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UNITARY INHIBITORY SYNAPTIC POTENTIALS IN THE GUINEA-PIG HIPPOCAMPUS *IN VITRO*

BY R. MILES AND R. K. S. WONG

From the Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550

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SUMMARY

1. Mechanisms involved in the generation of synaptic inhibition have been investigated by making simultaneous intracellular recordings from pairs of neurones in the CA3 pyramidal cell field of guinea-pig hippocampal slices.

2. Inhibitory post-synaptic potentials dependent on single presynaptic action potentials (i.p.s.p.s) and mediated through monosynaptic and disynaptic connexions have been identified. The recurrent nature of some hippocampal inhibition has been demonstrated by showing that activity in a single cell may initiate feed-back i.p.s.p.s onto itself. The observation of synchronous i.p.s.p.s in recordings from two cells illustrates the divergence of synaptic contacts made by inhibitory neurones.

3. The peak conductance change associated with an i.p.s.p. was in the range 5–9 nS and it reversed uniformly throughout its time course at membrane potentials between −73 and −80 mV. The shortest time-to-peak of synaptic potentials was approximately 3 ms and in this case the i.p.s.p. decayed with a time constant comparable to the passive membrane time constant of the post-synaptic neurone.

4. The peak amplitude of i.p.s.p.s fluctuated in a way consistent with the quantal release of inhibitory neurotransmitter.

5. Inhibitory neurones could fire bursts of action potentials not unlike those generated by pyramidal cells in this area.

6. A comparison of the conductance change associated with identified i.p.s.p.s with that associated with the maximal inhibitory post-synaptic potential resulting from electrical stimulation of fibre pathways suggested that, in the slice, a pyramidal cell is innervated by up to fifteen inhibitory neurones.

INTRODUCTION

Large, prolonged inhibitory potentials are observed in hippocampal pyramidal cells on stimulating afferent fibres. These events were first attributed to local synaptic interactions by Spencer & Kandel (1961) since they could also be evoked by selectively stimulating pyramidal cell axons. Andersen, Eccles & Loyning (1963) suggested that this feed-back inhibition involves an inhibitory neurone which makes synapses largely onto the soma of pyramidal cells. Recent evidence has suggested that

dendritically located synapses and feed-forward circuits (Fujita, 1979; Alger & Nicoll, 1982) also contribute to the generation of inhibition in the hippocampus.

Previous studies of inhibitory synaptic interactions have usually taken the form of recording, in a single neurone, synaptic responses to orthodromic or antidromic stimulation. However, this procedure often activates excitatory synapses as well as inhibitory synapses (Dingledine & Gjerstad, 1979; Ben-Ari, Krnjevic, Reiffenstein & Reinhardt, 1981; Alger & Nicoll, 1982). Furthermore, the interactions between cells involved in the generation of synaptic inhibition cannot be examined directly. An alternative approach is to look for inhibitory interactions in intracellular recordings made simultaneously from pairs of neurones. In this way inhibitory connexions between cells in the CA1 (Knowles & Schwartzkroin, 1981) and CA3 (MacVicar & Dudek, 1980) hippocampal fields have been demonstrated directly. However, discrete inhibitory potentials elicited through monosynaptic connexions have not been observed in these studies and the properties of the unitary synaptic event remain unclear.

In this report we have studied inhibitory synaptic mechanisms by making paired intracellular recordings in the CA3 region of the guinea-pig hippocampus *in vitro*. The results provide information on the properties of unitary i.p.s.p.s and the characteristics of the inhibitory neurones generating these events.

METHODS

Guinea-pigs weighing 200–300 g were stunned by a blow to the back and killed by cutting the neck so as to sever the jugular vein and carotid artery. The forebrain was quickly removed and one hippocampus dissected free. Its central portion was stuck to a small block with cyano-acrylate glue and placed underneath cold perfusing solution in a vibratome tissue slicer (Lancer Co.). Several transverse hippocampal slices of nominal thickness 400 μm were cut and transferred to a recording chamber. Slices were supported on nylon mesh with their lower surface in contact with a perfusing solution of the following composition (mM): NaCl, 124; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; $NaHCO_3$, 26 and D-glucose, 10. Their upper surface was exposed to a warmed, moistened atmosphere of 5% CO_2 in O_2, resulting in a pH of 7·4. The temperature of the chamber was maintained at 37 °C.

Recording electrodes were pulled from 1 mm diameter fibre-filled glass capillaries (F. Haer). They were filled with 3 M-K acetate and bevelled to a resistance of 60–90 MΩ before use. Signals were amplified by two high-input impedance amplifiers, with facilities for current injection through the recording electrode using an active bridge circuit, and for capacitance compensation (WPI M707). In some experiments fibre pathways were stimulated with bipolar tungsten electrodes of tip diameter 20 μm and separation 200 μm, using pulses of duration 100 μs and intensity up to 10 V. During an experiment signals were displayed on a digital oscilloscope (Nicolet 4562) and stored using a FM tape recorder (Vetter) for subsequent analysis.

Penetrations were made less than 200 μm apart in the stratum pyramidale and nearby stratum oriens of the CA3 region, with recording electrodes mounted on two independent manipulators (Zeiss, Jena) and approaching the slice from angles of about 15 deg on either side of the vertical. When stable recordings with membrane potentials greater than 60 mV and overshooting action potentials were obtained from two cells, the oscilloscope was set to trigger from action potentials in one neurone and the trace of the other neurone was examined for inhibitory interactions. The procedure was then reversed to detect connexions in the opposite direction. The membrane potential of the putative presynaptic neurone was controlled by maintained current injection to achieve a firing frequency of about one action potential per second. Membrane potentials were measured from the potential change when the electrode was withdrawn from the cell. Neuronal input resistance and time constant were determined from responses to hyperpolarizing current injections of intensity less than 0·5 nA and duration 80–120 ms.

The following abbreviations will be used:
i.p.s.p., inhibitory post-synaptic potential elicited by activity in a single inhibitory neurone;
population i.p.s.p., maximally evoked inhibitory post-synaptic potential dependent on activity in a population of inhibitory neurones.

RESULTS

Properties of unitary inhibitory synaptic potentials

This report is based on recordings from over 300 neurones in the CA3 region of guinea-pig hippocampal slices. At normal resting potentials of −60 to −70 mV spontaneous hyperpolarizing potentials such as those shown in Fig. 1*A* were seen in

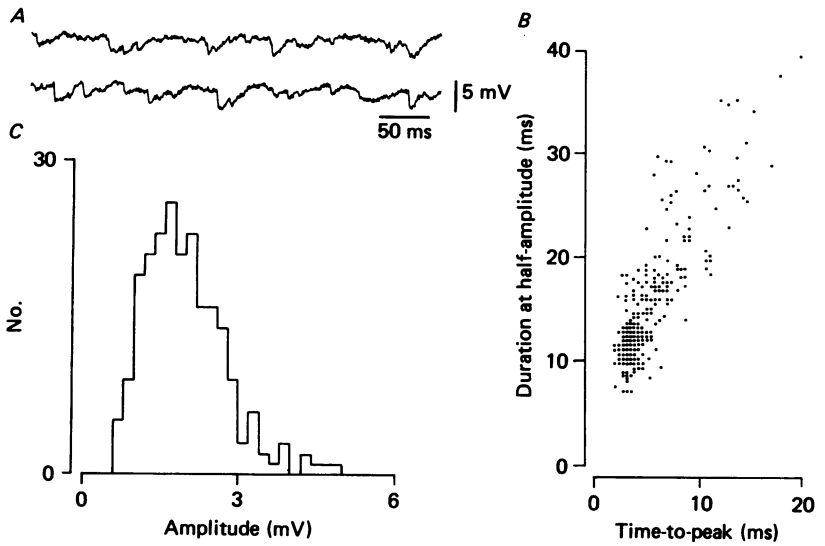

Fig. 1. *A*, records of spontaneous synaptic activity recorded from a CA3 pyramidal cell. *B*, time-to-peak of 223 inhibitory events plotted against their duration at half-amplitude. *C*, distribution of peak amplitudes of these events. Measurements were made of synaptic events with peak amplitude greater than −0·5 mV and which decayed by more than half their peak amplitude before another synaptic event occurred. The resting potential of this neurone varied between −62 and −64 mV and unusually few excitatory synaptic events were observed.

recordings from nearly all neurones. Such events appeared to reverse polarity during membrane hyperpolarization to potentials greater than −75 to −80 mV and were never observed in the presence of the γ-aminobutyric acid (GABA) antagonist picrotoxin (10^{-4} M). These synaptic potentials therefore seem likely to depend on the release of GABA from the synaptic terminals of inhibitory neurones.

The time-to-peak of spontaneous inhibitory potentials was typically 3–5 ms but, as shown in Fig. 1*B*, hyperpolarizing events with a longer time-to-peak were also sometimes observed. Since the rise time of a synaptic potential depends on its electrotonic distance from the recording site (Rall, 1967) it seems probable that inhibitory synapses are made at several different locations on the soma and dendritic processes of each neurone. Furthermore, the observation that many inhibitory

synapses are located on the soma of CA3 pyramidal cells (Gottlieb & Cowan, 1972; Ribak, Vaughn & Saito, 1978) suggests that the synapses responsible for the fastest rising inhibitory events were located on, or very close to, the soma. These synaptic potentials also had the shortest duration (Fig. 1*B*) and their decay resembled a single exponential function with a similar time constant to that of the cell membrane (15–40 ms). The amplitude of spontaneous inhibitory potentials detectable above the noise of the recording system (0·2–0·5 mV) ranged up to a maximum of about 4 mV. The distribution of amplitudes for one neurone is shown in Fig. 1*C*. However, further analysis of spontaneous synaptic events is difficult. The variation in times-to-peak suggests that they represent post-synaptic responses to transmitter released at more than one presynaptic site. Furthermore, synaptic events resulting from presynaptic action potentials cannot be differentiated from those due to spontaneous release of inhibitory transmitter (Brown & Johnston, 1980).

Simultaneous recordings from 129 pairs of neurones were examined for inhibitory interactions. In ten cases the observation of synchronous inhibitory synaptic potentials in both cells (Fig. 2) suggested that a spontaneously active, inhibitory neurone was presynaptic to both cells (Kandel, Frazier, Waziri & Coggeshall, 1967). This demonstrates the divergence of synaptic contacts made by an inhibitory neurone predicted by Andersen *et al.* (1963). Furthermore, it allows measurements to be made of some properties of an inhibitory post-synaptic potential dependent on activity in an identified inhibitory neurone (i.p.s.p.). I.p.s.p.s with the same time of onset occurred spontaneously in recordings from neurones shown in Fig. 2. Simultaneous i.p.s.p.s were also observed on some occasions following action potentials elicited in one neurone by depolarizing current injection. The reversal potential and peak conductance of the i.p.s.p. occurring in the other neurone was determined by manipulating its membrane potential. The assumption that a single inhibitory neurone was responsible for all simultaneous i.p.s.p.s was supported to some extent by the observation that their shape varied little. The mean time-to-peak was $3{\cdot}2 \pm 0{\cdot}6$ ms (mean $\pm$ S.D., $n = 52$) in one neurone and $3{\cdot}8 \pm 0{\cdot}9$ ms ($n = 52$) for the other neurone.

I.p.s.p.s identified in this way reversed uniformly throughout their time course at a mean membrane potential of 78 ± 6 mV (mean $\pm$ S.D., $n = 7$) determined from straight lines fitted to plots of i.p.s.p. amplitude against membrane potential (Fig. 2*C*). The peak i.p.s.p. conductance, $G_{\text{i.p.s.p.}}$, was obtained using the following relationship, derived from that of Ginsborg (1973)

$$\frac{1}{G_{\text{i.p.s.p.}}} = R_{\text{m}} \frac{V_{\text{m}} - V_{\text{rev}}}{V_{\text{i.p.s.p.}}} - 1,$$

where R_{m} is the input resistance of the neurone, V_{m} the membrane potential, $V_{\text{i.p.s.p.}}$ the peak amplitude of the i.p.s.p. and V_{rev} its reversal potential. Using direct measurements of R_{m} and values of $(V_{\text{m}} - V_{\text{rev}})/V_{\text{i.p.s.p.}}$ derived from the slope of plots such as that of Fig. 2*C*, the mean peak conductance was found to be $6{\cdot}7 \pm 2{\cdot}3$ nS ($n = 7$). The mean i.p.s.p. amplitude at resting potential in the post-synaptic cell was between 1 and 3 mV. For cell 2 of Fig. 2 it was $-2{\cdot}5 \pm 1{\cdot}1$ mV ($n = 16$) at membrane potentials between -62 and -66 mV. Two possibilities could account for the large amplitude and the wide fluctuation observed throughout the voltage range tested. More than

one inhibitory neurone may have contributed to the i.p.s.p. Alternatively, the amplitude of an i.p.s.p. dependent on an action potential in one inhibitory neurone may be about 2 mV with fluctuations resulting from variations in transmitter release.

Simultaneous recordings from an inhibitory neurone and a cell to which it was monosynaptically coupled confirmed that i.p.s.p.s could vary widely in amplitude.

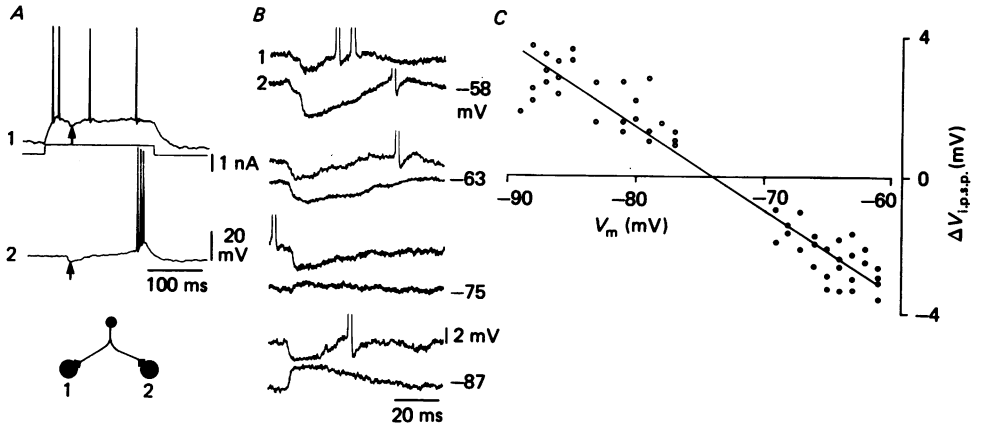

Fig. 2. Determination of reversal potential and peak conductance change for an identified i.p.s.p. The synchronicity of synaptic events, within 0·7 ms, in recordings from two cells, 1 and 2, was used to identify the action of a common inhibitory neurone. *A*, the simultaneous i.p.s.p. (arrows) sometimes followed action potentials elicited in cell 1 by current injection. Membrane potential of cell 2 was varied by hyperpolarizing and depolarizing current injection with the amplifier bridge balance repeatedly checked. *B*, simultaneous i.p.s.p.s at four different membrane potentials in cell 2. *C*, the peak amplitude, $\Delta V_{i.p.s.p.}$, of fifty-two i.p.s.p.s in cell 2 plotted against membrane potential, V_m. Events such as that shown in the upper traces of *B*, where two, temporally separate, i.p.s.p.s appeared in both traces, were excluded. A straight line, fitted by linear regression analysis, indicated that the i.p.s.p. reversed at approximately −74 mV. The slope of the straight line, 0·22, and the input resistance of cell 2, 24 MΩ, gave a value of 6·8 nS for the peak conductance change due to this i.p.s.p.

Two factors suggested that the inhibitory connexion between the cells shown in Fig. 3 involved only one synapse. The mean latency was $0{\cdot}7 \pm 0{\cdot}2$ ms ($n = 251$). Furthermore, no failures of synaptic transmission occurred at presynaptic firing frequencies less than about 100 Hz. The mean time-to-peak of the i.p.s.p. was $3{\cdot}5 \pm 0{\cdot}6$ ms, suggesting that the synapse was made either on or very close to the soma. If this was the case, the decay of potential following the peak of the i.p.s.p. should closely resemble that occurring at the post-synaptic site. Fig. 3*E* shows that the decay conformed closely to a single exponential function with time constant 18·2 ms, while the membrane time constant of the post-synaptic cell was 19·6 ms.

The i.p.s.p. reversed uniformly throughout its time course at a potential of about −77 mV determined graphically as in Fig. 2. The mean peak conductance change was found to be 6·2 nS using the same technique to derive a value for $(V_m - V_{rev})/V_{i.p.s.p.}$ and a directly measured value of 27 MΩ for neuronal input resistance. This falls within the range of conductances determined for i.p.s.p.s identified by their synchronous

occurrence in paired recordings. Another feature noted in analysis of simultaneous i.p.s.p.s was a wide fluctuation in amplitude at a particular post-synaptic membrane potential. The peak amplitude of 281 i.p.s.p.s varied between 0·6 and 4·2 mV (Fig. 3*B*), with a mean value of 2·2 ± 0·7 mV. Together with the multimodal shape

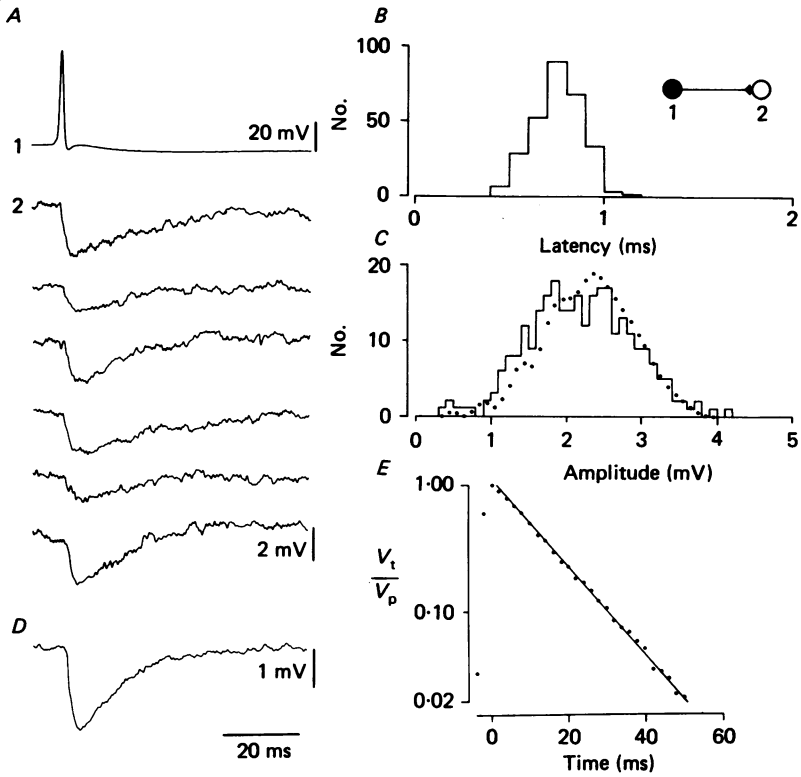

Fig. 3. Monosynaptic i.p.s.p. *A*, six responses in cell 2 to spontaneous single action potentials in cell 1. *B*, distribution of i.p.s.p. latencies, measured from the start of the upstroke of the presynaptic action potential. The mean latency was 0·7 ± 0·2 ms ($n = 281$). *C*, distribution of peak i.p.s.p. amplitudes. The mean amplitude was 2·1 ± 0·7 mV at post-synaptic resting potentials between −62 and −66 mV, and no failures of transmission were observed. The dotted curve is a binomial distribution with $q = 0{\cdot}46 \pm 0{\cdot}05$ mV, $n = 8$, and $p = 0{\cdot}58$. *D*, average of twenty i.p.s.p.s, triggered from the peak of the presynaptic action potential. *E*, semilogarithmic plot of the averaged time course of the i.p.s.p. Time $t = 0$ corresponds to the peak of the i.p.s.p. and the subsequent decay of the potential (V_t), normalized with respect to the peak potential (V_p), is plotted against time. Linear regression analysis showed that the i.p.s.p. decay was close to an exponential function with time constant of 18·2 ms.

of the i.p.s.p. amplitude distribution, this strongly suggests that a variable number of quanta of transmitter were released following each presynaptic action potential, as proposed by del Castillo & Katz (1954) at the frog neuromuscular junction. The distribution of i.p.s.p. amplitudes should then reflect both the amplitude (mean = q) and variability of the post-synaptic effect of a single quantum, and the fluctuations in the number of quanta released (mean = m). At neuromuscular synapses q is

commonly determined from the mean amplitude of activity-independent synaptic potentials. However, many synapses are made onto central neurones so q cannot be independently measured. In addition no transmission failures, which could be used to derive a value for m, were observed. However, using the variance technique (Hubbard, Llinas & Quastel, 1969), which assumes that the number of quanta released follows Poisson statistics, and correcting i.p.s.p. amplitudes for non-linear summation (Martin, 1955) values of 0·46 mV for q and 4·7 for m were derived.

It now seems likely that, unless artificially depressed, transmitter release follows binomial rather than Poisson statistics (Johnson & Wernig, 1971). Two parameters, n and p, rather than one, m, are needed to describe this distribution, and recent evidence suggests that n may correspond to a morphologically identifiable number of presynaptic terminals while p may be the probability of release at each terminal (Korn, Mallet, Triller & Faber, 1982). We found that a curve based on the binomial distribution (dotted curve in Fig. 3*C*) appeared to fit the i.p.s.p. amplitude distribution better than one based on a Poisson distribution. To generate this distribution we assumed that $m = n \times p$, that n was an integer and that q followed a normal distribution with standard deviation 10% of its mean value (cf Kuno & Weakly, 1972). These assumptions cannot be completely justified so the curve shown, where $n = 8$, $p = 0{\cdot}58$, and $q = 0{\cdot}46$ mV, indicates solely that a binomial distribution fitted the experimental data reasonably well.

Activation of inhibitory neurones

The identified i.p.s.p.s described here may have been activated through either feed-forward or recurrent pathways. In order to discriminate between these possibilities it is necessary to show whether a pyramidal cell can elicit an i.p.s.p. in another pyramidal cell through a disynaptic circuit involving an intercalated inhibitory neurone. The strength of such disynaptic pathways depends on the efficacy of transmission at excitatory synapses between pyramidal cells and inhibitory neurones. At other excitatory synapses it seems that many presynaptic cells must be synchronously activated to achieve a depolarization large enough to generate an action potential (Burke & Rudomin, 1977). The observation of apparently disynaptic i.p.s.p.s elicited by single action potentials in a pyramidal cell therefore suggests that excitatory synapses made onto inhibitory neurones may be extremely potent.

The inhibitory connexion between the two cells shown in Fig. 4 was considered to be disynaptic on the basis of its latency and the number of transmission failures. Approximately 20% of spontaneous action potentials in one cell were followed by an i.p.s.p. in the other neurone. Their mean latency was $3{\cdot}1 \pm 0{\cdot}7$ ms ($n = 61$). Assuming a delay at one synapse of 0·7 ms (Fig. 3) leads to the estimate that on average 1·7 ms elapsed between the onset of the excitatory post-synaptic potential (e.p.s.p.) and an action potential in the presumed inhibitory interneurone. Again the peak amplitude of this i.p.s.p. exhibited considerable, possibly quantal, fluctuations. Its mean value was $2{\cdot}7 \pm 1{\cdot}1$ mV and the corresponding peak conductance change was 9·5 nS. Another interesting feature of this i.p.s.p. was its long time-to-peak, $12{\cdot}2 \pm 3{\cdot}1$ ms, comparable to the slowest rising spontaneous i.p.s.p.s.

If synapses from pyramidal cells onto inhibitory neurones are as strong as these data suggest, it should be possible to see recurrent i.p.s.p.s in one neurone initiated

by action potentials in the same neurone, unless neuronal connectivity is such that inhibitory neurones never synapse on pyramidal cells from which they receive excitatory inputs. The recordings shown in Fig. 5 demonstrate that feed-back inhibitory circuits involving just two cells do exist. The simultaneous occurrence of spontaneous i.p.s.p.s in recordings from two neurones was again used to identify a

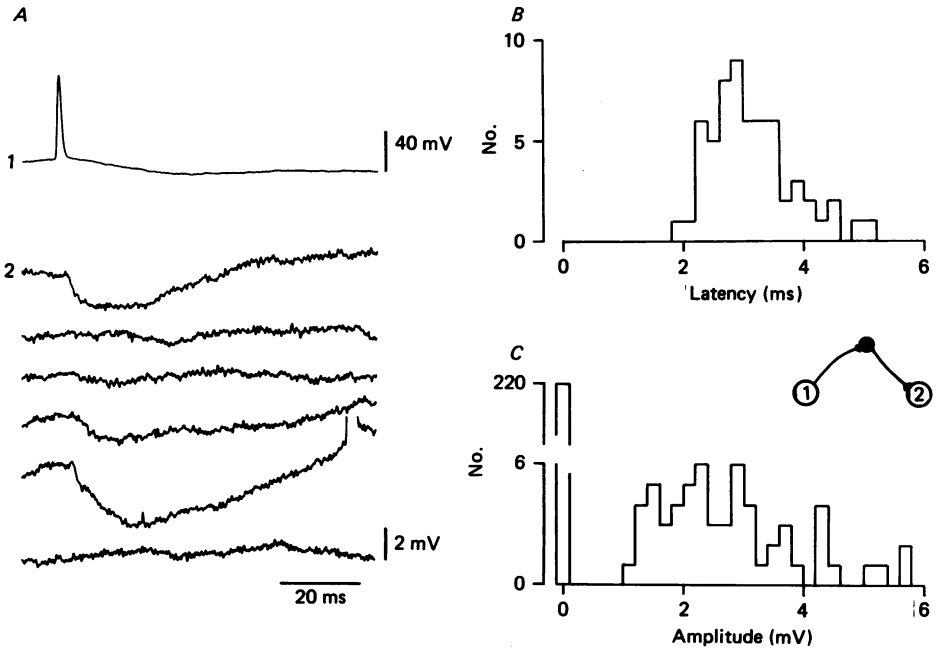

Fig. 4. Disynaptic i.p.s.p. *A*, six responses in cell 2, three i.p.s.p.s and three failures, to spontaneous single action potentials in cell 1. Note the comparatively long time-to-peak of this i.p.s.p. *B*, distribution of i.p.s.p. latencies, mean value $3{\cdot}1 \pm 0{\cdot}7$ ms ($n = 61$). *C*, distribution of peak amplitude for i.p.s.p.s evoked by 281 action potentials in cell 1. The 220 transmission failures which occurred are plotted at 0 mV. The mean amplitude of sixty-one i.p.s.p.s at post-synaptic resting potentials between 61 and 67 mV was $2{\cdot}7 \pm 1{\cdot}1$ mV.

single inhibitory cell which was presynaptic to both neurones. The simultaneous i.p.s.p. seemed on some occasions to follow spontaneously occurring single action potentials in one of the neurones. The distribution of latencies, from an action potential, of the identified i.p.s.p. exhibited a peak at about 7 ms (Fig. 5*B*). If the timing of i.p.s.p.s was unrelated to the occurrence of action potentials a flat distribution would be expected. A weaker coupling between the pyramidal cell and the postulated inhibitory neurone may explain the longer and more widely varying latency observed here than in the case of the disynaptically coupled neurones shown in Fig. 4. At this latency the conductance change due to the i.p.s.p. was superimposed on intrinsically generated conductance changes following an action potential, thus distorting its normal time course (compare middle and lower traces of Fig. 5*A*). This distortion made it difficult to assess its rise time. However, the mean rise time of the

recurrent i.p.s.p. in the simultaneously recorded neurone was $3{\cdot}4 \pm 0{\cdot}9$ ms ($n = 58$), comparable with that of the most frequently observed spontaneous i.p.s.p.s (Fig. 5*C*).

Similar distorted i.p.s.p.s occurred, with a probability higher than that expected by chance, within 15 ms of spontaneous action potentials in about 15% of neurones (see Fig. 6*C*). Thus it appears that the feed-back inhibition of a single cell initiated

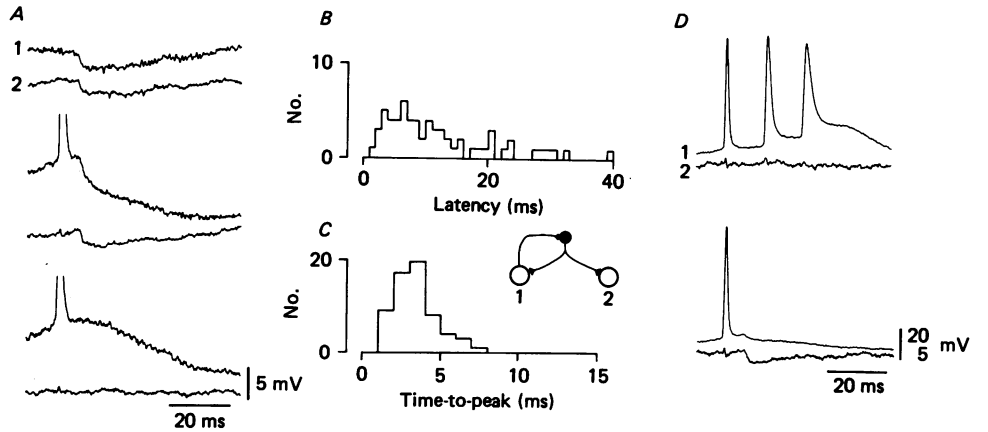

Fig. 5. Recurrent inhibition initiated by activity in a single pyramidal cell. *A*, simultaneous i.p.s.p.s, synchronous within 1 ms, occurred spontaneously in cells 1 and 2 (upper traces) suggesting that the same inhibitory neurone was presynaptic to both cells. On some occasions the simultaneous i.p.s.p.s closely followed spontaneous action potentials in cell 1 (middle traces). An instance when the simultaneous i.p.s.p. did not occur is also shown (lower traces). *B*, latency distribution of fifty-eight simultaneous i.p.s.p.s which occurred within 40 ms of 428 spontaneous single action potentials in cell 1. The distribution reached a peak at 5–10 ms after an action potential in cell 1. *C*, distribution of times-to-peak for the i.p.s.p. in cell 2; mean $= 3{\cdot}4 \pm 0{\cdot}9$ ms ($n = 58$). *D*, the recurrent i.p.s.p. was well timed to prevent intrinsically generated bursts. Upper traces show a burst in cell 1 when the simultaneous i.p.s.p. was not evoked. Lower traces show a single action potential in cell 1 followed with latency 7 ms by the simultaneous i.p.s.p.

by its own activity may be relatively common. An important consequence of this synaptic mechanism is shown in Fig. 5*D*. CA3 pyramidal cells often generate bursts of action potentials with spike separation of 10–20 ms. Recurrent i.p.s.p.s initiated in one pyramidal cell with latency approximately 7 ms, by single action potentials in the same cell, are perfectly timed to prevent the development of such intrinsic bursts. The elimination of this stabilizing mechanism, by inhibitory transmitter antagonists, has been shown to play a crucial role in the development of synchronized bursting activity in the hippocampus (Wong & Prince, 1979; Dingledine & Gjerstad, 1979).

Repetitive firing properties of inhibitory neurones

In the CA1 region of the hippocampus, a group of neurones with a different firing pattern from pyramidal cells has been shown to mediate inhibitory interactions (Knowles & Schwartzkroin, 1981). We have recorded from neurones with a similar firing pattern, where each action potential is followed by a prominent after-

hyperpolarization. As in the CA1 region, a train of action potentials in such a cell elicits a slowly developing inhibitory potential in coupled pyramidal cells (Fig. 6*A*). However, it appears that inhibitory neurones which can fire bursts of action potentials, not unlike those seen in pyramidal cells, are responsible for at least some of the sharply rising i.p.s.p.s which we have described.

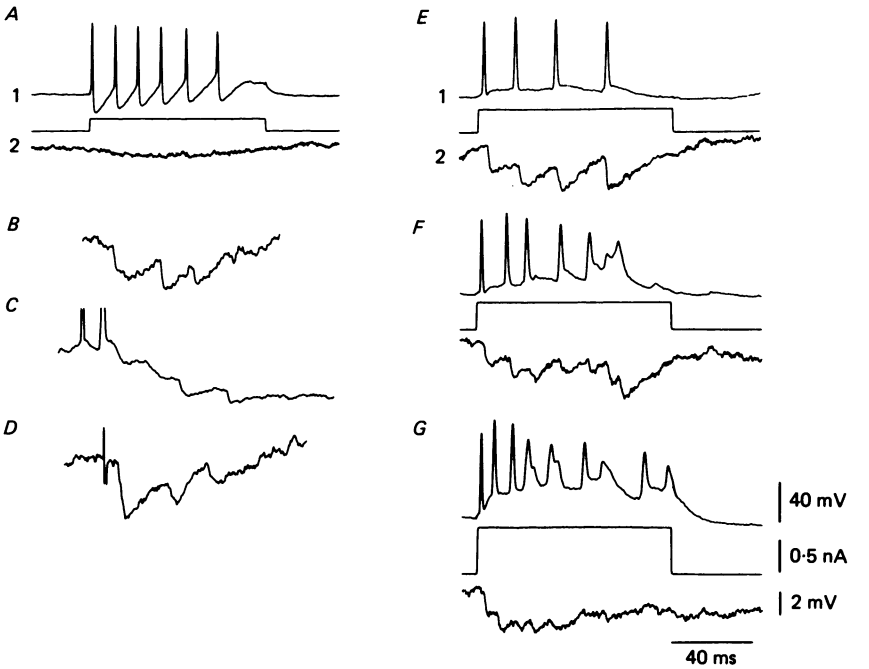

Fig. 6. Inhibitory neurones can fire bursts of action potentials. *A*, current injected into one neurone elicited a train of action potentials, each followed by a prominent after-hyperpolarization, which gave rise to a slowly developing hyperpolarizing potential in another simultaneously recorded neurone. *B–D*, bursts of inhibitory events were observed quite often. *B*, a group of three i.p.s.p.s occurring spontaneously. *C*, four i.p.s.p.s following spontaneous action potentials in another neurone. *D*, very weak stimulation of the CA1 stratum oriens elicits a burst of i.p.s.p.s. *E–G*, responses to current injected into the presynaptic neurone of a monosynaptically coupled pair of cells. Low current intensities elicited action potentials of about 2 ms duration. At higher intensities a slow depolarization developed and later spikes were broadened. Post-synaptic inhibitory responses were well maintained up to presynaptic action potential frequencies of about 100 Hz (*E* and *F*) but at higher frequencies the responses to later spikes in a burst were depressed or absent (*G*). Note that broadened spikes also elicited synaptic responses. In trace *F* the final presynaptic action potential elicited two i.p.s.p.s.

Spontaneous i.p.s.p.s (Fig. 6*B*), feed-back i.p.s.p.s dependent on activity in a single pyramidal cell (Fig. 6*C*), and apparently unitary i.p.s.p.s evoked by weak antidromic stimulation of CA3 axon collaterals in the CA1 region (Fig. 6*D*) sometimes appeared to occur in groups. These could either reflect a fortuitous temporal patterning of the activity of several inhibitory cells or could depend on inhibitory neurones firing in bursts of action potentials. Fig. 6*E–G* shows directly that some

inhibitory neurone can fire in bursts. Low intensity depolarizing current injected into the presynaptic neurone of a monosynaptically connected pair of cells elicited several action potentials of duration about 1·5 ms. At higher intensities the duration of successive spikes, which were superimposed on a slow depolarization, broadened to 4–6 ms, and the burst was followed by a prolonged after-hyperpolarization. This record cannot be differentiated from a typical pyramidal cell recording in the same area.

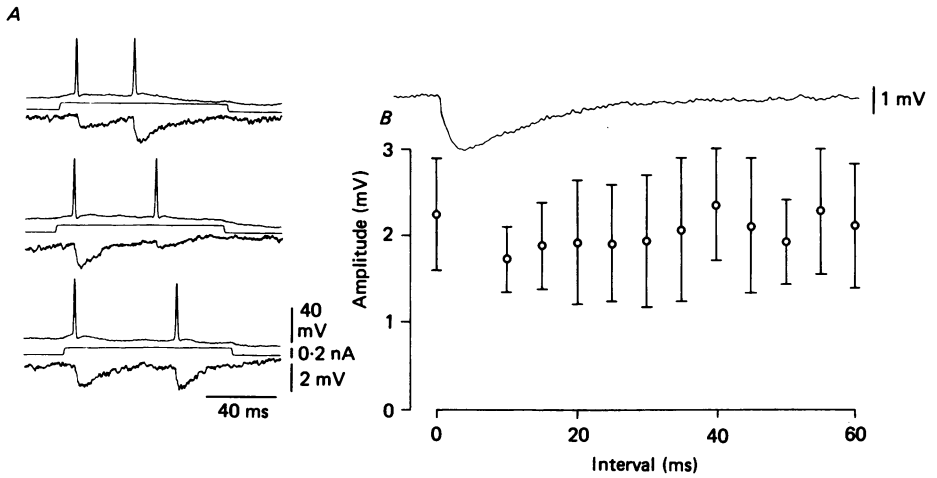

Fig. 7. Dependence of i.p.s.p. amplitude on interval. *A*, depolarizing pulses, of intensity sufficient to elicit more than one action potential, were injected into the presynaptic neurone at a frequency of 1 Hz. In single trials quantal fluctuations masked any interval dependence of the i.p.s.p. amplitude. *B*, averaging the amplitude of the second i.p.s.p. for different intervals following the control i.p.s.p. revealed a small but systematic effect. At the shortest interval, 10 ms, i.p.s.p. amplitude was depressed by about 20%. The depression recovered with time course similar to the decay of potential following the peak of the i.p.s.p. (shown above). Mean and standard deviation of the peak amplitude of the first i.p.s.p. ($n = 183$) are plotted at interval = 0 ms. Mean amplitude and standard deviation of second i.p.s.p.s occurring within 5 ms time intervals from 10 to 60 ms are plotted against i.p.s.p. separation. Each point derived from at least nine events.

How does repetitive presynaptic activity affect inhibitory transmission? This question was investigated in the monosynaptically coupled cells shown in Fig. 6 by measuring the amplitudes of i.p.s.p.s elicited by two presynaptic action potentials separated by different intervals (Fig. 7). Even at intervals as short as 10 ms the mean amplitude of the second i.p.s.p. was only depressed by 20% of its control value. A close correspondence between the recovery of the depression and the decay of the averaged control i.p.s.p. suggested that the depression may have been due to the non-linear summation of the two potentials (Martin, 1955). However, in the same pair of neurones inhibitory events were clearly depressed during the later stages of bursts of presynaptic action potentials at frequencies above 100 Hz (Fig. 6). This was not due to the membrane potential reaching the reversal level for the i.p.s.p. Another possibility is that broadened action potentials do not invade the axon. This was not the case since Fig. 6*F* shows that at lower firing frequencies such broadened action

potentials could elicit discrete i.p.s.p.s. The present results do not allow us to determine what other mechanisms may be involved.

Properties of the population i.p.s.p. evoked by stimulation of fibre pathways

It would be useful to compare the properties of unitary i.p.s.p.s with those of the maximal inhibitory post-synaptic potential observed when all inhibitory synapses onto one cell are synchronously activated (population i.p.s.p.). However, as Fig. 8

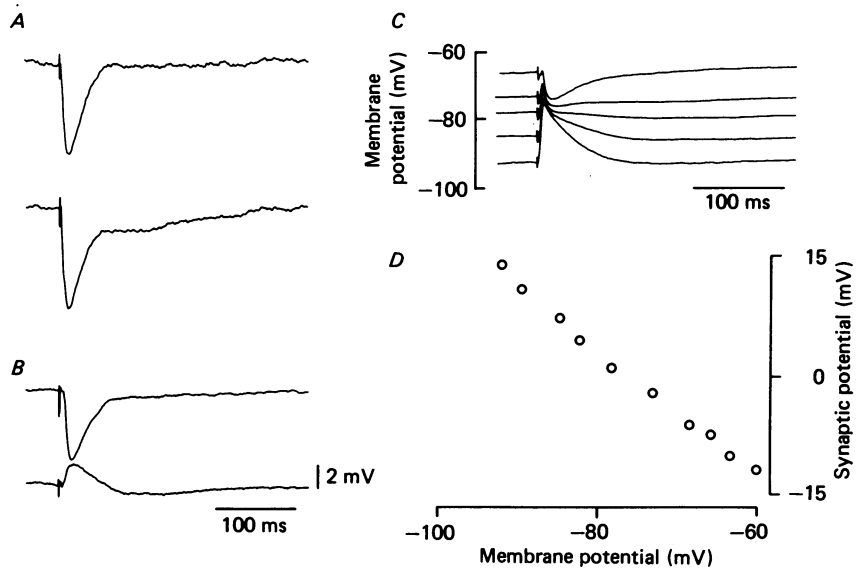

Fig. 8. Properties of the population i.p.s.p. elicited by electrical stimulation of fibre pathways. *A*, a second, higher threshold, hyperpolarizing potential with time-to-peak of 80–150 ms was elicited by mossy fibre stimulation. Upper trace shows response to submaximal stimulus, lower trace shows maximal response. *B*, responses to antidromic stimulation in the CA1 stratum oriens. In the normal solution a 'pure' population i.p.s.p. was evoked (upper trace). 15 min after changing to a solution containing 10^{-4} M-picrotoxin it was blocked and a depolarizing synaptic potential was revealed by the same stimulus (lower trace). *C*, voltage dependence of synaptic potentials elicited by mossy fibre stimulation. Membrane was hyperpolarized by maintained current injection with bridge balance checked repeatedly. All traces average of ten responses. *D*, potential change 13 ms after stimulus (time of peak hyperpolarization at resting membrane potential) plotted against membrane potential.

shows, it is difficult to selectively activate inhibitory synapses by electrical stimulation of fibre pathways without also activating other synapses.

When any orthodromic pathway to the CA3 region is stimulated excitatory synapses must necessarily be activated, even though the resulting synaptic potential may not be clearly evident at resting potential due to the onset of synaptic inhibition. In addition, at high stimulus intensities a second inhibitory potential may be activated (Alger & Nicoll, 1982; Thalman & Ayala, 1982). This potential (Fig. 8*A*) has a slow time course, reaching a peak at approximately 150 ms, and reverses at −85 to −90 mV. In order to activate pyramidal cells antidromically we looked for an axonal projection from CA3 which was anatomically separate from afferent fibres.

Such a group of axon collaterals projecting to the stratum oriens of the CA1 region has been described by Lorente de Nó (1934). On stimulating there, a monophasic hyperpolarizing synaptic potential was evoked (Fig. 8*B*). But when this potential was blocked by the GABA antagonist picrotoxin (10^{-4} M) an excitatory synaptic potential was revealed, which may depend on the existence of recurrent connexions between CA3 neurones (Miles & Wong, 1983*b*).

Measurements of the properties of the population i.p.s.p. elicited by electrical stimulation must therefore be compromised by the presence of these other synaptic potentials. Nevertheless, data obtained from seventeen cells are presented here for comparison. The time from onset to the peak of the hyperpolarization due to the population i.p.s.p. ranged from 8 to 15 ms. The total duration of the population i.p.s.p. could not be measured due to the presence of the slow inhibitory potential, but when submaximally evoked it appeared to last for 50–80 ms. The peak amplitude of the population i.p.s.p. measured at resting potentials of −60 to −70 mV was 4–12 mV. Population i.p.s.p.s, measured at the time of the peak hyperpolarization at resting potential, reversed at potentials between −75 and −82 mV. Fig. 8*C* shows the largest amplitude population i.p.s.p. we recorded. The peak conductance change due to this population i.p.s.p., derived as described previously, was 105 nS. In seven further neurones values ranged between 28 and 72 nS.

DISCUSSION

In this report paired intracellular recordings have been used to study synaptic inhibition in the CA3 region of the hippocampal slice. We have been able to derive values for the conductance and reversal potential of unitary i.p.s.p.s. In addition some properties of inhibitory neurones, including their firing pattern and the way they are recruited during normal activity, have been determined. Several factors have facilitated these studies. Simultaneous recordings may be obtained, with some difficulty, from both pre- and post-synaptic neurones at an inhibitory synapse. Synaptic potentials dependent on a single presynaptic action potential are of sufficiently large amplitude (at resting potential in the post-synaptic neurone) that deconvolution techniques (Edwards, Redman & Walmsley, 1976; Faber & Korn, 1982) are not needed to resolve them. The excitatory synapses made onto inhibitory neurones also seem to be strong, so that disynaptic interactions underlying the recruitment of i.p.s.p.s may be examined. Intrasomatic recordings can measure the synaptic potential with little decrement due to electrotonic spread since inhibitory synapses often terminate on the soma of the post-synaptic neurone (Andersen *et al.* 1963; Gottlieb & Cowan, 1972). Finally, the transmitter mediating inhibitory synaptic interactions (which is likely to be GABA) has a reversal potential sufficiently close to resting potential that values for the peak synaptic conductance may be directly determined.

Properties of unitary i.p.s.p.s

Two reports have described unitary events at inhibitory synapses in the mammalian C.N.S. (Jankowska & Roberts, 1972; Kuno & Weakly, 1972). In both cases inhibitory neurones in the spinal cord were activated indirectly and unitary i.p.s.p.s

of amplitude up to 0·4 mV were recorded in motoneurones. In contrast, the mean amplitude of the identified i.p.s.p.s we have described ranged between 1·3 and 3·1 mV at post-synaptic potentials between −60 and −70 mV. What factors are responsible for the large amplitude of inhibitory potentials in the hippocampus? One difference may be the neuronal input resistance: that of CA3 hippocampal pyramidal cells is typically 20–40 MΩ which compares with 2–5 MΩ for cat spinal motoneurones (Burke & Rudomin, 1977). Another factor determining the amplitude of a synaptic potential is the associated change in membrane conductance. Although the conductance due to a motoneurone i.p.s.p. is not known, the conductance of unitary e.p.s.p.s elicited by impulses in single Ia axons has recently been measured using voltage-clamp techniques (Finkel & Redman, 1983). Here a peak conductance change of about 5 nS was associated with an e.p.s.p. of peak amplitude about 0·1 mV. The present results show that, despite a smaller driving force, a similar conductance change (5–9 nS) associated with the i.p.s.p. in a pyramidal cell produced significantly larger events.

The observation of fluctuations in the peak amplitude of i.p.s.p.s suggests that the inhibitory transmitter is released in the quantal fashion described at other synapses (del Castillo & Katz, 1954; Kuno & Weakly, 1972; Korn *et al.* 1982). It appears that the statistical factors governing transmitter release at this synapse are such that at low frequencies a presynaptic action potential invariably elicits an i.p.s.p. In this respect too, inhibitory synapses in the hippocampus seem to be more powerful than those in the spinal cord where significant numbers of transmission failures are observed (Kuno & Weakly, 1972). The experiment illustrated in Fig. 7 suggests that release statistics were not appreciably altered for the second of two i.p.s.p.s separated by short intervals. Furthermore, a clear depression of post-synaptic responses to repetitive presynaptic activity was only apparent at frequencies above 100 Hz (Fig. 6*G*). Thus the behaviour of a single inhibitory synapse appears not to account for the profound depression of the electrically evoked population i.p.s.p. which is observed during repetitive stimulation at 10 Hz (Ben-Ari, Krnjevic & Reinhardt, 1979).

Properties of inhibitory neurones

It is commonly assumed that inhibitory neurones in the hippocampus are physiologically different from pyramidal cells. Unlike pyramidal cells, the neurones shown by Knowles & Schwartzkroin (1981) to mediate inhibitory interactions in the CA1 region were spontaneously active with a pronounced after-hyperpolarization following each action potential. The post-synaptic response to repetitive activity in these neurones is a slow hyperpolarization rather than a rapidly rising synaptic potential. In this study the properties of the slowly developing inhibition (Fig. 6*A*) have not been examined in detail. They may reflect discrete i.p.s.p.s generated at distal dendritic sites, or alternatively their generation may involve a different neurotransmitter or post-synaptic receptor. However, we have shown that single action potentials in another type of inhibitory neurone give rise to discrete i.p.s.p.s. Unexpectedly, action potentials were followed by a depolarizing after-potential so that membrane depolarization resulted in the generation of a burst of action potentials like those generated by pyramidal cells (Fig. 6*E–G*). It seems that the firing pattern of a neurone cannot be used to predict its post-synaptic actions.

Andersen *et al.* (1963) proposed that hippocampal inhibitory neurones are activated

by pyramidal cell axon collaterals and make divergent synaptic connexions back onto many pyramidal cells. The observation of simultaneous i.p.s.p.s in recordings from pairs of pyramidal cells demonstrates the divergence of synaptic contacts made by a single inhibitory neurone. We have also shown that recurrent inhibition may operate at the single cell level (cf. van Keulen, 1981) in that action potentials in one cell could initiate feed-back i.p.s.p.s in the same cell (Fig. 5). Together with the observation of disynaptically mediated i.p.s.p.s (Fig. 4), this suggests that single excitatory synapses made onto inhibitory neurones can be strong enough to cause the neurone to fire. Our previous studies have shown that excitatory connexions between pyramidal cells are also extremely powerful and, in the presence of GABA antagonists, may contribute to an uncontrolled spread of excitation (Miles & Wong, 1983*a*). The tight coupling of pyramidal cells to inhibitory neurones allows inhibition to effectively regulate the transmission of activity across excitatory synapses during normal function.

Comparison between unitary i.p.s.p.s and the population i.p.s.p.

The population i.p.s.p. recorded in hippocampal pyramidal cells *in vivo* is of large amplitude and long duration compared to that recorded in spinal motoneurones (Eccles, 1969). The long duration may be partly explained by a prolongation of GABA-dependent synaptic potentials by barbiturate anaesthesia (Nicoll, Eccles, Oshima & Rubia, 1975) and by the presence of a second slower inhibitory synaptic potential (Alger & Nicoll, 1982; Thalman & Ayala, 1982). Can the summation of unitary i.p.s.p.s, with similar properties to those described here, account for the initial fast phase of the electrically evoked population i.p.s.p. The finding of similar reversal potentials and the observation that both spontaneous i.p.s.p.s and the fast population i.p.s.p. were blocked by picrotoxin, suggest that the same neurotransmitter, GABA, mediates both events.

Both the duration of the unitary i.p.s.p. and the extent to which inhibitory neurones fire repetitively will influence the duration of the population i.p.s.p. In the goldfish Mauthner cell, i.p.s.p. duration appears to be predominantly determined by transmitter channel kinetics (Faber & Korn, 1982). In contrast, the present results suggest that in the hippocampus the membrane time constant of the post-synaptic cell governs the time course of the unitary i.p.s.p. This appears to be incompatible with the finding that a significant conductance increase accompanies the decay of the electrically evoked population i.p.s.p. (Dingledine & Langmoen, 1980). However, this result may be explained by the presence of the second slow phase of the evoked population i.p.s.p., which is insensitive to picrotoxin or bicuculline (Fig. 8, Alger & Nicoll, 1982). The absence of a slow component in unitary events provides further evidence that different transmitter actions mediate the fast and slow components of the electrically evoked population i.p.s.p. The repetitive firing of inhibitory neurones in response to stimulation of fibre pathways would also be expected to prolong the population i.p.s.p. (Andersen *et al.* 1963; Finch & Babb, 1977). Although membrane depolarization could elicit bursts of action potentials in inhibitory neurones we have no direct evidence that similar bursts accompanied the electrically evoked population i.p.s.p. However, the observation of bursts of i.p.s.p.s in response to weak antidromic stimulation (Fig. 6*D*) is consistent with this hypothesis.

We have suggested that the different amplitudes of unitary i.p.s.p.s in hippo-

campal neurones and in spinal motoneurones may be due to different input resistances of the two types of cell. The amplitude of the population i.p.s.p. is also influenced by the number of inhibitory neurones which make synapses onto a cell. This number may be estimated by comparing the conductance change associated with an i.p.s.p. with that for the population i.p.s.p. The mean value for the conductance change of fifteen identified i.p.s.p.s was 6 nS and the largest value for the evoked population i.p.s.p. was 105 nS (cf. values between 40 and 160 nS derived using voltage-clamp techniques (Brown & Johnston, 1983)). It appears that, in a hippocampal slice, up to fifteen inhibitory neurones make synapses onto a particular pyramidal cell. This number may well underestimate neuronal connectivity in the intact hippocampus. In comparison, approximately seventy inhibitory neurones are thought to contribute to the population i.p.s.p. recorded in spinal motoneurones (Jankowska & Roberts, 1972). A final estimate may be made if the conductance of GABA channels on cultured hippocampal neurones, 20 pS (Segal & Barker, 1984), is comparable with that of post-synaptic channels at the inhibitory synapses examined here. Then, with a mean i.p.s.p. conductance change of 6 nS it appears that approximately 300 channels are opened following an action potential in the inhibitory neurone.

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