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VARIATION IN STRENGTH OF INHIBITORY SYNAPSES IN THE CA3 REGION OF GUINEA-PIG HIPPOCAMPUS *IN VITRO*

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SUMMARY

1. Simultaneous recordings were made from inhibitory cells located close to the stratum pyramidale and from pyramidal cells in the CA3 region of guinea-pig hippocampal slices, to examine inhibitory synaptic interactions.

2. The average amplitude of inhibitory postsynaptic potentials (IPSPs) initiated by single action potentials at different synapses varied between 0·3 and 2·6 mV. Experiments were performed to investigate the source of this variation.

3 Unitary IPSPs reversed at similar potentials to the first phase of the synaptic inhibition elicited by afferent fibre stimulation. IPSPs evoked by single action potentials or repetitive inhibitory cell firing were suppressed by picrotoxin, a γ-aminobutyric acid ($GABA_A$) receptor antagonist.

4. The time to peak and amplitude of averaged IPSPs were not related as predicted if amplitude variations resulted simply from different electrotonic locations of inhibitory terminals.

5. Transmission failures could be resolved at connections which generated small averaged IPSPs, but were not apparent at connections where averaged IPSPs were large.

6. IPSPs elicited by the same inhibitory cell in several pyramidal cells were of similar amplitude. The amplitudes of simultaneous IPSPs impinging on pairs of neighbouring pyramidal cells were positively correlated.

7. Thus, the variation in efficacy of inhibitory synapses may result from differences in transmitter release from different inhibitory cells and not from postsynaptic factors.

INTRODUCTION

Anatomical studies suggest that inhibitory cells in the hippocampus are diverse. They have different morphologies, axonal arborization patterns and are located in different layers of the hippocampus (Ramon y Cajal, 1911; Lorente de Nó, 1934; Ribak, Vaughn & Saito, 1978; Tombol, Somogyi & Hadju, 1978; Somogyi, Nunzi, Gorio & Smith, 1983). Several peptides (Kohler & Chan-Palay, 1982; Kosaka,

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Kosaka, Tateishi, Hamaoka, Yanaihara, Wu & Hama, 1985; Somogyi, Hodgson, Smith, Nunzi, Gorio & Wu, 1984) and other molecules including calcium-binding proteins (Katsumaru, Kosaka, Heizmann & Hama, 1988; Sloviter, 1989) are present in different groups of γ-aminobutyric acid (GABA)-containing cells suggesting that functionally distinct subsets of inhibitory cells may exist.

This anatomical diversity suggests physiological differences may exist in cellular interactions which underly synaptic inhibition in the hippocampus. The inhibitory transmitter GABA is known to have several postsynaptic actions. At least two channels, one permeable to Cl^- ions and one to K^+ ions, are coupled to GABA receptors (Curtis, Duggan, Felix & Johnston, 1970; Newberry & Nicoll, 1985; Thalmann, 1988). These two channels may mediate fast and slow components of the inhibitory postsynaptic potential (IPSP) evoked in pyramidal cells by afferent stimulation. However the relation between inhibitory cell firing and the two components of the IPSP is not clear. GABA released by one group of inhibitory cells might mediate both phases of the afferent IPSP (Dutar & Nicoll, 1988) or different groups of inhibitory cells might be involved. Lacaille & Schwartzkroin (1988) proposed that different cells generate the two components of the IPSP in the CA1 region. Inhibitory cells of the stratum lacunosum generate the slower K^+-mediated phase (Lacaille & Schwartzkroin, 1988), while the Cl^--dependent fast IPSP depends on inhibitory cells located in stratum pyramidale and oriens (Knowles & Schwartzkroin, 1981).

Unitary IPSPs generated by inhibitory cells of the stratum pyramidale in the CA3 region were previously shown to have diverse properties (Miles & Wong, 1984). Some inhibitory cells evoked small IPSPs that were easily detected only when presynaptic cells fired repetitively (cf. Knowles & Schwartzkroin, 1981; Lacaille & Schwartzkroin, 1988). In contrast single action potentials in other inhibitory cells elicited large IPSPs. The difference might originate postsynaptically, possibly due to activation of different receptors or to variation in the electrotonic location of inhibitory terminals, or presynaptic factors might be involved (Miles & Wong, 1984). To resolve this question the properties of IPSPs initiated by CA3 stratum pyramidale inhibitory cells were examined in greater detail. Now I report that IPSP shape, voltage dependence and sensitivity to $GABA_A$ receptor antagonists, suggest these cells initiate an inhibition mediated exclusively by Cl^-. However the average amplitude of unitary IPSPs varies considerably between inhibitory connections. This fluctuation in synaptic strength may result from variations in GABA release from different presynaptic cells.

METHODS

Experiments were done on transverse hippocampal slices, of thickness 400 μm, prepared with a vibratome from guinea-pigs (200–300 g) which were killed by cervical dislocation. Slices were supported on nylon mesh in a recording chamber with their lower surface in contact with a solution of composition (in mM): NaCl, 124; KCl, 4; $CaCl_2$, 2·5; $MgCl_2$, 2; $NaHCO_3$, 26 and D-glucose, 10. Their upper surface was exposed to a warmed (37 °C), moistened atmosphere of 5% CO_2 in 95% O_2 resulting in a pH of 7·4.

Electrodes for intracellular recording were made from fibre-filled glass tubing. In most experiments electrodes were filled with 3 M-potassium acetate (pH adjusted to 7·2) and bevelled to a resistance of 40–90 MΩ before use. In other experiments electrodes were filled with 3 M-KCl

(resistance 20–40 MΩ) in order to shift the reversal potential of chloride-mediated IPSPs to positive potentials and so increase driving force. At least 10 min was allowed for intracellular chloride to equilibrate in these recordings. When Cl^--filled electrodes were used, synaptic excitation was suppressed using the excitatory amino acid antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and DL-2-amino-5-phosphonovalerate (APV, both from Tocris Neuramin, Essex, England). The $GABA_A$ antagonist picrotoxin (PTX, from Sigma) was used in some experiments.

Membrane potentials were measured using an amplifier (Axoclamp 2) operated in current clamp mode. One electrode was used to record from pyramidal cells in the stratum pyramidale of the CA3 region. Another electrode entered the slice within about 500 μm of the first to record from inhibitory cells located in the stratum pyramidale or in a region of thickness about 100 μm on either side of it. Depolarizing current pulses were injected every 1 or 2 s to make presynaptic cells fire single action potentials or to fire repetitively. Maintained hyperpolarizing current was applied if needed to prevent firing between pulses. Afferent fibres were stimulated using electrical pulses of duration 50–100 μs passed between bipolar tungsten electrodes.

Capacitative coupling artifacts between the two recording electrodes were suppressed as described previously (Miles, 1990) and postsynaptic signals were filtered at 1 kHz. Membrane potentials were measured from the potential change when the electrode was removed from a cell. Membrane input resistance and time constant were measured from responses to hyperpolarizing current injections of intensity 0·5 nA and duration 50–100 ms. Responses in one cell to hyperpolarizing current injected into the other were also examined to test for the existence of electrotonic coupling.

Voltage signals were stored on videotape using a modified pulse code modulation device (Neurodata Instruments). They were analysed after analog-to-digital conversion (Tekmar Labmaster) with 12 bit resolution at a rate between 0·05 and 0·5 ms per point and storage in the hard disc of an IBM-XT computer (Miles, 1990). Potentials were measured semi-automatically and averaged with a program written by Shuo Huang of the Department of Electrical Engineering at Columbia University. Statistical analysis was done and figures made with spreadsheet and graphics programs (Lotus Development Corp.). Histograms of IPSP and noise distributions were constructed from periods when IPSPs were stable as judged from running averages of thirty events (Miles, 1990). Coefficients of variation for IPSP distributions were corrected for the variation of the noise distribution (Ropert, Miles & Korn, 1990).

RESULTS

Two types of inhibitory interactions?

Figure 1 shows two interactions between inhibitory cells located close to the stratum pyramidale and CA3 pyramidal cells. In one case single presynaptic action potentials evoked inhibitory postsynaptic potentials of amplitude about 2 mV (Fig. 1*A*). In the other interaction (Fig. 1*B*) repetitive presynaptic firing was needed to evoke a postsynaptic hyperpolarization that could be easily detected. In this report I ask whether pre- or postsynaptic factors underly the differences in inhibitory interactions. Thirty-four synapses made by thirty inhibitory cells located close to stratum pyramidale were examined. IPSP latencies, measured from the peak of the presynaptic action potential, were less than 1·2 ms suggesting all connections were mono-synaptic (mean latency $0{\cdot}9 \pm 0{\cdot}2$ ms).

Unitary IPSPs – amplitude

A unitary IPSP was resolved at each synapse by averaging postsynaptic responses to single presynaptic action potentials. Figure 2 shows averages obtained from the same connections as in Fig. 1. The distribution of amplitudes for averaged IPSPs from thirty-four synapses, with postsynaptic cells at potentials between -60 and -70 mV, is shown in Fig. 2*C*. The amplitude of averaged unitary IPSPs ($n =$

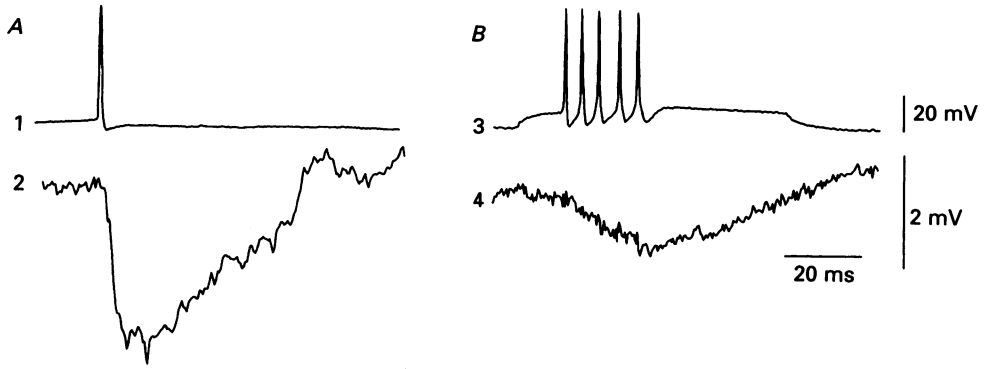

Fig. 1. Two inhibitory interactions between CA3 neurones. *A*, single action potentials in cell 1 evoked IPSPs of amplitude about 2 mV and time to peak 7 ms in cell 2. *B*, five action potentials in cell 3 elicited a slower hyperpolarization, of amplitude about 1 mV and time to peak 25 ms, in cell 4.

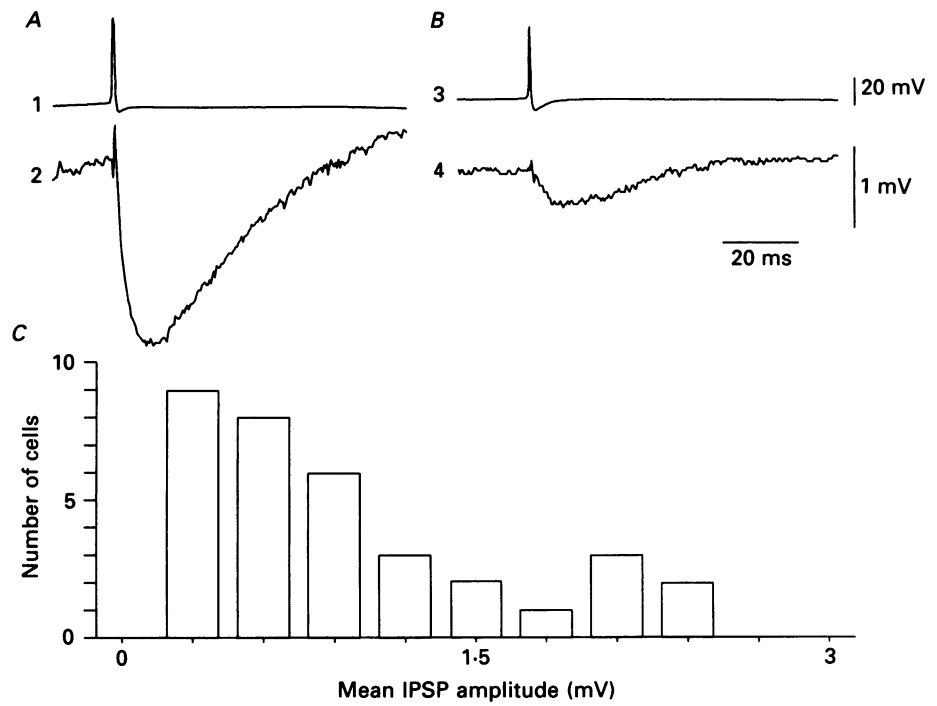

Fig. 2. Variation in amplitude of unitary IPSPs. *A* and *B*, averages of IPSPs initiated by single presynaptic action potentials ($n = 40$) at the same inhibitory connections illustrated in Fig. 1. The amplitude of the IPSP in *B* was about six times smaller than that in *A*, but the time courses of unitary events were similar. *C*, the distribution of amplitudes for averaged unitary IPSPs at thirty-four connections.

100–500) ranged between 0·3 and 2·6 mV and had a mean value of $1{\cdot}4 \pm 0{\cdot}9$ mV (mean $\pm$ S.D.). This ninefold fluctuation is greater than that observed for unitary EPSPs elicited at synapses between CA3 cells. The variation in averaged EPSP

amplitude at thirty-six excitatory connections between pyramidal cells (Miles & Wong, 1986 and R. Miles, unpublished observations) was less than threefold (range 0·5–1·2 mV, mean ± S.D., 0·7 ± 0·3 mV).

Differences in IPSP amplitude might result from varying recording conditions, such as differences in postsynaptic input resistance or driving force for ion flux

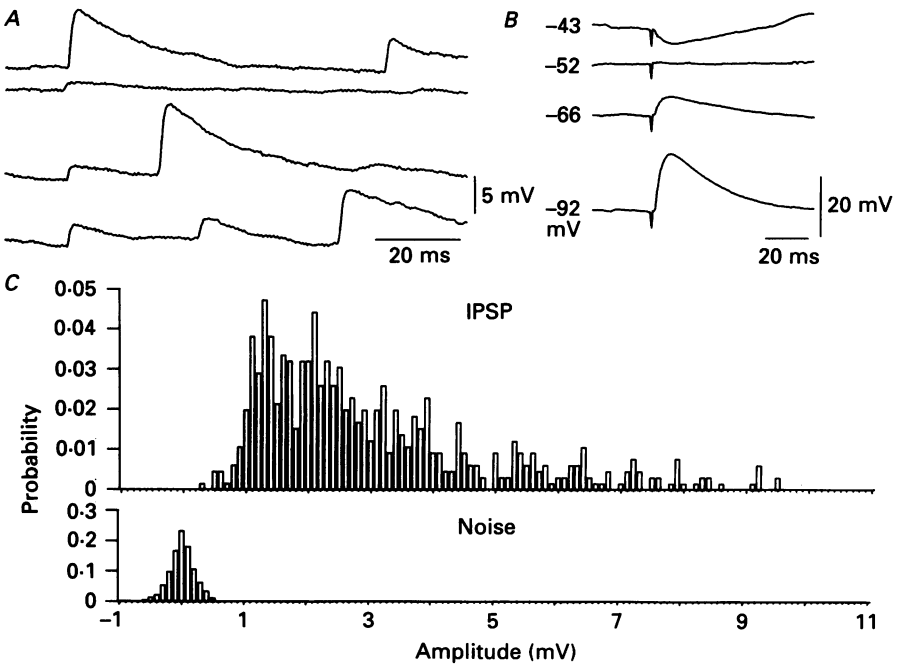

Fig. 3. Variation in amplitude of IPSPs in one CA3 neurone. *A*, depolarizing synaptic events recorded with an electrode containing 3 M-KCl in the presence of 10 μM-CNQX and 100 μM-APV. *B*, the voltage dependence of synaptic responses to local stimulation with a bipolar electrode. The uniform reversal of the response, close to −50 mV, suggests synaptic excitation was suppressed. *C*, amplitude distribution for 1200 IPSPs recorded over a period of 5·3 min at potentials close to 40 mV depolarized from the IPSP reversal. The amplitude distribution for noise, from recording system and membrane sources, is shown below.

through synaptic channels. To eliminate these sources of variation, IPSPs impinging on single CA3 cells were examined (Fig. 3). Spontaneous IPSPs were recorded using electrodes filled with KCl ($n = 28$ cells). Excitatory synaptic events were suppressed (Fig. 3*B*) with the excitatory amino acid antagonists CNQX (10 μM) and APV (100 μM). Depolarizing events recorded at potentials between −80 and −100 mV were then presumed to be IPSPs. Their amplitude fluctuated widely (Fig. 3). Histograms of IPSP amplitudes were typically skewed, with more events of small amplitude. The spread of these distributions was assessed by their coefficient of variation after the coefficient of variation of the noise was excluded. It ranged between 0·69 and 1·03. This was larger than the range of fluctuations in IPSP amplitude at single connections. The coefficient of variation for IPSP amplitudes at single connections, also corrected for noise, varied between 0·16 and 0·58 (see also

Figs 4 and 9). This suggests there is a wide fluctuation in the efficacy of inhibitory synapses which terminate on a single pyramidal cell.

Unitary IPSPs – voltage dependence

Figure 4 shows another way in which the efficacy of different inhibitory synapses was assessed. The voltage dependence of unitary IPSPs was compared with that of the maximal synaptic inhibition elicited by mossy fibre stimuli (cf. Jankowska & Roberts, 1972). Stimulus intensity was adjusted to give a maximal inhibitory response in the pyramidal cell. At these intensities a single action potential was evoked in most (10/14) inhibitory cells. Three inhibitory cells fired multiple action potentials (2–5) and the other cell did not fire. Variation of inhibitory cell membrane potential suggested their firing was limited by a biphasic synaptic inhibition (Misgeld & Frotscher, 1986) similar to that seen in pyramidal cells.

At all synapses tested ($n = 14$), the polarity of IPSPs reversed completely close to the apparent reversal potential of the first component of afferent inhibition. Summed synaptic events elicited by three to six presynaptic action potentials at frequencies of 30–100 Hz also reversed at the same potential in three cells examined (not shown). The relation between amplitude and membrane potential deviated from linearity at hyperpolarized potentials for both unitary IPSPs and the inhibitory component of afferent responses (Hamill, Bormann & Sakmann, 1983; Collingridge, Gage & Robertson, 1984). This precluded comparison of the slope conductance of unitary and afferent IPSPs. An index of inhibitory synaptic efficacy was therefore obtained by dividing the mean unitary IPSP amplitude by the amplitude of the first phase of the afferent IPSP at 12–15 mV depolarized from reversal. Unitary IPSPs from fourteen connections generated between 2·3 and 12·6% of the afferent inhibition.

Unitary IPSPs – pharmacology

The reversal potential of these IPSPs suggests they were mediated by Cl^- channels. Application of the $GABA_A$ antagonist picrotoxin confirmed this hypothesis. Picrotoxin (50 μM) completely suppressed unitary IPSPs at all ($n = 4$) inhibitory connections tested (Fig. 5). The amplitudes of unitary IPSPs before blockade ranged from 0·7 to 2·3 mV suggesting that activation of Cl^--channels was responsible for both large and small IPSPs. Furthermore in the presence of picrotoxin there was no response to repetitive inhibitory cell discharge at frequencies up to 100 Hz (Fig. 5*B*). Differences in postsynaptic receptors apparently do not underly the variation in IPSP amplitude.

Unitary IPSPs – shape

Variation in electrotonic distance from inhibitory terminals to the somatic recording site might contribute to the variation in IPSP amplitudes. If IPSP shape was solely dependent on variation in electrotonic distance, events originating at distant sites should be smaller with a slower time course than somatic IPSPs (Rall, 1967). However, IPSP time to peak showed a weak tendency to increase rather than decrease with amplitude (not shown). A small but systematic increase in IPSP time to peak with amplitude was also apparent for spontaneous events in eight of eleven neurones recorded with Cl^--filled electrodes.

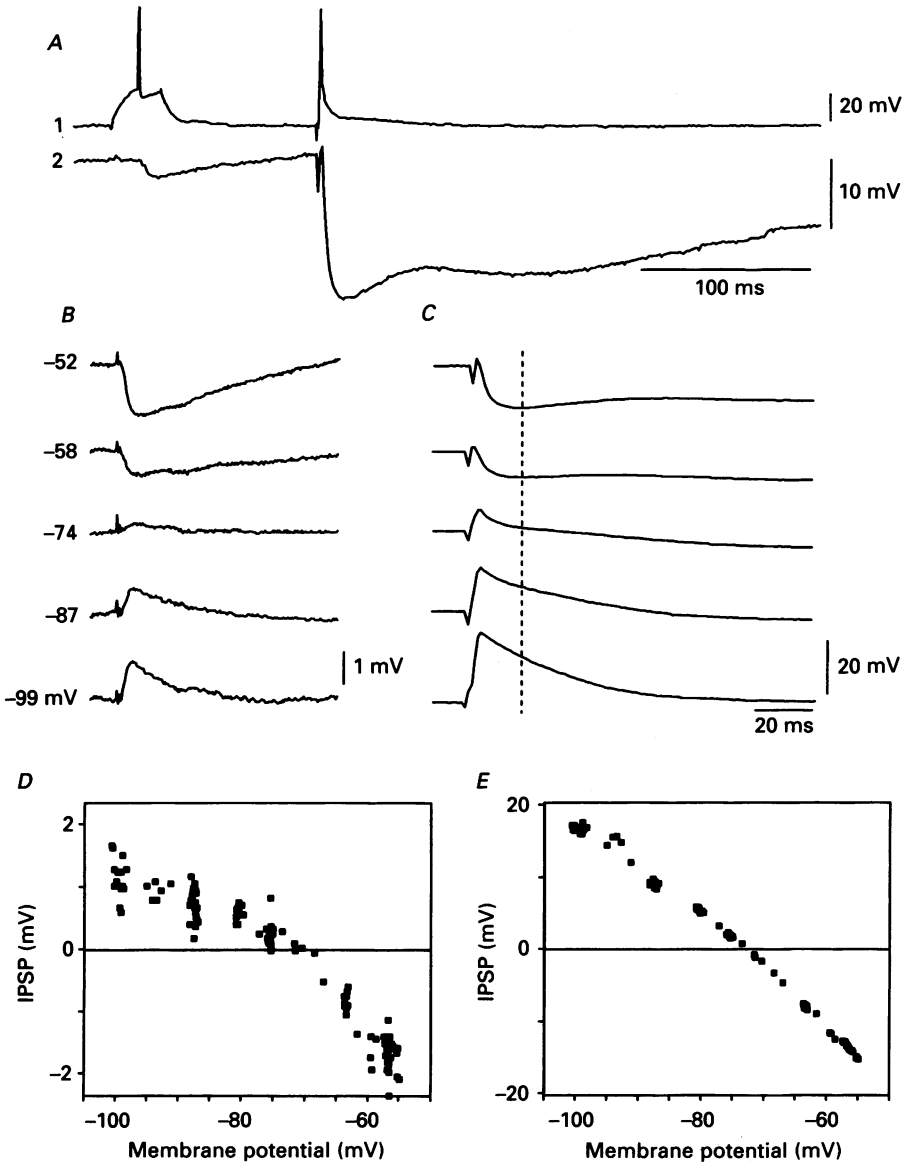

Fig. 4. Voltage dependence of a unitary IPSP compared with that of the maximal IPSP evoked by afferent stimulation. *A*, inhibitory cell 1 was made to fire single action potentials by injecting depolarizing pulses from a maintained hyperpolarizing current. Mossy fibre stimulation was adjusted to elicit the maximal afferent response. The membrane potential of cell 2 was varied by maintained current injection. *B* and *C* show averaged ($n = 12$) unitary IPSPs and afferent responses in cell 2 at different potentials. The polarity of the unitary IPSP reversed at about -72 mV, close to the apparent reversal potential of the first phase of the afferent IPSP. *D* and *E* show the potential dependence of the unitary and the afferent IPSP, measured 18 ms after the stimulus (dotted line in *C*). At -58 mV this cell generated 9% of the afferent inhibition.

Unitary IPSPs – presynaptic variation

If postsynaptic factors cannot account for the variation in mean IPSP amplitude, presynaptic differences may exist. Fluctuations in IPSP amplitude were examined to assess the possibility that GABA release varies at different synapses. Figure 6 shows

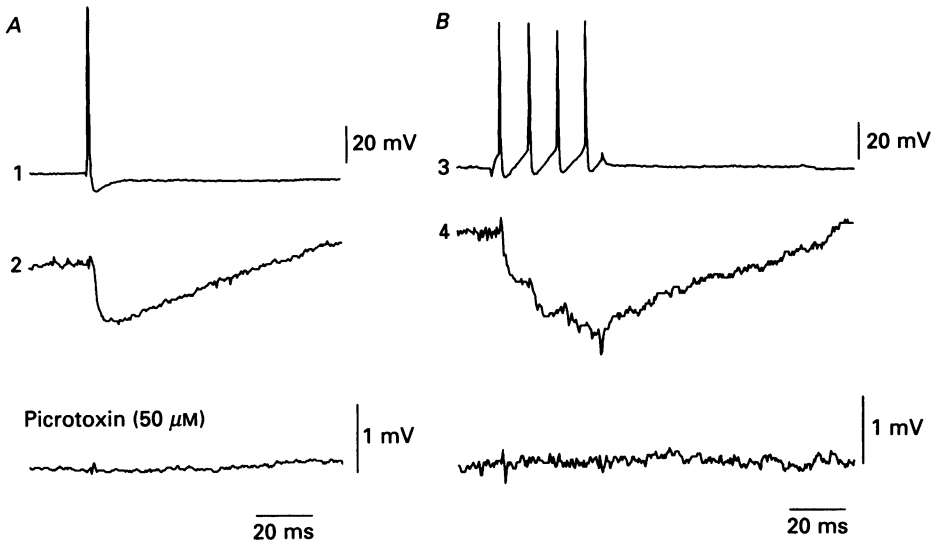

Fig. 5. Unitary IPSPs are mediated by $GABA_A$ receptors. Picrotoxin (50 μM) suppressed both large IPSPs evoked by single action potentials (*A*) and summed IPSPs evoked by bursts of presynaptic action potentials (*B*). Postsynaptic traces are averages of at least forty responses.

IPSPs generated at a weak inhibitory synapse, where the mean IPSP amplitude was 0·5 mV, and at a strong inhibitory synapse, with mean amplitude 2·2 mV. At the strong synapse all presynaptic spikes elicited an IPSP (Fig. 6*A*), whereas at the weak synapse some action potentials appeared to elicit no postsynaptic response (Fig. 6*B*). IPSP amplitudes from the strong synapse did not overlap with the distribution of membrane and recording system noise (Fig. 6*C*). Selective averaging of the largest and smallest 10% of responses at this synapse (Fig. 6*E*) produced IPSPs of larger and smaller amplitudes. In contrast there was substantial overlap of the IPSP and noise histograms constructed for the weak inhibitory synapse (Fig. 6*D*) and for another twelve inhibitory connections. Selective averaging of the largest and smallest 10% of IPSPs at this connection produced different results (Fig. 6*F*). The largest responses gave an event that was similar in shape but smaller than IPSPs from the strong synapse. Averaging the smallest 10% of responses at this and five other weak connections gave a response with no trace of an IPSP. At the remaining seven synapses a small IPSP was resolved. The flat average implies that at least 10% of presynaptic action potentials generated no postsynaptic response at six of the weak connections. This points to a difference in transmitter release at weak and strong inhibitory synapses.

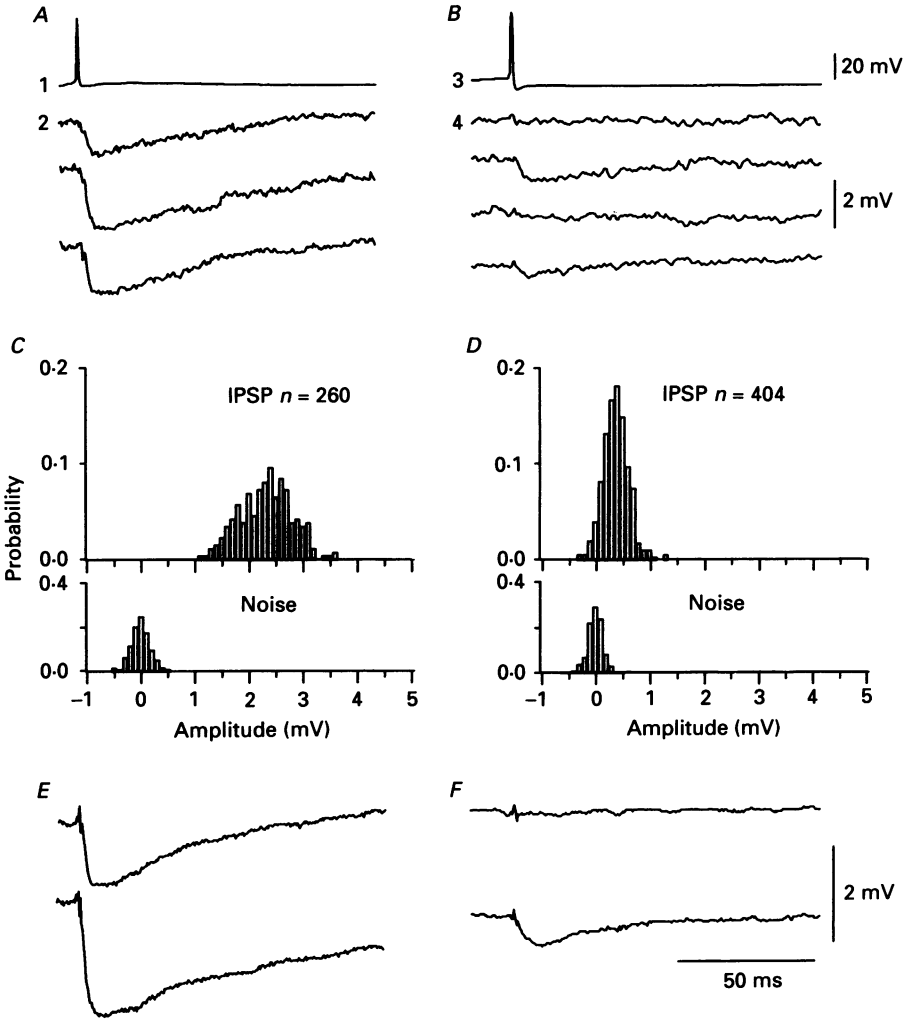

Fig. 6. Synaptic transmission fails at weak but not strong inhibitory synapses. *A* and *B* show IPSPs from a strong inhibitory synapse (mean amplitude 2·2 mV) and a weak inhibitory synapse (mean amplitude 0·5 mV). *C*, the distribution of IPSP amplitudes at the strong synapse did not overlap with the distribution of noise. *D*, at the weak synapse the IPSP and noise distributions did overlap and some individual traces in *B* appeared to be transmission failures. *E* and *F*, averages of the smallest and largest 10% of responses show transmission failures occurred at the weak synapse (number of traces averaged $n = 40$) but not at the strong synapse ($n = 26$).

Do single inhibitory cells generate IPSPs of similar strength?

If the variation in inhibitory synaptic efficacy originates presynaptically, synaptic strength might be specific to all connections made by one inhibitory cell or it might differ at various connections made by the same cell. This issue was examined by comparing IPSPs initiated by the same inhibitory cell in several pyramidal cells (Fig. 7). On two occasions, a recording from one inhibitory cell was maintained while

records from three postsynaptic cells were obtained sequentially. Both the mean and distribution of IPSP amplitudes in the postsynaptic cells were similar. Mean IPSP amplitudes from the inhibitory interactions shown in Fig. 7 were 0·9, 1·1 and 1·1 mV. In the other sequential recording of three inhibitory interactions, the mean IPSP amplitudes were 0·4, 0·6 and 0·5 mV.

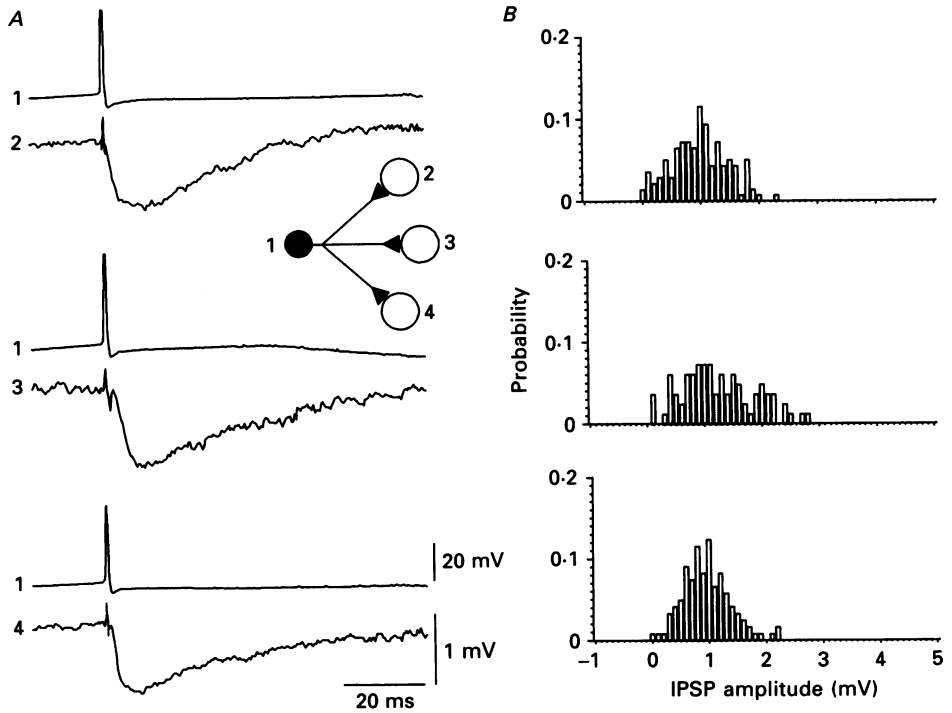

Fig. 7. One inhibitory cell evokes IPSPs of similar amplitude in three postsynaptic cells. *A*, shows averages of IPSPs elicited by inhibitory cell 1 in pyramidal cells 2, 3 and 4. Recordings from cells 2, 3 and 4 were made sequentially while that from cell 1 was maintained. *B* shows histograms of IPSP amplitudes from the three postsynaptic cells. The mean amplitude of the IPSP in cell 2 was 0·9 ± 0·5 mV (mean ± S.D., $n = 139$), in cell 3 it was 1·1 ± 0·6 mV ($n = 86$) and in cell 4 it was 1·1 ± 0·4 mV ($n = 120$).

Further insight into the specificity of inhibitory synaptic strength was sought by comparing the amplitudes of spontaneous IPSPs occurring simultaneously in two pyramidal cells (Fig. 8). When recordings were made from cells separated by more than 800 μm, few synaptic events occurred simultaneously ($n = 4$ cell pairs). In contrast, when electrodes entered the slice less than 200 μm apart ($n = 8$ cell pairs) 58–95% of spontaneous synaptic events occurred with less than 1 ms difference in their time of onset (Fig. 8*A* and *B*). Records were made in the presence of CNQX and APV so simultaneous IPSPs probably reflected firing of single inhibitory cells rather than two inhibitory cells firing synchronously in response to a common excitatory input. The absence of responses in one cell to hyperpolarizing current injected into the other cell suggested that these neurones were not electrotonically coupled. If single inhibitory cells make synapses of similar strength with all the cells they

innervate then the amplitudes of simultaneous IPSPs in pairs of pyramidal cells should be correlated.

In the recordings shown in Fig. 8, 78% of IPSPs in cell 2 occurred simultaneously with an IPSP in cell 1. Amplitudes of the remaining events (see the second and third

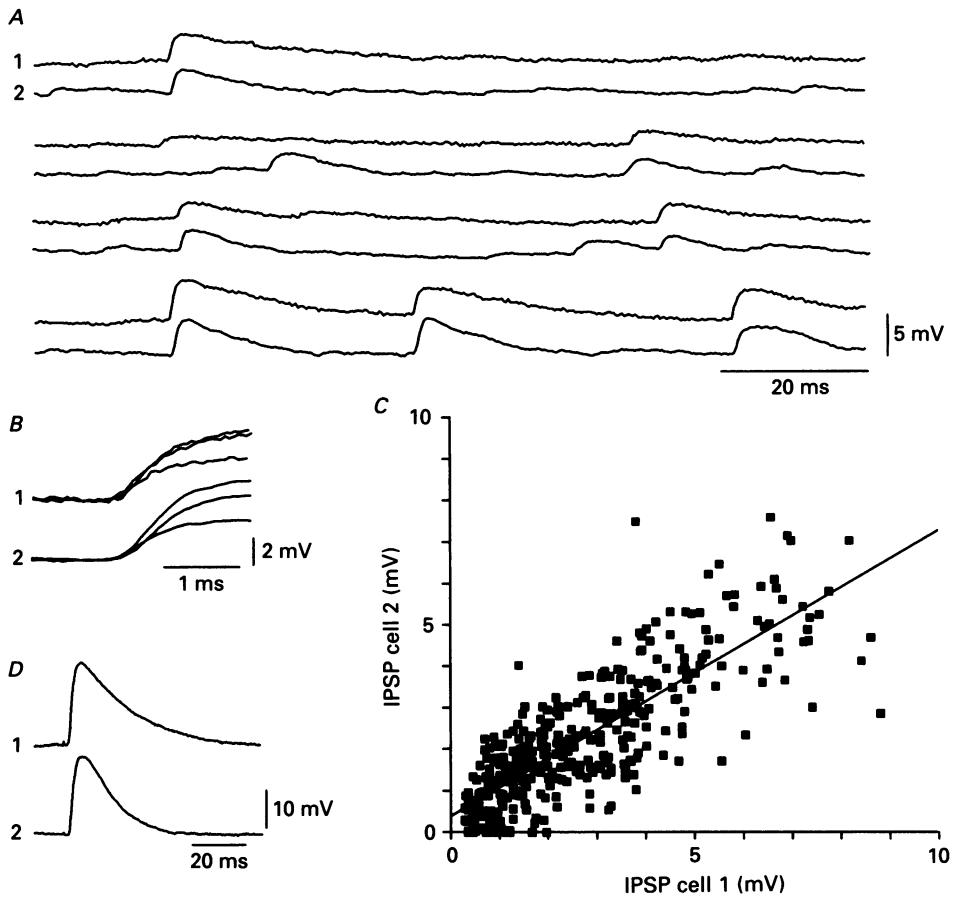

Fig. 8. Synchronous IPSPs in neighbouring pyramidal cells. *A*, depolarizing IPSPs recorded with KCl electrodes from two pyramidal cells in the presence of CNQX (10 μM) and APV (100 μM). Many IPSPs were synchronous in both cells, with time of onset (*B*) differing by less than 1 ms. *C* shows the amplitude of 385 IPSPs in cell 1 (recorded over 3·5 min) plotted against the corresponding potential change in cell 2. IPSP amplitude in cell 1 was quite well correlated with that of events in cell 2. The straight-line fit gave a relation: IPSP cell 1 = 0·69 × IPSP cell 2 + 0·39 (R^2 = 0·71). *D*, local electrical stimulation elicited a smaller, faster decaying response in cell 2 than in cell 1 suggesting differences in driving force might underly the deviation from a unity slope.

pairs of traces from Fig 8*A*) varied over a wide range. They probably originate in part from cells which made synapses with cell 2 but not cell 1. Ninety-five per cent of the IPSPs recorded in cell 1 occurred simultaneously with an IPSP in cell 2. The remaining, isolated events in cell 1 were small (Fig. 8*A* trace 2 and Fig. 8*D*). Small isolated IPSPs might originate in two additional ways. Firstly, transmission failures

(Fig. 6) may occur at one synapse made by an inhibitory cell which contacts both neurones. Secondly, action potential-independent exocytosis of GABA (Ropert *et al.* 1990) would not be expected to occur simultaneously from different presynaptic terminals.

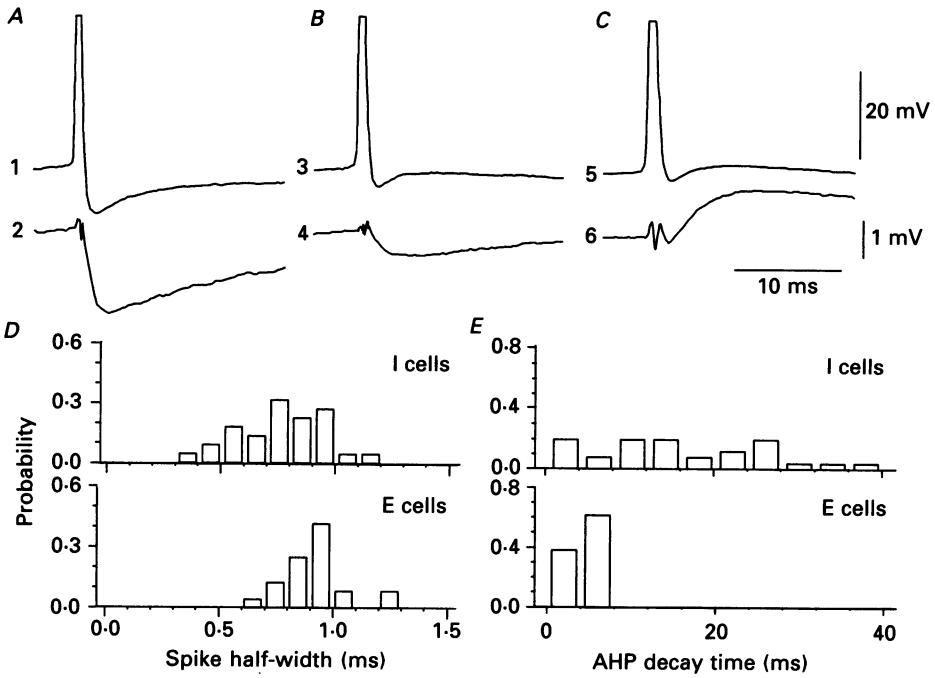

Fig. 9. Action potentials of inhibitory and excitatory cells. Averaged traces ($n = 40$) from pre- and postsynaptic cells at two inhibitory synapses (*A* and *B*) and one excitatory synapse (*C*). Presynatic traces are enlarged to show after-potentials. Action potentials of inhibitory cell 1 were faster than those in excitatory cell 5. They were followed by a large, slowly decaying AHP, whereas in the excitatory cell a short AHP was succeeded by a depolarizing after-potential. The properties of action potentials in inhibitory cell 3 were between those of cells 1 and 5. *D*, distribution of action potential durations at half-height for thirty inhibitory cells (I) and twenty-five excitatory cells (E). *E*, distribution of times for 90% decay of AHPs in inhibitory and excitatory cells.

When the amplitudes of correlated IPSPs in cells 1 and 2 were compared (Fig. 8*C*) an approximately linear relation emerged. Similar relations were apparent for the amplitudes of synchronous IPSPs in another eleven CA3 cell pairs. Typically the scatter of points increased with IPSP amplitude, presumably since IPSP amplitude fluctuations are larger for stronger synapses (Fig. 6). However large events in one cell were rarely correlated with small IPSPs in another neurone as would be expected if single inhibitory cells made both strong and weak synapses with different postsynaptic cells. The slope of straight lines fitted to these plots was not always 1, possibly due to differences in the driving force for Cl^- ions. In this case the amplitude of the summed IPSP elicited by local stimulation with tungsten electrodes was smaller in cell 2 (Fig. 8*D*). Simultaneous IPSPs were also, in general, smaller in cell 2 than in cell 1.

Presynaptic firing pattern and postsynaptic efficacy

While the efficacy of inhibitory connections varies, single inhibitory cells seem to make synapses of similar strength with different pyramidal cells. Variations in the shape of inhibitory cell action potentials were also apparent (Figs 2, 5 and 6). It

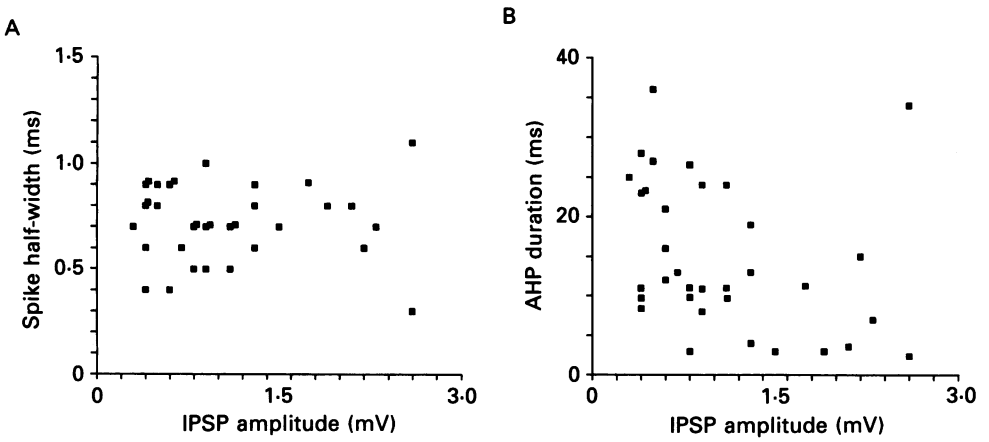

Fig. 10. There was not a good correlation between presynaptic firing pattern and inhibitory synaptic efficacy. *A*, averaged IPSP amplitude from thirty-four connections plotted against the half-width of action potentials in the presynaptic cell. *B*, IPSP amplitude plotted against the time for 90% decay of the AHP in the presynaptic cell.

would be interesting if synaptic efficacy were correlated with the electrical properties of the presynaptic cell. The shape of presynaptic action potentials was examined to test this hypothesis (Fig. 9). Short action potentials with large prolonged after-hyperpolarizations (AHPs) have been used as criteria to identify inhibitory cells in hippocampus and cortex. The duration of presynaptic action potentials and the duration of spike after-hyperpolarizations (AHPs) were measured. The duration of AHPs was measured rather than AHP amplitude since it was less sensitive to variation in membrane potential. These parameters measured from identified inhibitory cells were also compared with values from twenty-five cells which evoked mono-synaptic EPSPs in CA3 neurones (Miles & Wong, 1986).

The duration of inhibitory cell action potentials, measured at half-amplitude, varied between 0·3 and 1·0 ms and the mean value was $0{\cdot}7 \pm 0{\cdot}2$ ms ($n = 30$). For excitatory cells, the mean action potential duration was $0{\cdot}8 \pm 0{\cdot}1$ ms ($n = 25$). There was significant overlap in the distributions, although some inhibitory cells clearly had shorter duration action potentials than any excitatory neurone (Fig. 9*D*). The time taken for decay of the AHP discriminated between inhibitory and excitatory cells (Fig. 9*E*). A fast AHP reached a maximum about 2 ms after an excitatory cell action potential and was succeeded by a depolarizing after-potential (Wong & Prince, 1981). The mean time for 90% decay of the AHP was $4{\cdot}2 \pm 1{\cdot}3$ ms. In contrast the mean time for AHP decay in inhibitory cells was 21 ± 8 ms. However in some inhibitory cells AHP decay was as fast as in excitatory cells.

These findings show inhibitory cell action potentials vary in shape (Miles & Wong,

1984) and overlap to some extent with action potential shape in identified excitatory neurones. Finally, I asked whether these properties of inhibitory cell action potentials were correlated with the efficacy of their inhibitory synapses. Figure 10*A* shows the duration of somatic action potentials was not well correlated with mean IPSP amplitude. There was a tendency for inhibitory cells with more rapidly decaying AHPs to generate larger IPSPs (Fig. 10*B*) but one cell with a slow AHP clearly evoked a large IPSP.

DISCUSSION

This report sought to uncover factors underlying differences in the properties of IPSPs evoked in CA3 pyramidal cells by a group of inhibitory cells located close to the stratum pyramidale. At single inhibitory synapses, IPSPs fluctuated in amplitude (Fig. 6). At different inhibitory synapses, the mean amplitude of IPSPs varied over a range of about 9 times (Figs 2 and 3). This variation in efficacy appears not to originate postsynaptically as suggested previously (Miles & Wong, 1984) but may instead arise from variation in transmitter release. Further, synaptic strength appears to be quite specific to connections made by individual inhibitory cells (Figs 7 and 8).

Cl^-- and K^+-mediated IPSPs in CA3 pyramidal cells

Inhibitory postsynaptic potentials evoked in CA3 pyramidal cells by stratum pyramidale inhibitory cells reversed completely at potentials close to the apparent reversal of the first phase of the afferent evoked IPSP (Fig. 4). Furthermore, inhibitory events elicited by single spikes or repetitive firing were suppressed by picrotoxin (Fig. 5). Transmitter liberated by these inhibitory neurones then seems to act exclusively on $GABA_A$ receptors coupled to Cl^- channels.

Inhibitory cells recorded in this study probably correspond to basket and chandelier cells (Ramon y Cajal, 1911; Lorente de Nó, 1934; Tombol *et al.* 1978; Somogyi *et al.* 1983). The axon terminals of these neurones largely face the soma and initial segment of CA3 pyramidal cells (Somogyi *et al.* 1983; Katsumaru *et al.* 1988; Kawaguchi & Hama, 1988). They do not contact distal apical dendrites where K^+-mediated synaptic inhibition appears to be generated (Newberry & Nicoll, 1985; Dutar & Nicoll, 1988). If another set of inhibitory neurones generates the late, K^+-mediated phase of afferent inhibition in CA3 pyramidal cells (cf. Lacaille & Schwartzkroin, 1988) then different inhibitory cells are coupled to different types of GABA receptor. This contrasts with synapses in *Aplysia* (Kehoe, 1972), *Tritonia* (Getting, 1981) and *Xenopus* (Dale & Roberts, 1985) and excitatory connections formed between hippocampal cells in culture (Forsythe & Westbrook, 1988) where single presynaptic neurones release transmitter that activates multiple postsynaptic receptors.

Variation in efficacy of inhibitory synapses

The variation in amplitude of unitary IPSPs (Fig. 2) may have resulted in part from differences in driving force and postsynaptic membrane properties. However, differences persisted when unitary IPSP amplitudes were compared to the maximal IPSP induced in the same cell by afferent stimulation (Fig. 4). Furthermore there

was a wide variation in amplitude of IPSPs recorded in single pyramidal cells (Fig. 3). Small events were dominant, both in the amplitude distributions for IPSPs evoked by single inhibitory cells (Fig. 2) and for spontaneous IPSPs (cf. Jankowska & Roberts, 1972; Korn & Faber, 1990; Ropert *et al.* 1990).

The variation in amplitude of averaged IPSPs expressed as a coefficient of variation (c.v.) was 0·65. This is greater than the variation observed in mean amplitudes of recurrent EPSPs in the CA3 region (c.v. = 0·32). However inhibitory synaptic events from other systems show similar variabilities. IPSPs evoked in the goldfish Mauthner cell by an identified population of glycinergic cells (Korn, Faber & Triller, 1986) and IPSCs in buccal ganglion cells of *Aplysia* evoked by cholinergic neurones (Gardner, 1986) have c.v. values of 0·8 and 0·9 respectively and a value of 0·9 was derived from IPSPSs elicited by Ia inhibitory cells in cat spinal motoneurones (Jankowska & Roberts, 1972).

What is the mechanism for variation in inhibitory synaptic efficacy?

Inhibitory postsynaptic potentials were mediated by Cl^--channels (Figs 4 and 5) so different postsynaptic receptors did not account for the variation in inhibitory synaptic efficacy. Differences in location of inhibitory terminals seemed not to be involved, since IPSP time to peak tended to increase with amplitude. The origin of this effect is not clear. Another postsynaptic factor, which was not examined, is that the conductance change due to a single quantum of inhibitory transmitter might differ at synapses of different strengths. However since single pyramidal cells received both weak and strong inhibitory inputs (Figs 3 and 8) this implies that miniature IPSPs in one cell should vary widely in amplitude. This seems not to be the case for tetrodotoxin-resistant events in CA1 neurones (Ropert *et al.* 1990).

Another observation suggested variation in synaptic efficacy was at least partly presynaptic in origin. Selective averaging revealed that transmission failures occurred at some weak inhibitory synapses but not at strong synapses (Fig. 6). Differences in release underly variation in efficacy at frog (Grinnell & Herrera, 1980) and crayfish (Bittner, 1968) neuromuscular junctions and at inhibitory synapses made on the Mauthner cell (Korn *et al.* 1986). In contrast, EPSP efficacy at Ia afferent synapses on spinal motoneurones was reported to depend on the size of postsynaptic cells due to the effect of cell size on input resistance (Mendell & Hennemann, 1971). However, EPSPs elicited by different Ia fibres in the same motoneurone have more recently been shown to vary in amplitude over a 10-fold range (Clamann, Hennemann, Luscher & Mathis, 1985).

Variation in transmitter release at inhibitory synapses might result from differences in the number of release sites or in the probability of GABA release or in both. Attempts to distinguish between these alternatives by analysis of IPSP amplitude fluctuations were not presented since, with potassium acetate-filled electrodes, the size of inhibitory quanta may not be much larger than the noise. Both filling postsynaptic cells with Cl^- (Figs 3 and 8) and using low-resistance suction electrode techniques (Edwards, Konnerth, Sakmann & Takahashi, 1989) may improve the ratio of quantal size to noise so that this question may be resolved.

Alternatively labelling inhibitory cells would allow the number of terminals to be compared with synaptic efficacy. Basket and chandelier cells, in hippocampus or

cortex, make between three and thirty-five terminals on postsynaptic pyramidal cells (Somogyi *et al.* 1983; Kisvarday, Martin, Whitteridge & Somogyi, 1985; Kisvarday, Martin, Friedlander & Somogyi, 1987). This variation in terminal numbers is close to the range of inhibitory synaptic efficacies. However variation in the probability of release may also contribute to differences in inhibitory synaptic efficacy. Both direct (Fig. 7) and indirect (Fig. 8) observations suggested single inhibitory cells made connections of similar efficacies with different pyramidal cells. Then if the number of release sites alone determined synaptic efficacy, one inhibitory cell might form a similar number of terminals with all postsynaptic cells. Alternatively, release probability may vary to ensure the release of a similar amount of transmitter at different connections made by a single inhibitory cell.

Inhibitory circuitry in the CA3 region

This work shows CA3 pyramidal cells receive inhibitory inputs of varying strengths (Figs 2, 3 and 8). Comparison of the amplitude of unitary events with that of the maximal inhibition evoked by afferent stimuli suggests single pyramidal cells receive ten to fifty inhibitory inputs. Some inhibitory cells tend to make stronger synapses and others weaker synapses (Figs 2 and 6). However inhibitory synaptic efficacy was not correlated with presynaptic action potential shape (Fig. 10). Electrical properties seem not to define subsets of stratum pyramidale inhibitory cells of different strengths.

Synaptic inhibition controls the spread of activity via recurrent synapses between CA3 pyramidal cells and thus the degree of synchrony in population firing (Traub, Miles & Wong, 1989). Our modelling studies suggest that if two pyramidal cells are connected by a di-synaptic excitatory pathway and a functional but weak inhibitory pathway, firing may be transmitted although with a longer latency. Activity may then escape along excitatory pathways with a weak inhibition in parallel, while activity is blocked in other pathways where inhibitory synapses are strong. This effect may partly underly the generation of weakly synchronous population activities in the presence of $GABA_A$-mediated inhibition.

Records from pairs of pyramidal cells revealed an interesting spatial aspect of inhibitory synaptic connectivity (Fig. 8). Neighbouring cells received many simultaneous IPSPs while distant cells had very few common IPSPs. The anatomical substrate for this effect may be that inhibitory cell axons are highly divergent but ramify in a restricted area (Ramon y Cajal, 1911; Lorente de Nó, 1934; Tombol *et al.* 1978; Kosaka *et al.* 1985; Kawaguchi & Hama, 1988). With overlapping inhibitory innervation from cells which make synapses of similar strength, neighbouring pyramidal cells will receive spontaneous IPSPs of highly correlated amplitude and timing. Modulating recurrent inhibition should then cause nearly identical changes in the fine structure of inhibition which impinges on localized clusters of pyramidal cells. Further studies may reveal how precisely inhibition controls the activity of local groups of pyramidal cells.

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