (50 μM) and 2-hydroxysaclofen (200 μM) were added to the solution in some experiments. CPP, CNQX, and 2-hydroxysaclofen were obtained from Tocris Neuramin (Bristol, UK). All other chemicals were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Recording electrodes were pulled from fibre-filled glass capillaries to a resistance of 40–80 MΩ and usually filled with 3 mM potassium acetate. In experiments in which cells were prepared for fluorescence imaging, the tips of recording electrodes were filled with 4 % Lucifer Yellow (LY) dilithium salt, dissolved in distilled water, and backfilled with 1 mM Li_2SO_4. Simultaneous penetrations were made with electrodes controlled by separate manipulators. In experiments in which interneurones were injected with LY only one intracellular impalement per slice was performed.

Signals were amplified by high impedance amplifiers with facilities for capacitance compensation and for current injection through the recording circuit using an active bridge circuit (Axoclamp-2A, Axon Instruments, Burlingame, CA, USA). Bipolar tungsten electrodes of tip diameter 20 μm and separation 200 μm were used to stimulate neurones with electrical pulses of 100–300 μs. The stimulating electrode was placed either spanning the hippocampal fissure in the perforant path area or in the mossy fibre pathway. Signals were displayed on a digital oscilloscope (Nicolet 4562; Nicolet instrument Corporation, Madison, WI, USA) and stored digitally on videotape.

In experiments in which neurones were injected with LY, cells were impaled and cellular properties were briefly analysed. LY was injected intracellularly using pulses of $-0{\cdot}5$ to 2·0 nA for 2–15 min. After injection, the slices remained in the chamber for 10–90 min. Slices were then fixed overnight between two pieces of filter paper by immersion in 3 % paraformaldehyde, pH 7·0. Slices were then dehydrated in a series of alcohol rinses and cleared in methylsalicylate before being examined with a Nikon epifluorescence microscope equipped with appropriate filters and photographic accessories.

Results are given as means ± S.D. throughout.

RESULTS

Properties of synchronized IPSPs

Intracellular recordings were obtained from principal cells of all regions of the hippocampal slice. These included CA3 and CA1 pyramidal cells and dentate granule cells. Addition of 4-AP (75 μM) to the perfusion solution elicited synchronized epileptiform bursting in all recorded regions. These bursts were blocked upon introduction of the fast EAA receptor blockers CPP (10 μM) and CNQX (10 μM) to the solution. Under this condition two types of spontaneous synaptic events persisted. These were (1) large amplitude inhibitory events and (2) smaller amplitude IPSPs (Fig. 1*A*).

The large amplitude events occurred spontaneously and synchronously, at frequencies of 0·125–0·25 Hz, in all principal cells recorded. They were invariably triphasic in appearance with a mean peak amplitude of 7·22 ± 0·64 mV (mean ± S.D.) and a rise time of 55·87 ± 14 ms ($n = 25$ events in 3 cells; Michelson & Wong, 1991). Because these large amplitude events occurred simultaneously in all principal cells, they will be referred to as triphasic synchronized IPSPs. The smaller amplitude IPSPs were desynchronized events occurring independently in simultaneously recorded neurones. The mean rise time of these events was 7·82 ± 2·51 ms and their mean amplitude was 1·16 ± 0·57 mV ($n = 37$ events in 4 cells at -62 mV membrane potential). These values correspond to those reported for unitary IPSPs (Miles & Wong, 1984).

Effects of picrotoxin on the triphasic synchronized IPSP

The addition of picrotoxin altered several aspects of the spontaneous synaptic activity recorded in the presence of 4-AP and EAA blockers. Within 5 min of adding picrotoxin to the bath, the frequency of occurrence of the triphasic synchronized IPSP began to decrease. The rhythmicity of the event became quite irregular during the drug wash-in period, and in some cells (three out of seven), the event was completely suppressed for periods of up to 5 min. Within 10 min, in all cells, the rhythm at which the synchronized event occurred stabilized at a frequency which was approximately 50 % of the level before the addition of picrotoxin (PTX; Fig. 1*B*). Synchronized hyperpolarizing events persisted in picrotoxin and continued to occur spontaneously in all regions of the hippocampus.

PTX also abolished the fast unitary IPSPs described above. In their place, on occasion, we observed another type of randomly occurring hyperpolarizing event in the baseline (Fig. 1*A*). These smaller amplitude events had a significantly slower rise time than the unitary $GABA_A$-mediated IPSPs (251·97 ± 78·03 ms, $n = 40$ events in 4 cells at −62 mV membrane potential) and decreased in amplitude upon membrane hyperpolarization. Additional experiments showed that these spontaneous events disappeared upon the introduction of 2-hydroxysaclofen (SAC; 200 μM) to the perfusion solution (Fig. 1*A*). These events probably represent unitary synaptic potentials mediated by the activation of postsynaptic $GABA_B$ receptors. The synchronized event (mean amplitude 6·1 ± 0·24 mV) generated in the presence of PTX was about 5-fold larger than the smaller amplitude events (mean amplitude 1·30 ± 0·39 mV, $n = 40$ events in 4 cells at −62 mV membrane potential).

The synchronized IPSP observed in the presence of PTX could be distinguished in three ways from that which occurred prior to the wash-in of PTX. Firstly, synchronized events recorded in the presence of PTX had a slower rise time than the events observed before PTX (235·13 ± 68·84 ms, $n = 15$ events in 3 cells *vs.* 55·87 ± 14·0 ms, $n = 25$ events in 3 cells). Secondly, as noted above, events recorded in PTX occurred at a consistently slower rhythm compared with the rhythm observed before PTX application. Thirdly, PTX-resistant synchronized events reversed uniformly at a membrane potential of about −94 mV (Fig. 2). This reversal potential corresponds to

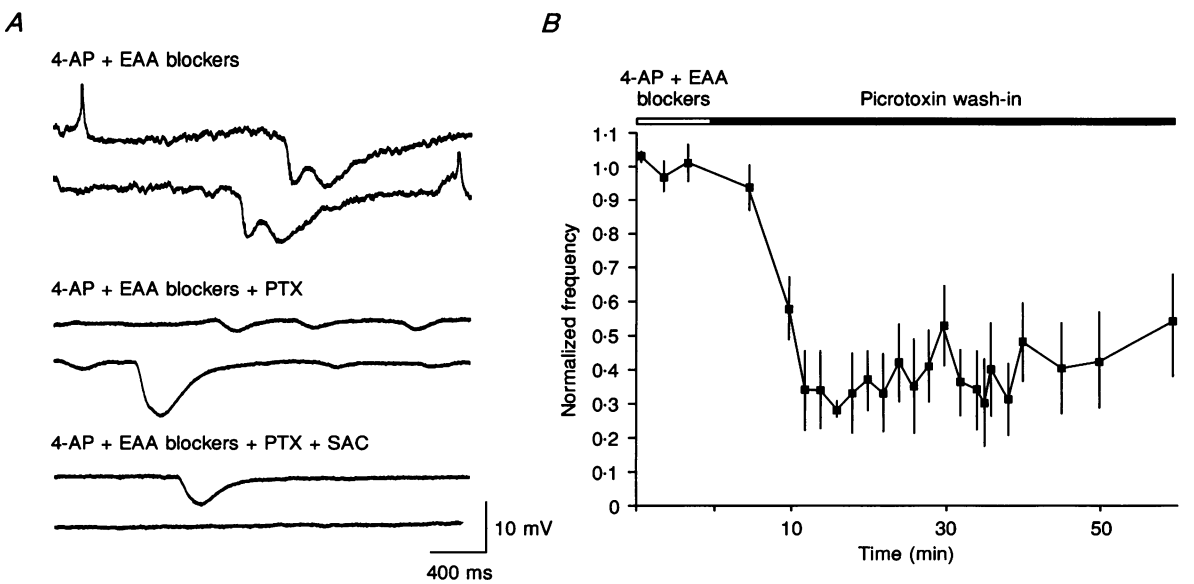

Figure 1. Effects of picrotoxin (PTX) and 2-hydroxysaclofen (SAC) on the synchronized IPSP in a CA3 pyramidal cell

A, in the presence of 4-AP and EAA blockers, the synchronized IPSP was composed of three components corresponding to the fast $GABA_A$ response, the depolarizing $GABA_A$ response and the slow $GABA_B$ response (upper traces). In the same cell, 1 h after wash-in of PTX, the synchronized event becomes monophasic, and there are smaller amplitude, slow IPSPs randomly occurring along the baseline (middle traces). SAC (200 μM) blocked the smaller amplitude IPSPs and reduced the amplitude of the synchronized IPSP (lower traces). Spike bursts in top traces were truncated for figure production. *B*, the effect of PTX on the rhythm of the synchronized IPSP. Data were normalized across seven cells. The synchronized event which was observed in the presence of PTX was generated at a consistently slower frequency than the event recorded in 4-AP and EAA blockers alone.

that of the third component of the triphasic synchronized IPSP generated before the addition of PTX and most probably represents the synchronized activation of postsynaptic $GABA_B$ receptors. For this reason, the large amplitude IPSPs occurring after the application of PTX will be referred to as monophasic synchronized IPSPs.

Intracellular recordings from the hilar interneurones

To examine the generation mechanisms underlying the triphasic and monophasic synchronized IPSPs, we attempted to make intracellular recordings in inhibitory GABAergic interneurones in the hilar region. Immunocytochemical studies show that the density of interneurones in this area is increased, and that they constitute about 60 % of the population of neurones in the region (Seress & Ribak, 1983). Previous studies indicate that a majority of hilar neurones show rhythmic bursting activity which occurred synchronously within a population of hilar cells and in phase with triphasic synchronized IPSPs in the principal cells (Michelson & Wong, 1991). Triphasic synchronized IPSPs were never observed in bursting hilar neurones. Hyperpolarization of the neurones during the synchronized burst revealed a large amplitude depolarizing synaptic event sustaining the burst.

Addition of PTX to the bath ($n = 14$ cells) blocked the synchronized burst and the underlying depolarizing event (Fig. 3*A*) in a subpopulation of the bursting hilar neurones. We termed the group of hilar neurones in which synchronized bursting activity was blocked by PTX as type I hilar neurones. The results suggest that synchronized bursts in type I hilar neurones are sustained by depolarizing postsynaptic responses mediated by $GABA_A$ receptors (Alger & Nicoll, 1982*b*; Wong & Watkins, 1982; Michelson & Wong, 1991). Figure 3*B* also shows that, in some instances, monophasic synchronized IPSPs began to appear in type I hilar neurones following the addition of PTX. Obviously, monophasic synchronized IPSPs were not generated by type I hilar neurones.

A second group of hilar neurones which exhibited rhythmic bursts before PTX continued to fire bursts of action potentials after PTX was added to the bath (type II hilar neurones; Fig. 3*C*). In these neurones ($n = 12$), PTX suppressed the depolarizing wave underlying synchronized

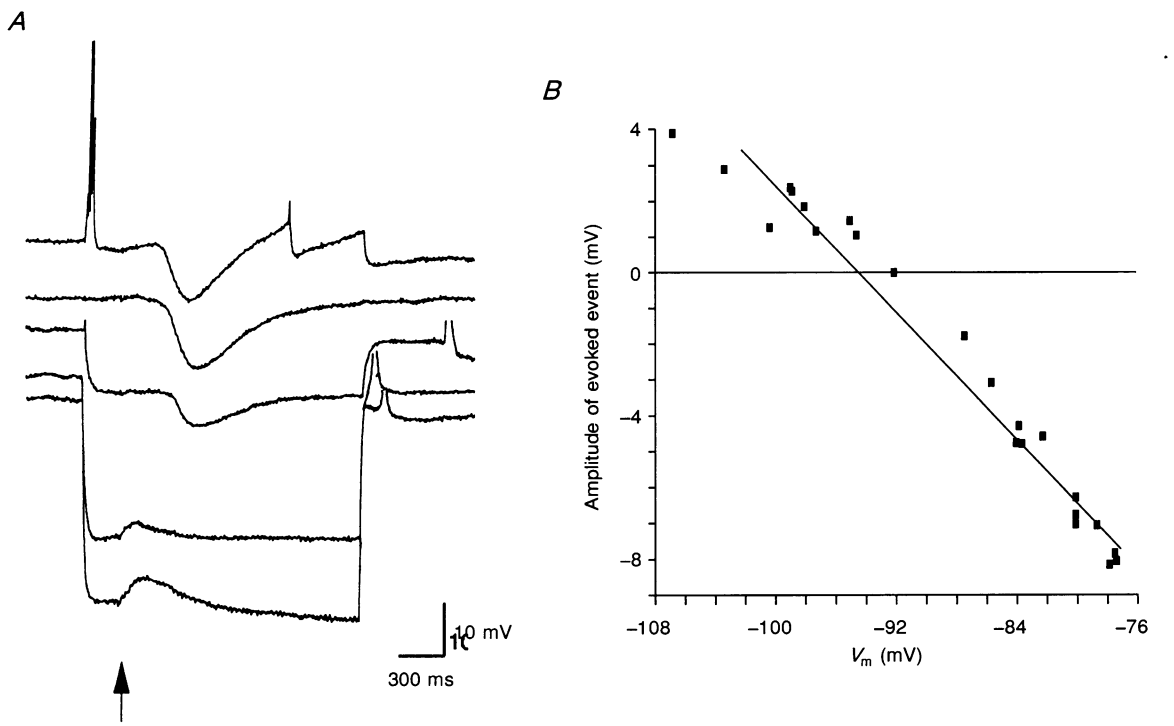

Figure 2. Reversal potential estimations for the monophasic synchronized event in a CA3 pyramidal cell after the addition of PTX to the bath

4-AP, CPP and CNQX were also present. *A*, membrane potential was altered by current injection into the cell and the synchronized event was evoked (arrow) via a stimulating electrode placed spanning the hippocampal fissure. Stimulus intensity was increased when the event was close to reversal, causing the response to be time locked to the stimulus at a shorter latency. The procedure did not affect the amplitude of the event (Aram *et al.* 1991) but allowed easier identification of the evoked event. *B*, plot relating the amplitude of the evoked event to the membrane potential (V_m).

bursting and reduced the number of action potentials associated with each burst. However, bursting activity itself was not blocked. In addition, the interval between the rhythmic synchronized bursts was increased in the same manner as was the rhythm of the synchronized IPSPs in principal cells, after PTX was added to the bath. The bursts continued to occur coincident with the synchronized monophasic IPSPs in pyramidal cells.

Generation of synchronized burst firing in type II hilar neurones following PTX

The burst events in type II hilar neurones recorded in PTX consisted of trains of action potentials lasting 300–800 ms which arose abruptly from baseline with no preceding or underlying membrane depolarization (Fig. 4). Hyperpolarization of the cell by current injection through the recording electrode reduced the spontaneous firing rate; however, synchronized burst firing persisted. With additional hyperpolarization, full amplitude action potentials were blocked, uncovering attenuated spikes (3–10 mV), and bursts of these attenuated spikes occurred in the hilar cell simultaneous with the monophasic synchronized IPSPs in principal cells. Finally, with sufficient hyperpolarization of the hilar cells, all spiking activity associated with synchronized IPSPs in principal cells was blocked. In no instance was a synaptic depolarization sustaining the burst firing uncovered in type II hilar neurones after PTX. Such an underlying EPSP would have occurred if the bursts in type II hilar neurones were sustained by chemical excitatory synapses, as was the case in type I hilar cells coupled by GABAergic excitation mediated via $GABA_A$ receptors (as shown in Fig. 3*A* and *B*).

Properties of burst-firing hilar cells

These results show that synchronized bursts in hilar cells are sustained by at least two different mechanisms – one dependent on chemical transmission mediated by GABA and the other most probably independent of chemical transmission. Additional data revealed that these bursts were generated in two classes of neurones distinguishable by their action potential patterns.

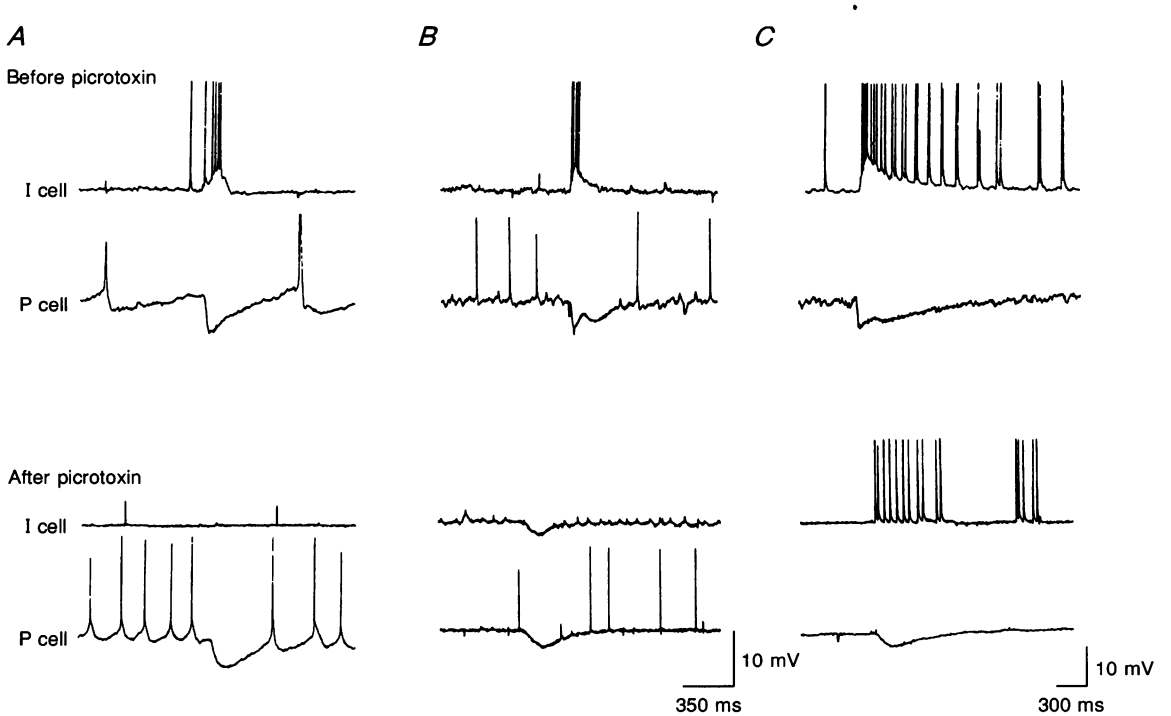

Figure 3. Hilar neurones which burst in 4-AP, CPP and CNQX exhibit different responses following PTX application

Traces illustrate simultaneous intracellular recordings of bursting hilar neurones (I cells) and CA3 pyramidal cells (P cells). *A*, some bursting hilar neurones become silent after PTX application and do not contribute to the generation of the monophasic synchronized IPSP. *B*, some bursting hilar neurones exhibit the monophasic synchronized IPSP after PTX, coincident with the same event in a CA3 pyramidal cell. Cells exhibiting responses similar to those shown in *A* and *B* were referred to as type I hilar neurones. *C*, some hilar neurones which burst before PTX application continued to fire in bursts after PTX. These bursts were correlated with the monophasic synchronized IPSP in pyramidal cells. These cells were termed type II hilar neurones.

For type II hilar neurones, in which synchronized bursts continued to occur in PTX, the action potential patterns that occurred between the synchronized bursts varied depending on the resting potential. At sufficiently hyperpolarized levels, attenuated spikes of no larger than 10 mV were recorded (Fig. 5*B*). Depolarization of the cell from this level caused spontaneous burst firing in the cell. These bursts differed from the synchronized bursts in that: (1) they were blocked upon hyperpolarization before synchronized bursts were suppressed; (2) they had shorter durations (<200 ms) than synchronized bursts (>300 ms); and (3) their spontaneous rate of occurrence was much higher than that of the synchronized bursts. With additional depolarization, spontaneous firing in type II hilar neurones consisted mainly of single action potentials. Most action potentials occurred rhythmically, arising from a gradual membrane depolarization (pacemaker potential). However, some spikes also arose abruptly from baseline or from subthreshold levels, such as during a spike after-hyperpolarization (Fig. 6).

Figure 5*A* shows the spontaneous activity recorded from a cell where burst firing was sustained by synaptic depolarizations, as in the case of type I hilar cells coupled by the depolarizing $GABA_A$ mechanism. In these cells, increasing levels of depolarization increased the frequency of action potential firing without, in general, eliciting spontaneous bursting at any level. Conversely, increasing levels of hyperpolarization gradually suppressed cell firing. Attenuated spikes were never observed in type I hilar cells. In addition, action potentials arising abruptly from baseline were also not detected in these cells.

Morphology of burst-firing hilar neurones

Hilar neurones were filled with the fluorescent dye Lucifer Yellow in order to examine their morphology. Previous studies (Michelson & Wong, 1991) indicated that the somata of some type I hilar neurones which formed excitatory $GABA_A$-mediated recurrent connections were located subjacent to the superior blade of the dentate gyrus. They were multipolar sparsely spiny neurones with dendritic trees which arborized extensively within the hilus, primarily parallel to the plane of the superior blade. The dendrites of these neurones only rarely crossed into the granule cell layer. Morphologically, these cells resembled hilar neurones which are positively immunoreactive for glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA, suggesting that they were GABAergic interneurones (Kosaka, Wu & Benoit, 1988).

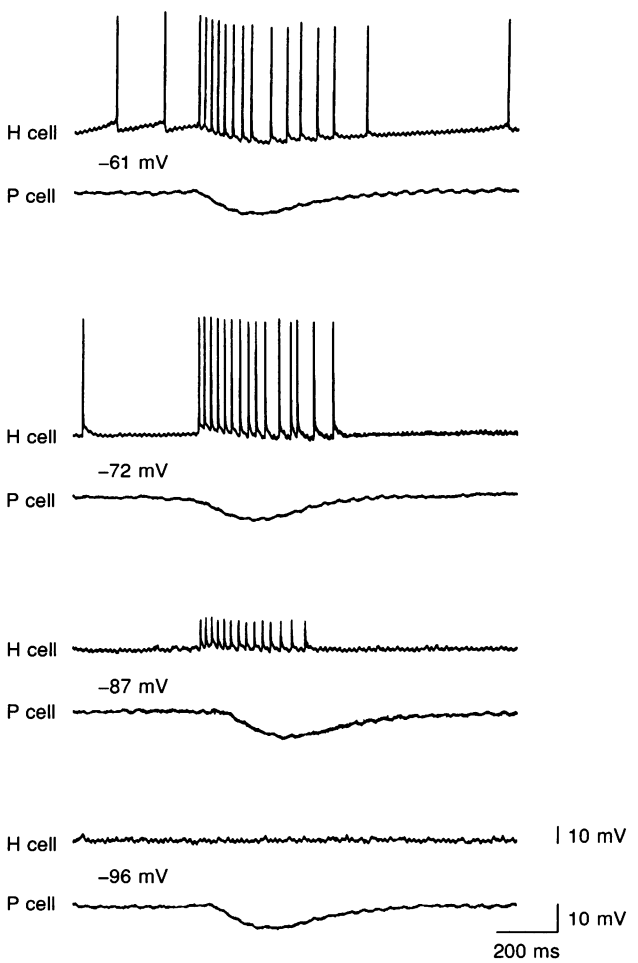

Figure 4. Simultaneous intracellular recordings from a type II hilar neurone and a CA3 pyramidal cell in the presence of 4-AP, CPP, CNQX and PTX
The hilar cell (H cell) was progressively hyperpolarized by current injection to the membrane potentials shown. The membrane potential of the CA3 pyramidal cell (P cell) remained at −65 mV throughout the experiment. Type II hilar neurones exhibited bursts of action potentials coincident with the monophasic synchronized IPSPs in pyramidal cells. This hilar neurone also received input from neurones generating the monophasic synchronized IPSP (upper trace). Hyperpolarization reduced spontaneous firing but does not block the synchronized burst event. Further hyperpolarization blocked the production of full amplitude action potentials and revealed spikes with attenuated amplitudes. With additional hyperpolarization, all spiking activity was blocked. Note that hyperpolarization does not uncover depolarizing synaptic potentials sustaining the burst in type II hilar neurones.

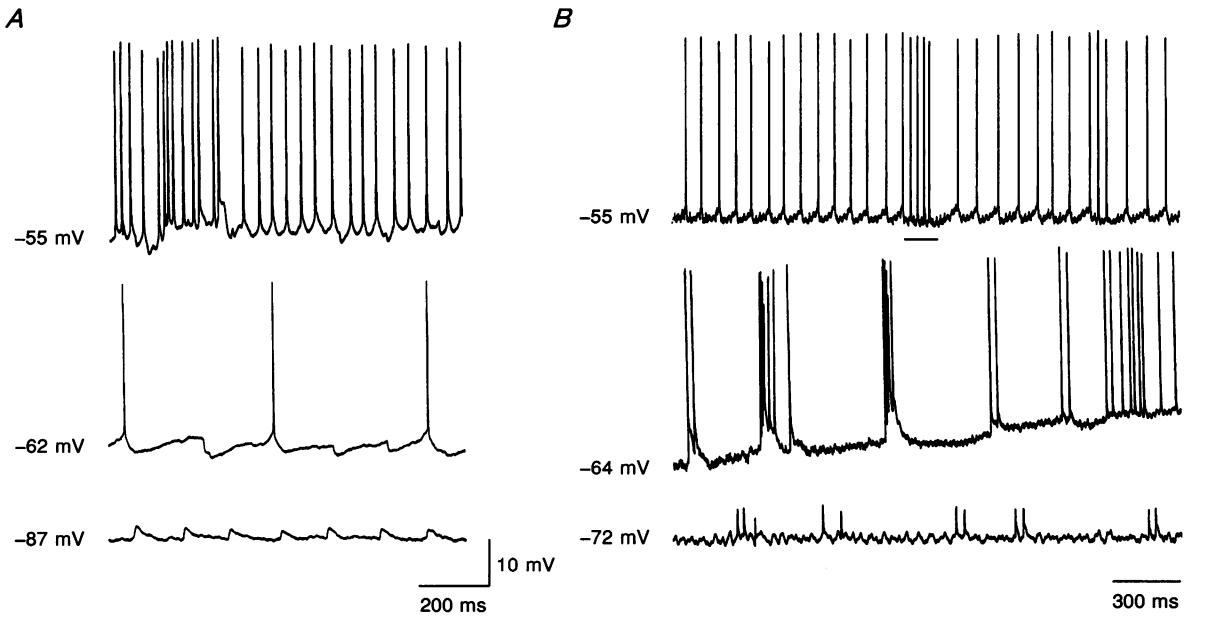

Figure 5. Comparison of the firing patterns of type I and type II hilar neurones
A, type I hilar neurones fired single action potentials which decreased in frequency as the cell was hyperpolarized until firing was completely suppressed. Spontaneous unitary IPSPs were recorded in this cell. With sufficient hyperpolarization (−87 mV), the unitary IPSPs reversed and became depolarizing. *B*, at resting potential (−55 mV) type II hilar neurones exhibited single action potentials arising from a gradual depolarization and spikes which arose abruptly from baseline, indicated by the line. When slightly hyperpolarized (−64 mV), these cells fired in short-duration bursts. With increased hyperpolarization, spontaneous action potentials were blocked, and spikes with truncated amplitudes were revealed.

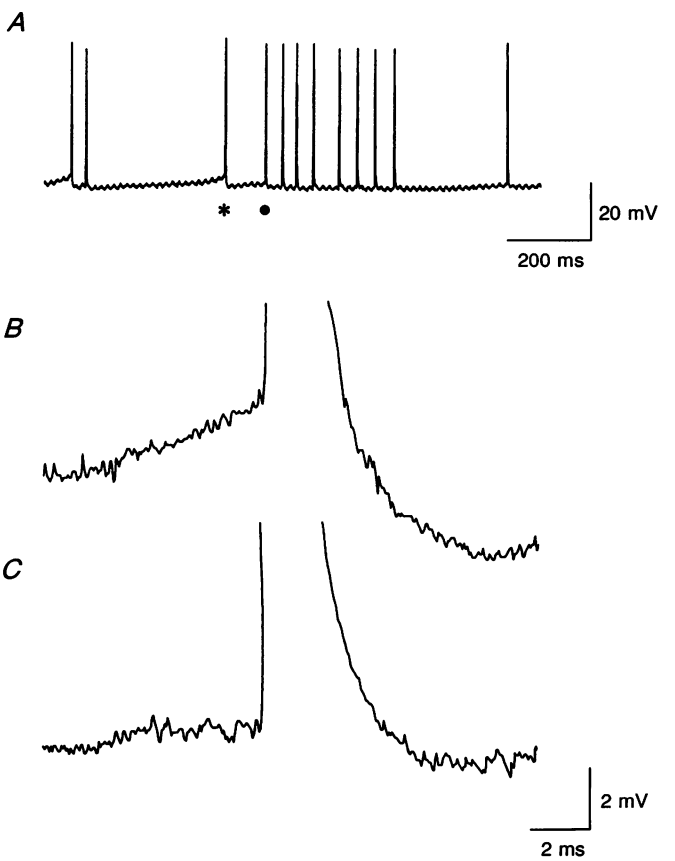

Figure 6. Comparison of spike firing thresholds in type II hilar neurones
A, cells exhibited spikes which fire from a gradual depolarization (asterisk) and spikes which arise abruptly from baseline without a preceding depolarization (dot). *B*, spike marked by asterisk in *A*, on expanded time base. *C*, spike marked by dot in *A*, on expanded time base. Spikes which arose abruptly from baseline were not observed in type I hilar neurones.

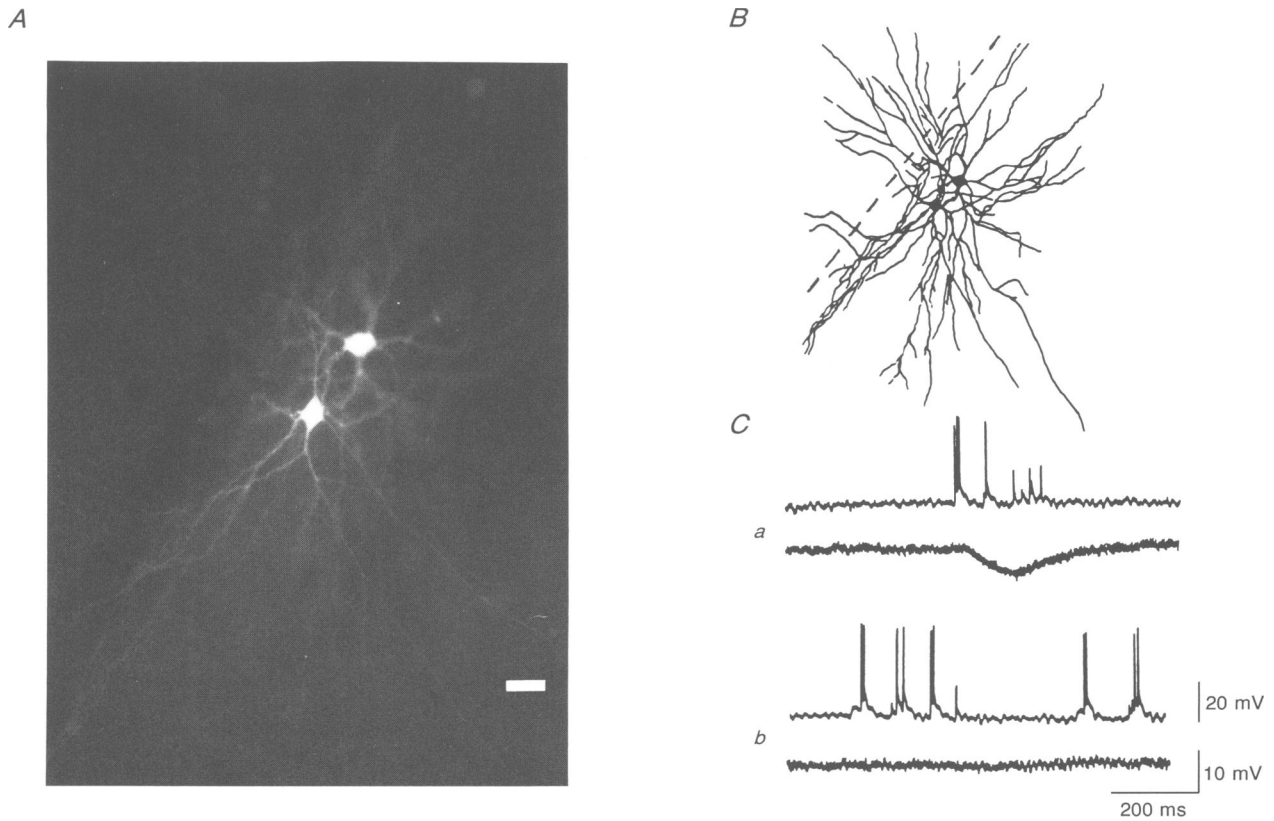

Figure 7. Type II hilar neurones exhibit dye coupling
A, two hilar neurones were labelled following injection of LY into one type II hilar neurone. Calibration bar, 25 μm. *B*, neurones traced from slides of the fluorescent images of hilar neurones filled intracellularly with LY shown in *A*. The dashed line represents the inner border of the dentate granule cell layer. *C*, recordings of the impaled hilar neurone shown in *A*. The type II hilar neurone showed bursts which correlated with the monophasic synchronized IPSP in a simultaneously recorded CA3 pyramidal cell (*a*) and spontaneous bursts (*b*).

In the present study, type II hilar neurones which continued to burst in the presence of 4-AP, EAA blockers and PTX ($n = 7$) shared morphological features that distinguished them from the type I hilar neurones described above. The cell bodies of some of these neurones could also be located subjacent to the superior blade of the dentated gyrus. These neurones were sparsely spiny multipolar cells with dendritic arborizations which ramified extensively within the hilus. However, they also sent several dendritic branches through the granule cell layer and into the molecular layer of the dentate. In addition, in four out of seven experiments where LY was injected into one type II hilar cell, more than one cell was marked by LY. Dye coupling was observed between the injected cell and one to three others. The dye coupling was always observed between multipolar sparsely spiny cells; coupling between these cells and granule cells or spiny mossy cells was not observed. One example of dye coupling between hilar neurones is shown in Fig. 7. In no instance was dye coupling observed in type I hilar neurones in which bursting was blocked by PTX.

DISCUSSION

The experiments in the present study demonstrate that the inhibitory circuit in the hippocampus is more complex than previously understood. Earlier studies indicated that inhibitory cells are activated by recurrent and feedforward pathways (Andersen, Gross, Lømo & Sveen, 1975; Alger & Nicoll, 1982*a*). Both these pathways employ glutamatergic synapses to activate inhibitory neurones, implying that the activation of the inhibitory circuit could not occur in the absence of glutamate-mediated excitation.

In the present study we confirmed our previous findings that recruitment of inhibitory neurones could occur following blockade of fast glutamatergic neurotransmission, resulting in the generation of synchronized IPSPs in all principal cells in the hippocampus. The triphasic synchronized IPSPs are generated by GABAergic neurones which are simultaneously burst firing. These inhibitory cells are recurrently connected and were recruited to burst fire synchronously via the depolarizing action of GABA on $GABA_A$ receptors (Michelson & Wong, 1991).

Following blockade of this GABA-mediated synchronization process by PTX, a monophasic synchronized IPSP continued to be generated in principal cells, which had similar characteristics to the potassium-mediated synchronized IPSP reported by others (Misgeld, Bijak, Brunner & Dembrowsky, 1992). The results of the present study indicate that a subpopulation of interneurones (type II hilar neurones) burst fire to generate the monophasic synchronized IPSP. However, in contrast to type I hilar neurones, the bursts in these interneurones are not sustained by an EPSP, suggesting that these cells synchronized their firing through a process which is not mediated via known mechanisms involving chemical synapses.

The inhibitory circuit recruitment processes were facilitated by the presence of the convulsant compound 4-aminopyridine. 4-AP is a unique convulsant, in that it can elicit hyperexcitation in cortical neurones without compromising inhibitory processes (Voskuyl & Albus, 1985; Segal, 1987; Rutecki, Lebeda & Johnston, 1987; Perreault & Avoli, 1991). The enhancement of synaptic neurotransmission in the presence of 4-AP could have facilitated the occurrence of the synaptically mediated depolarizing GABA response among hilar neurones. The fact that the depolarizing GABA response in hilar neurones is synaptic differentiates it from the depolarizing GABA response in pyramidal cells, which is observed only following exogenous GABA application (Alger & Nicoll, 1982*b*; Wong & Watkins, 1982). Other investigators have demonstrated that large amplitude IPSPs occur in pyramidal cells in the presence of 4-AP and EAA receptor antagonists (Muller & Misgeld, 1991; Perreault & Avoli, 1992). Large amplitude IPSPs were also elicited when zinc was added to the perfusion solution (Xie & Smart, 1992; Lambert, Levitin & Harrison, 1992). These studies also show that the IPSPs persisted in ionotropic glutamate receptor blockers.

The action potential firing patterns suggest that spikes from type II hilar neurones were generated from ectopic initiation sites. These neurones frequently exhibited spikes which arose directly from baseline without any preceding depolarization, intermingled with spontaneous action potentials arising from pacemaker potentials. In contrast, type I hilar neurones exhibited only action potentials arising from a preceding pacemaker depolarization. In addition, type II hilar neurones exhibited attenuated action potentials which were only revealed following membrane hyperpolarization. Attenuated spikes were also observed on occasion in principal cells and type I hilar neurones (Perreault & Avoli, 1989; Aram *et al.* 1991). However, we observed only in type II hilar neurones the coincident occurrence of bursts of attenuated spikes with the monophasic synchronized IPSPs in principal cells. LY injection into a type II hilar neurone frequently resulted in the staining of more than one cell. The fluorescent dye Lucifer Yellow is known to cross gap junctions and is an indicator of such functional connections between neurones (Stewart, 1978; Dudek, Andrew, MacVicar, Snow & Taylor, 1983; Connors, Benardo & Prince, 1984). Together, these results raise the possibility that this subset of interneurones is coupled by gap junctions.

Other studies have demonstrated that gap junctions exist between interneurones in the hippocampus (Kosaka, 1983; Kosaka & Hama, 1985), particularly in the hilus (Katsumaru, Kosaka, Heizmann & Hama, 1988). Gap junctions are found specifically on the subgroup of interneurones which stain positively for the calcium-binding protein parvalbumin (Katsumaru *et al.* 1988). Parvalbumin-containing hilar neurones have dendrites which pass out of the hilus and into the granule cell and molecular layers of the dentate gyrus (Kosaka, Katsumaru, Hama, Wu & Heizman, 1987), similar to the dendritic arborization observed in the type II hilar neurones in this study. It seems possible that the parvalbumin-containing interneurones may be electrotonically coupled and thus may also be involved in the generation of the monophasic synchronized IPSPs in the present experiments.

Of the two glutamate-independent recruitment processes observed in the inhibitory circuit, the more dominant synchronization mechanism appears to be the mechanism mediated through the recurrent excitatory GABA response. This synchronization process paced the rhythm for the spontaneous burst firing of interneurones producing the triphasic synchronized IPSP. The second recruitment process, which generated the slower, monophasic synchronized IPSP was entrained by the excitatory $GABA_A$ mechanism. Before the addition of PTX, the synchronized IPSP was always triphasic; in no instance did the slower monophasic synchronized event occur independently. In the presence of PTX, the rhythm became destabilized as the depolarizing GABA response was blocked. The second synchronization process then emerged, independently occurring at a consistently slower rhythm. This finding suggests that the two events are generated by separate mechanisms which can synchronously activate the inhibitory circuit independently. The finding that, prior to the addition of PTX the synchronized event is invariably triphasic and occurs at a faster rhythm than the monophasic event does independently, suggests that the depolarizing GABA synchronization mechanism is the primary synchronizing process.

The mechanism underlying the depolarizing GABA response remains unclear. Some investigators (Misgeld & Lux, 1986) have suggested that the depolarizing GABA response results from a reversed chloride gradient in the pyramidal cell dendrites. However, it is also possible that the depolarizing GABA response is mediated by a separate receptor complex (Wong & Watkins, 1982; see also Nicoll, Malenka & Kauer, 1990, for review). We have demonstrated that unitary EPSPs and IPSPs can be recorded in bursting hilar neurones in the presence of CPP

and CNQX, and that both these synaptic responses can be blocked by PTX (Michelson & Wong, 1991). Similarly, application of GABA elicited both depolarizing and hyperpolarizing responses in the dendrites and the somata of pyramidal cells (Wong & Watkins, 1982). Thus, while the mechanism for the generation of the depolarizing GABA response is not known, the data show that distinct regions of the same cell can produce each of these responses separately.

The implications of these findings are severalfold. Firstly, this study indicates that the inhibitory circuit extends beyond the feedforward and feedback recuitment pathways previously described. Recruitment of inhibition does not solely depend upon glutamate-mediated excitation from pyramidal cells; interneurones are capable of recruiting neighbouring interneurones via synaptically mediated depolarizing GABA responses or via non-chemical means of neurotransmission. It is clear that these non-glutamate-dependent recruitment mechanisms exist for inhibitory neurones throughout the hippocampus. Secondly, the present findings, and those of others (Amaral, 1978; Kawaguchi & Hama, 1988; Williams & Lacaille, 1992) suggest that both a structural and a functional heterogeneity exist within the hippocampal interneurone population. Heterogeneity is also suggested by the differential sensitivity of specific interneurone subtypes to ischaemic or excitotoxic insult (Sloviter, 1987; Obenhaus, Esclapez & Houser, 1993). Further research should elucidate the role of these synchronization processes in the differential activation of structurally and functionally identified inhibitory neurone subtypes during normal and pathophysiological conditions.

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