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SYNAPTIC EXCITATION OF INHIBITORY CELLS BY SINGLE CA3 HIPPOCAMPAL PYRAMIDAL CELLS OF THE GUINEA-PIG *IN VITRO*

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SUMMARY

1. In simultaneous recordings from pairs of neurones in hippocampal slices from guinea-pigs, single action potentials fired by CA3 pyramidal cells could initiate inhibitory postsynaptic potentials (IPSPs) in nearby pyramidal cells.

2. The latencies of these IPSPs could be as short as 3 ms. However, they were mediated disynaptically via chemical, excitatory synapses, since inhibitory coupling was suppressed by an excitatory amino acid antagonist.

3. The properties of excitatory synapses made onto inhibitory cells were examined to assess the basis for this strong coupling. Inhibitory cells were identified either by showing that they inhibited another cell or by their characteristic firing pattern.

4. Excitatory postsynaptic potentials (EPSPs) elicited by single pyramidal cell action potentials had a mean amplitude of 1–4 mV and a time to peak of 1·5–4 ms. In most cases they decayed with a time constant similar to that of the inhibitory cell membrane.

5. EPSP amplitude increased with hyperpolarization of the postsynaptic membrane. Membrane polarization had little effect on EPSP shape.

6. EPSPs fluctuated in amplitude and transmission sometimes failed, suggesting transmission was quantal and that few quanta were released.

7. When presynaptic cells were made to fire bursts of action potentials, EPSPs in inhibitory cells were initially potentiated.

8. EPSPs could cause inhibitory cells to fire. The interval between pre- and postsynaptic spikes could be as short as 2·5 ms and the probability of spike transmission could be as high as 0·6. Some inhibitory cells which received feedback excitation were also excited in feedforward fashion by mossy fibre stimuli.

9. One pyramidal cell could activate several disynaptic inhibitory pathways terminating on another pyramidal cell. This suggests that excitatory synapses made by pyramidal cell axon collaterals onto inhibitory cells are divergent.

10. This strong, divergent excitation of inhibitory cells ensures recurrent inhibition is sufficiently widespread, rapid and potent to control the spread of activity by recurrent excitatory connections between CA3 pyramidal cells.

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INTRODUCTION

Afferent stimuli elicit disynaptic IPSPs with short latencies in cells from many regions of the brain, including spinal motoneurones (Eccles, Fatt & Koketsu, 1954), thalamic neurones (Andersen & Sears, 1964) and pyramidal cells in cortex (Phillips, 1959) and hippocampus (Spencer & Kandel, 1961). This suggests that excitatory synapses made onto inhibitory cells reliably cause the inhibitory cells to fire. One factor that may ensure effective coupling is that inhibitory cells often have a low firing threshold (Ross, Cleveland & Haase, 1975; Schwartzkroin & Mathers, 1978). Less is known about another important factor: the effectiveness of synapses that excite the inhibitory cells.

Hippocampal inhibitory cells are excited by afferent synapses (Buszaki, 1983) and by recurrent synapses made by axon collaterals of nearby pyramidal cells (Andersen, Eccles & Loyning, 1964; Knowles & Schwartzkroin, 1981). Since single CA3 pyramidal cells can initiate IPSPs in nearby pyramidal cells (Miles & Wong, 1984) it appears that the feedback excitation of inhibitory cells may be very strong.

In the present experiments feedback excitation was examined directly by recording from CA3 pyramidal cells and inhibitory cells that they excited in guinea-pig hippocampal slices. Unitary EPSPs were large with a fast time course and often induced postsynaptic firing. The properties of these EPSPs were also compared with those of recurrent EPSPs evoked by single CA3 pyramidal cells in other pyramidal cells (Miles & Wong, 1986). Since the presynaptic cells were from the same population while the postsynaptic cells differed, this allowed pre- and postsynaptic factors controlling synaptic efficacy to be assessed.

METHODS

Transverse hippocampal slices, of 400 μm thickness, were prepared with a vibratome from guinea-pigs (weight 200–300 g) killed by cervical dislocation (Miles & Wong, 1984). 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; $MgCl_2$, 2; $NaHCO_3$, 26; and D-glucose, 10. Their upper surface was exposed to a warmed (37 °C), moistened 5% CO_2 in O_2 atmosphere resulting in a pH of 7·4.

Intracellular recording electrodes were made from fibre-filled glass tubing (F. Haer). They were filled with 3 M-potassium acetate (pH adjusted to 7·2) and bevelled to a resistance of 40–90 MΩ before use. The excitatory amino acid antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), obtained from Tocris Neuramin, was used in some experiments.

Membrane potentials were measured using an amplifier (Axoclamp 2) operated in the current-clamp mode. Pyramidal cells were recorded from the stratum pyramidale of the CA3 region. Inhibitory cells were often penetrated in a region of thickness about 100 μm on either side of the stratum pyramidale. The separation between the points at which electrodes recording from pre- and postsynaptic cells entered the slices was less than 500 μm. Presynaptic cells were made to fire single action potentials or to fire repetitively by injecting depolarizing current pulses every 1 or 2 s. Maintained hyperpolarizing current was applied when needed to prevent firing between pulses. Afferent fibres were stimulated with bipolar tungsten electrodes using electrical pulses of duration 50–100 μs.

Capacitative coupling artifacts between recording electrodes were apparent in the trace from a postsynaptic cell when the presynaptic cell fired an action potential. They were suppressed by electrically subtracting a differentiated, amplified presynaptic signal from the postsynaptic voltage (cf. Miles & Wong, 1986). Postsynaptic signals were filtered at 1 kHz. These procedures affected the time to peak of synaptic events by less than 5%. Membrane potentials were measured from the

potential change on withdrawal of the electrode from a cell. Membrane input resistance and time constant were measured from responses to hyperpolarizing current injections of intensity 0·5 nA and duration 100 ms.

Voltage signals were stored on videotape using a modified pulse code modulation device (Neurodata Instruments). For subsequent analysis they were stored in the hard disc of an IBM-XT computer after analog-to-digital conversion (Tekmar Labmaster) with 12-bit resolution at a rate of between 0·05 and 0·5 ms per point. Analysis programs (written by Shuo Huang of the Department of Electrical Engineering at Columbia University) allowed capture, measurement and selective averaging of pre- or postsynaptic traces. Subsequent statistical analysis was done and figures were made with spread-sheet and graphics programs (Lotus Development Corp.).

Quantal analysis

Attempts were made to extract values for the quantal parameters N, p and q at these excitatory synapses. The number of quanta released was assumed to follow binomial statistics (del Castillo & Katz, 1954; Johnson & Wernig, 1971). The number of release sites, N, was allowed to take only an integer value (McLachlan, 1978). Release was assumed to occur with equal probability, p, at each release site (Korn, Triller, Mallet & Faber, 1981). The postsynaptic action of a single quantum, q, was assumed to be normally distributed with a standard deviation Δq (del Castillo & Katz, 1954).

A period was selected when postsynaptic responses were stable, judged by the absence of a long-term trend in running averages of thirty responses. Histograms of EPSP and noise amplitude distributions were constructed from this period. An initial estimate for the quantal parameters was obtained from three measurements made on these distributions. They were the mean EPSP amplitude, the variance of the EPSP amplitude distribution (minus the variance of the noise distribution) and the proportion of transmission failures. Transmission failures were first estimated visually and were then checked by selectively averaging traces which appeared to represent failures.

Then for a binomial distribution:

$$\text{Mean} = Npq,$$

$$\text{Variance} = Npq^2(1-p).$$

The proportion of failures is the first binomial term:

$$\text{Failures} = (1-p)^N.$$

The following expression in one variable can then be obtained.

$$\frac{\text{Variance}}{(\text{Mean})^2}\log_e(\text{Failures}) = \frac{(1-p)}{p}\log_e(1-p).$$

This expression was solved arithmetically to give a value for p. Values for N and q were then calculated. However, this procedure rarely gave an integer value for N and the value of Δq was not known. Therefore the values for N, q and p were used as initial estimates in a search procedure to optimize the fit between the experimental EPSP distribution and the binomial distribution. N was varied in integer steps, p was varied in steps of 0·01 and q was varied in steps of 0·01 mV. A Gaussian distribution for each q value was made with Δq varying in steps of 1% of q. Values of q were not corrected for non-linear summation (Martin, 1955). The resulting distribution was then convoluted with the experimental noise distribution. The sum of the squares of differences between this distribution and the experimental EPSP distribution was minimized to arrive at final values for N, p, Δq and q.

RESULTS

Disynaptic IPSPs

Frequently, action potentials in one CA3 pyramidal cell initiated IPSPs in a nearby pyramidal cell (Fig. 1). When firing in one cell was used to trigger an average of membrane potential fluctuations in a simultaneously recorded neurone, inhibitory interactions, which could be reciprocal, were detected in about 30% of cell pairs tested.

Although coupling could be very strong, several factors suggested the inhibitory interactions were not mediated monosynaptically. As shown in Fig. 1, a high-frequency burst of presynaptic action potentials usually elicited a single IPSP. Furthermore, IPSP latency fluctuations and transmission failures seemed in-

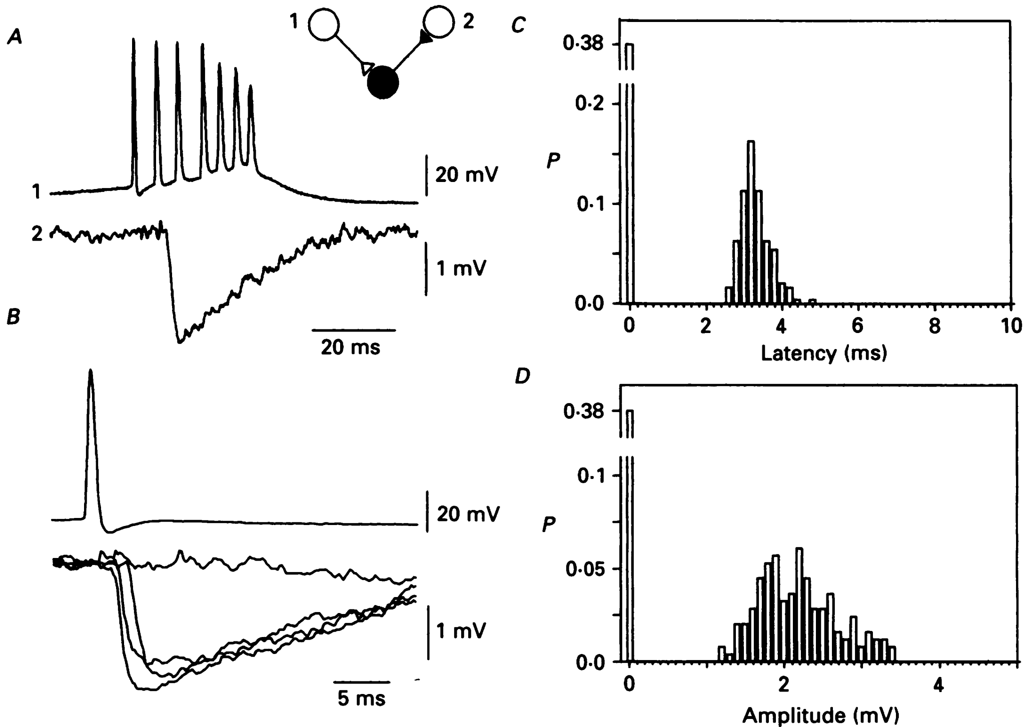

Fig. 1. An inhibitory interaction between two CA3 pyramidal cells. *A*, a burst of seven action potentials in cell 1 evoked a single IPSP in cell 2. *B*, the latency of IPSPs elicited in cell 2 by single spikes in cell 1 fluctuated and transmission sometimes failed. *C*, distribution of latencies for IPSPs elicited in cell 2 by 243 spikes in cell 1. The ninety-two transmission failures that occurred are plotted at 0 mV. Mean IPSP latency was $3{\cdot}3 \pm 0{\cdot}4$ ms (mean ± S.D., $n = 151$). *D*, amplitude probability distribution for IPSPs. The mean amplitude was $2{\cdot}2 \pm 0{\cdot}6$ mV.

consistent with monosynaptic coupling. These parameters were measured from responses to at least 200 single action potentials in ten of the most strongly coupled cell pairs. The mean IPSP latency was $3{\cdot}5 \pm 0{\cdot}7$ ms (mean ± S.D., $n = 10$) and the probability that an IPSP was evoked was $0{\cdot}63 \pm 0{\cdot}18$. The mean amplitude of averaged IPSPs was $1{\cdot}9 \pm 0{\cdot}6$ mV. Transmission of IPSPs of this amplitude rarely fails at monosynaptic connections (Miles & Wong, 1984, and unpublished observations). However, if these interactions are disynaptic, and involve pyramidal cell excitation of an inhibitory cell which then inhibits another pyramidal cell, then the excitatory coupling appears to be both rapid and potent.

Electrotonic coupling can mediate a fast transmission of excitation between neurones. Although physiological evidence for electrotonic connections in the hippocampus is limited to coupling between excitatory cells (MacVicar & Dudek,

1981), gap junctions have been identified anatomically on hippocampal inhibitory cells (Katsumaru, Kosaka, Heizmann & Hama, 1988). To discriminate between electrotonic and chemical excitation of inhibitory cells an excitatory amino acid antagonist (Drejer & Honore, 1988) was applied. In two cases the compound 6-

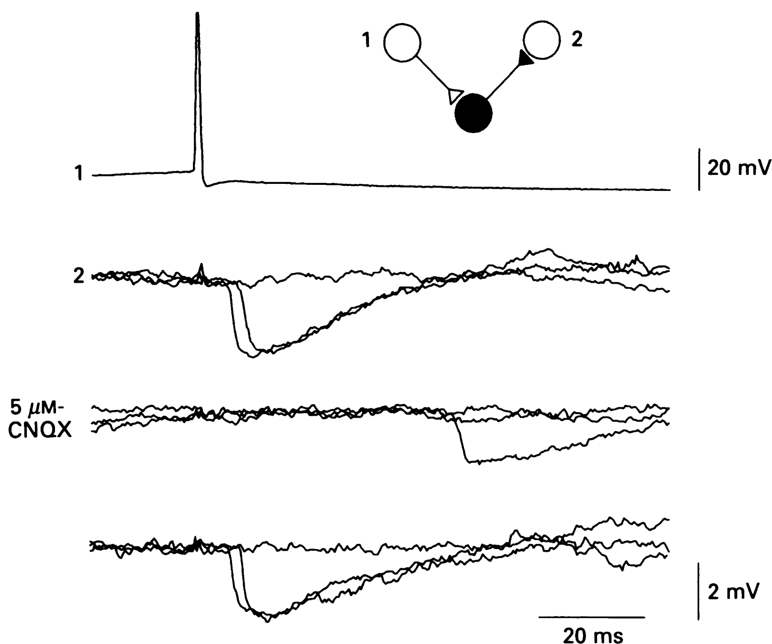

Fig. 2. An excitatory amino acid antagonist suppresses inhibitory coupling between two pyramidal cells. The upper trace from cell 2 shows three responses – two IPSPs and one transmission failure – evoked by single action potentials in cell 1. The inhibitory coupling was suppressed, although spontaneous IPSPs were not blocked, in the presence of 5 μM-CNQX (middle traces). Coupling was re-established on return to the control solution (lower traces).

cyano-7-nitroquinoxaline-2,3-dione (CNQX, 5 μM) completely suppressed strong inhibitory interactions between CA3 cells. Figure 2 shows that when the inhibitory coupling was blocked, spontaneous IPSPs were not suppressed. This experiment excludes both electrotonic coupling and the possibility that single inhibitory connections with a long axonal conduction delay might account for these interactions. Further experiments were performed to examine directly the properties of excitatory synapses onto inhibitory cells.

EPSPs in inhibitory cells

Neurones were identified as inhibitory if they inhibited another cell (Fig. 3) or if their action potentials were followed by a hyperpolarization with duration longer than 10 ms (Schwartzkroin & Mathers, 1978; Knowles & Schwartzkroin, 1981). The properties of unitary EPSPs recorded in inhibitory cells that were identified directly ($n = 2$) or indirectly ($n = 10$) were similar.

Twelve EPSPs initiated by pyramidal cells in inhibitory neurones were examined. Unitary EPSPs, initiated by single presynaptic spikes, were averaged with the postsynaptic cell maintained in a subthreshold potential range. EPSP latency, measured from the peak of the presynaptic action potential to the onset of the

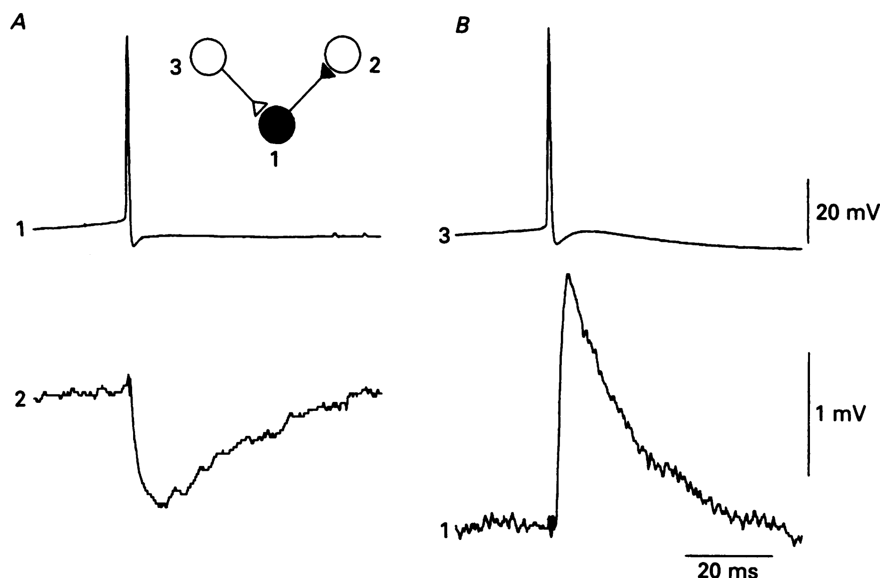

Fig. 3. Monosynaptic EPSPs in an identified inhibitory cell. *A*, cell 1 inhibited cell 2. An average of the IPSPs in cell 2 initiated by single action potentials in cell 1 ($n = 40$) is shown. The electrode was then removed from cell 2 and inserted into cell 3. *B*, cell 3 excited cell 1. Average of EPSPs evoked by single action potentials in cell 3 ($n = 40$).

postsynaptic event, was in all cases less than 1·2 ms. The mean EPSP amplitude was 1·9 ± 0·8 mV (mean ± S.D., $n = 12$) at postsynaptic potentials between −60 and −70 mV. The mean time to peak of EPSPs was 2·6 ± 0·8 ms and their mean duration at half-amplitude was 9·8 ± 4·2 ms.

These EPSPs are larger and have a faster time course than recurrent EPSPs elicited by CA3 pyramidal cells in other pyramidal cells (Miles & Wong, 1986). The mean amplitude of thirty-one unitary recurrent EPSPs initiated by pyramidal cells in other pyramidal cells was 0·8 ± 0·3 mV (mean ± S.D., $n = 31$) and their mean time to peak was 8·1 ± 2·6 ms. Postsynaptic membrane properties might contribute to these differences. The mean input resistance of the inhibitory cells that received EPSPs was 53 ± 12 MΩ and their mean time constant was 9 ± 3 ms. The mean input resistance of postsynaptic pyramidal cells was 29 ± 8 MΩ and their mean time constant was 32 ± 9 ms.

A short electrotonic distance between the, presumably somatic, recording site and the location of excitatory terminals could also contribute to a rapid EPSP time course. The electrotonic location of synaptic sites was assessed by comparing EPSP waveform with membrane responses to current injection (Rall, 1967). In Fig. 4 an averaged EPSP and the potential decay after injecting a small depolarizing current into the postsynaptic cell are plotted against time. Both potential waveforms

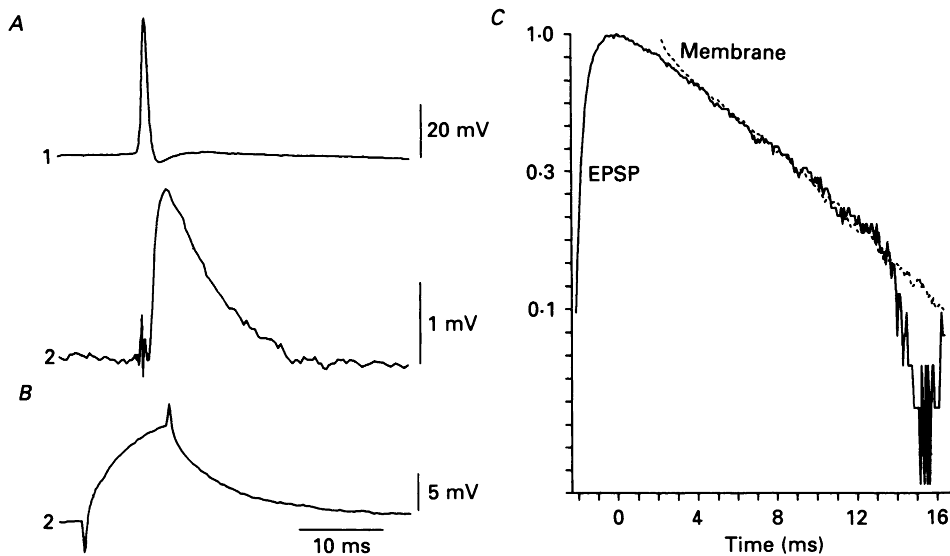

Fig. 4. Comparison of EPSP decay with membrane response to current injection. *A*, EPSPs in inhibitory cell 2, elicited by single pyramidal cell spikes in cell 1, were averaged ($n = 80$). *B*, responses to 0·2 nA, 10 ms depolarizing current injections into cell 2 were averaged ($n = 80$). *C*, both voltage traces were normalized to the same peak amplitude and plotted on a logarithmic scale against time. Time $t = 0$ is at the peak of the EPSP. The membrane response to current injection is positioned to show the similar time course of both traces. The slowest time constant of the membrane response was found to be 7·8 ms from a least-squares exponential fit ($R^2 = 0{\cdot}99$).

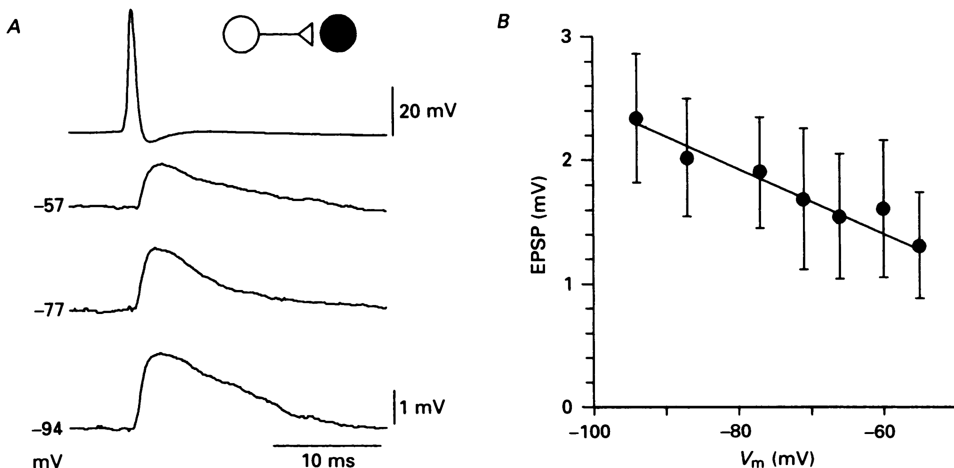

Fig. 5. Voltage dependence of an EPSP in an inhibitory cell. *A*, averaged EPSPs recorded while the postsynaptic cell was held at the potentials indicated with maintained current injection. EPSP shape did not change in a consistent fashion with membrane potential. *B*, EPSP peak amplitude increased monotonically with membrane hyperpolarization. The points plotted represent mean and standard deviation for at least thirty EPSPs recorded at each potential. The straight line fitted to these points had a slope of $-0{\cdot}02$ and the intercept on the membrane potential axis was -7 mV.

decayed with similar time constants, suggesting that synaptic terminals were located close to the recording site. The decay of recurrent EPSPs recorded in nine of twelve inhibitory cells was well approximated by a single-exponential function. The mean time constant for the EPSPs was 8·9 ± 2·9 ms and in the same cells the final

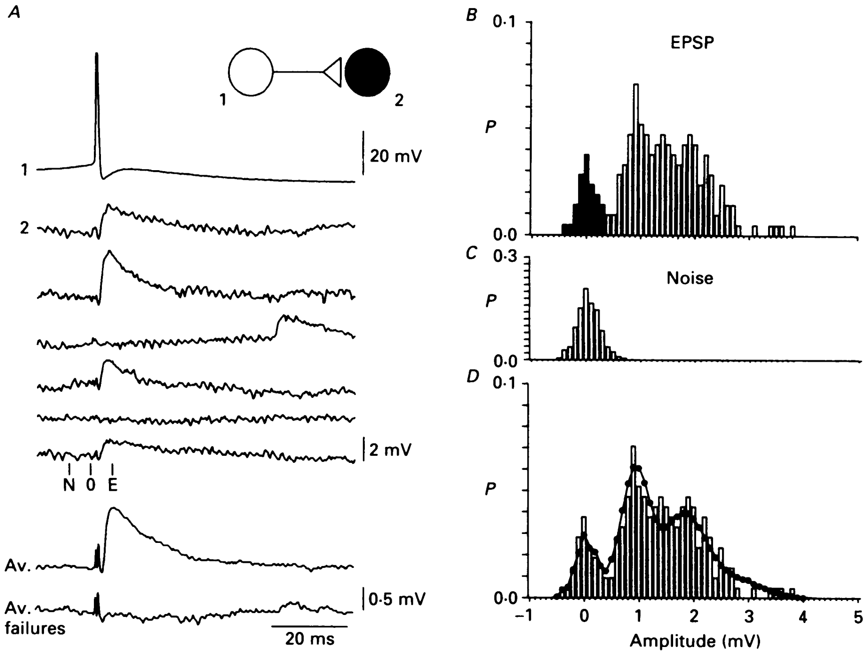

Fig. 6. EPSP amplitude fluctuations. *A*, six responses in an inhibitory cell, cell 2, elicited by single spikes in cell 1. An averaged EPSP is shown below. The amplitude difference between points 0, before the EPSP, and E, the peak of the averaged EPSP, was measured from 211 responses to generate the EPSP amplitude distribution (*B*). Amplitude differences between points N, and 0, with the same time difference, generated the noise histogram (*C*). There was a peak close to 0 mV (shaded) in the EPSP distribution. The lowest trace of *A* (Av. failures) is an average of these traces ($n = 38$). A simple binomial distribution was constructed using values of $N = 3$, $p = 0·48$ and $q = 0·9 \pm 0·1$ mV. Convolution with the experimental noise distribution yielded the curve shown in *D*.

membrane time constant was 9·2 ± 3·2 ms. In two of the other cells averaged EPSPs decayed faster than the membrane time constant. In these cases the rapid EPSP decay probably resulted from longer latency IPSPs that were evident in some single responses. EPSP decay in another cell (Fig. 9), where IPSPs were not apparent, was not well fitted by a single-exponential function.

EPSP voltage dependence

The voltage dependence of six unitary EPSPs was examined (Fig. 5). The postsynaptic membrane was held at different potentials and at least thirty EPSPs were collected at each one. The peak amplitude of averaged EPSPs increased approximately linearly with postsynaptic hyperpolarization and EPSP shape showed

no consistent changes with membrane potential. The peak somatic conductance change during EPSPs was estimated (Ginsborg, 1973) using values for neuronal input resistance and the slope of the EPSP–voltage relation and assuming that EPSPs reversed close to 0 mV. Peak conductance values between 0·7 and 2·2 nS were obtained.

EPSP amplitude fluctuations

EPSP amplitude fluctuated at all synapses examined, but there was little variation in their shape. Figure 6*A* shows six responses evoked by single presynaptic action potentials at one connection. In one response transmission appeared to fail. Apparent transmission failures were observed at all excitatory synapses on inhibitory cells.

The distribution of EPSP amplitudes and the distribution of noise, from recording system and membrane sources, were plotted in histogram form (Fig. 6*B*). A peak, centred around 0 mV, in the EPSP amplitude distribution, which mirrored the peak of the noise histogram, seemed likely to represent failures of transmission. Responses corresponding to these measurements were averaged. The flat averaged trace (Av. failures, Fig. 6*A*) confirmed that transmission failures occurred. At this connection, the probability of transmission failure was about 0·15, and at other excitatory connections made on inhibitory cells it varied between 0·05 and 0·20. Transmission also fails at excitatory synapses between CA3 pyramidal cells with a probability of between 0·02 and 0·25 (thirty-one connections).

Estimates for the probability of transmission failure together with an optimization procedure (see Methods) were used to derive values for the quantal parameters N, p, q and Δq. At five synapses, EPSP amplitude was stationary for long enough that histograms of the distribution of EPSP and noise amplitudes could be constructed from at least 200 responses (Fig. 6*B* and *C*). Figure 6*D* shows the result of convoluting a distribution constructed with $N = 3$, $p = 0{\cdot}48$ and $q = 0{\cdot}9 \pm 0{\cdot}1$ mV, with the experimentally measured noise distribution (Fig. 6*C*). At the five synapses, the optimization procedure yielded values for N of between 2 and 6, with a mean value for p of $0{\cdot}53 \pm 0{\cdot}14$ (mean ± S.D., $n = 5$) and for q of $0{\cdot}9 \pm 0{\cdot}2$ mV. It was apparent at some connections, however, that fits obtained with several different values for N were almost equally good.

EPSP frequency dependence

The ability of CA3 pyramidal cells to discharge bursts of several action potentials, at an interval of 5–12 ms, has several consequences for synaptic transmission. Firstly, the security of transmission is enhanced if single spikes do not reliably evoke EPSPs. Secondly, temporal summation occurs when the interval between presynaptic spikes is shorter than the time course of a unitary EPSP. Figure 7*A* shows that temporal summation occurs at excitatory synapses onto CA3 inhibitory cells. However, the effect is less than at recurrent synapses between pyramidal cells (Fig. 7*B*) where EPSPs have a slower time course (Miles & Wong, 1986). Thirdly, repeated presynaptic firing may facilitate or depress EPSPs.

EPSP facilitation was examined in four connections. Responses evoked by a second presynaptic action potential occurring at an interval of 8–12 ms were facilitated by

47 ± 15 %. When individual responses were examined the apparent number of failures was reduced suggesting the effect originated presynaptically. A similar facilitation in response to presynaptic bursts of action potentials occurs at excitatory synapses between pyramidal cells (Fig. 7, Miles & Wong, 1986). Furthermore at CA3

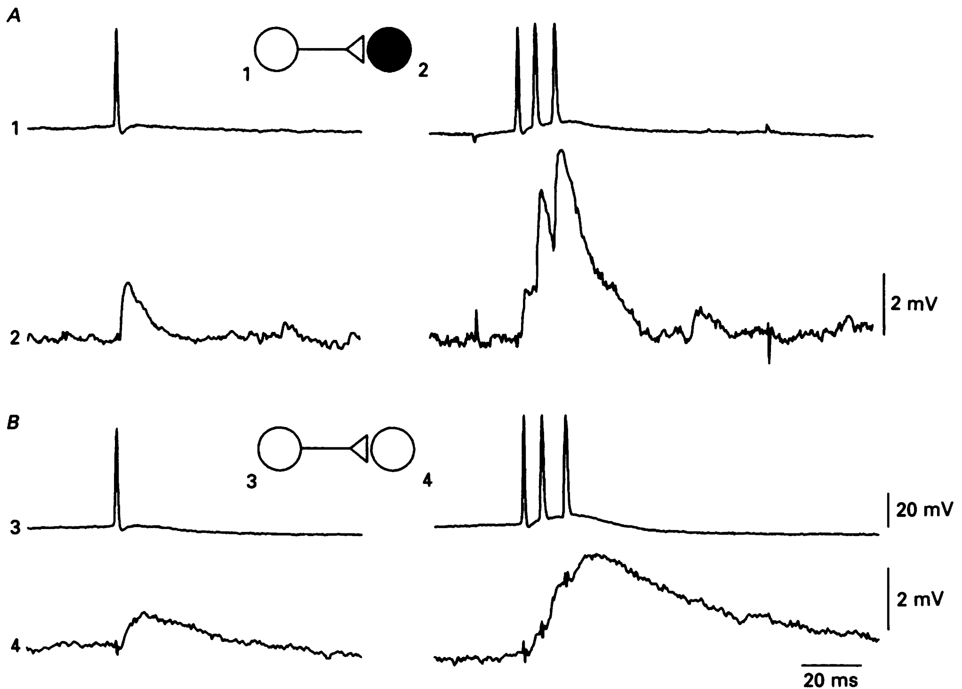

Fig. 7. Comparison of EPSPs evoked in inhibitory cells and pyramidal cells by single presynaptic action potentials and bursts. *A*, EPSPs initiated in an inhibitory cell. *B*, EPSPs evoked in a pyramidal cell. Note the difference in amplitude and time course of the unitary EPSPs. The extent of temporal summation in responses to three presynaptic spikes differs at the two synapses but facilitation of the second and third EPSPs appears to be similar. Representative traces with no apparent transmission failures are shown.

axon collateral synapses made onto both pyramidal cells and inhibitory cells, facilitation may decline during a longer burst of presynaptic spikes (not shown).

Recurrent inhibitory circuits

These results show that EPSPs initiated by pyramidal cells in inhibitory cells have a time to peak of about 3 ms and an amplitude of about 2 mV. Can they account for the short-latency disynaptic IPSPs such as that shown in Fig. 1? If so, single pyramidal cell action potentials must cause inhibitory cells to fire with similar probability and shorter latency than the observed disynaptic IPSPs. In all connections examined unitary EPSPs elicited inhibitory cell firing at resting potential. The probability that single pyramidal cell spikes elicited inhibitory cell discharge was 0·41 at the synapse shown in Fig. 8. The distribution of intervals between pre- and postsynaptic spikes is shown in Fig. 8*B*. In this case the mean interval was 2·9 ± 0·7 ms, and the shortest interval measured at any of the twelve

synapses was $2{\cdot}1 \pm 0{\cdot}5$ ms. Thus large, fast EPSPs and a low postsynaptic firing threshold ensure efficient transmission of recurrent inhibition.

In addition to feedback excitation from pyramidal cell collateral synapses, inhibitory cells in the hippocampus may be excited directly by afferent stimulation

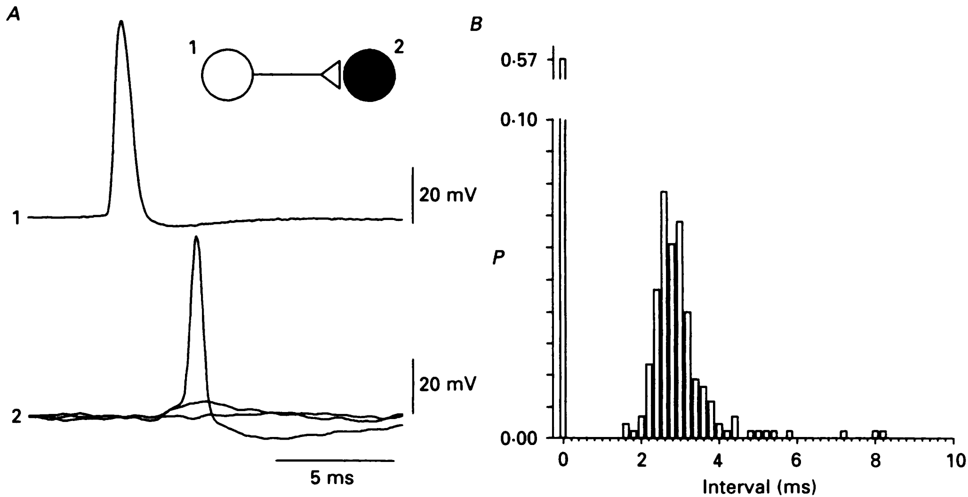

Fig. 8. Spike-to-spike transmission at an excitatory synapse on an inhibitory cell. *A*, cell 1 excites cell 2. Three traces show one transmission failure, one EPSP and an EPSP that caused postsynaptic firing. *B*, latency distribution (measured from peak to peak) for spike–spike transmission. Action potentials were evoked in cell 2 by 175 out of 426 spikes in cell 1. Their latency ranged between 1·7 and 8·2 ms with a mean value of $2{\cdot}9 \pm 0{\cdot}7$ ms (mean ± S.D., $n = 175$).

(Buszaki, 1983; Frotscher, 1985). It has not been clear whether distinct sets of CA3 inhibitory cells are excited differently or whether some inhibitory cells receive both feedback and feedforward excitation. This question was examined by recording responses in an inhibitory cell and a pyramidal cell that excited it, while systematically varying the intensity of mossy fibre stimulation. Figure 9 shows an inhibitory cell that received feedback excitation from a pyramidal cell. The threshold intensity of mossy fibre stimulation which elicited an EPSP in the inhibitory cell was lower than that for the pyramidal cell. At higher stimulus intensities, EPSP latency in the inhibitory cell was shorter than for synaptic events in the pyramidal cell by about 2 ms. A lower threshold and shorter latency afferent response suggested that three of four inhibitory neurones tested received both feedforward and feedback excitation.

Since inhibitory interactions between pyramidal cells were readily detected (Fig. 1) disynaptic inhibition seems to be highly divergent. Divergence of these disynaptic circuits depends on the divergence of inhibitory axons, which is known to be high (Somogyi, Nunzi, Gorio & Smith, 1983; Schwartzkroin & Kunkel, 1985), and on the divergence of excitatory synapses onto inhibitory cells. Figure 10 shows indirectly that excitatory synapses onto inhibitory cells are divergent.

If pyramidal cells each excite a single inhibitory cell, disynaptic IPSPs cannot

be larger than monosynaptic IPSPs. However, if one pyramidal cell excites several inhibitory cells which inhibit another pyramidal cell, several disynaptic IPSPs might be detected. The largest IPSPs evoked in the disynaptic interaction of Fig. 10 generated about 30 % of the inhibition evoked by maximal afferent stimulation. This

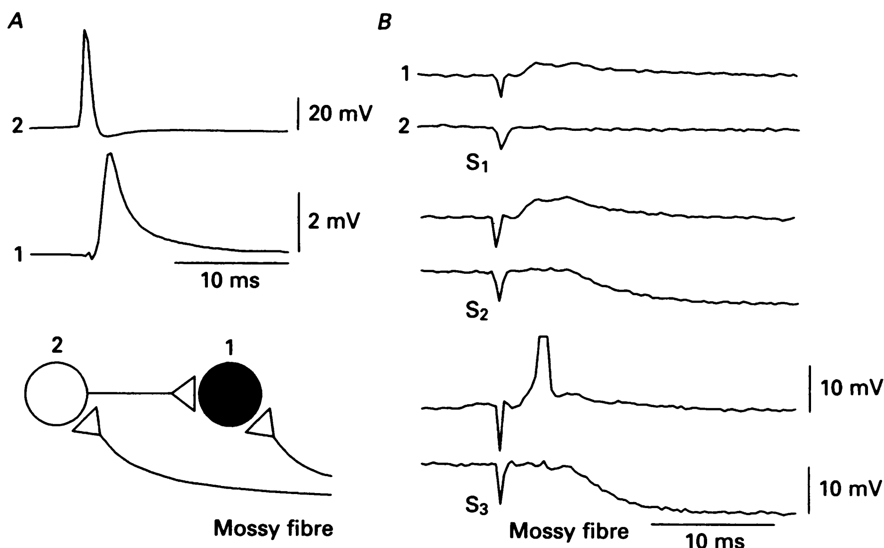

Fig. 9. The same inhibitory cell is excited by feedback and feedforward pathways. *A*, pyramidal cell 2 elicited an EPSP in inhibitory cell 1. *B*, responses of cells 1 and 2 to mossy fibre stimuli. Stimulus intensity was increased in a graded fashion in the three sets of traces (S_1, S_2 and S_3). The inhibitory cell received feedforward excitation, since synaptic events were elicited at lower intensity and with shorter latencies than in the pyramidal cell.

is several times the efficacy of the strongest monosynaptic unitary IPSPs which evoke only about 12 % of the maximal afferent inhibition (R. Miles, unpublished observations). Two other disynaptic IPSPs generated more than 15 % of the maximal afferent inhibition.

A detailed examination of disynaptic responses elicited by this pyramidal cell suggested that at least three recurrent pathways were involved. About 20 % of single spikes evoked no response and the IPSP amplitude distribution had several peaks (Fig. 10*B*). Clear differences were apparent in the latency and time to peak of IPSPs selected from three different regions of the distribution. The smallest events (0·7–1·5 mV) had mean latencies of $4{\cdot}2 \pm 0{\cdot}9$ ms and time to peak of $8{\cdot}7 \pm 2{\cdot}4$ ms while events with medium amplitudes (2·5–3·3 mV) had latencies of $3{\cdot}1 \pm 0{\cdot}5$ ms and times to peak of $3{\cdot}1 \pm 0{\cdot}5$ ms. Averages of small and medium amplitude IPSPs (Fig. 10*B*) show these differences which suggest that at least two different inhibitory pathways were activated. Further examination of the largest disynaptic IPSPs (4·2–6·0 mV) sometimes revealed two components in their rising phase. Neither component matched the smallest IPSPs, so either one cell firing twice or firing in two inhibitory cells could be responsible. Since no inflexion was detected in other equally large events two cells must have been involved. In this case one pyramidal cell axon

apparently diverged to excite three inhibitory cells which then converged to inhibit the second pyramidal cell.

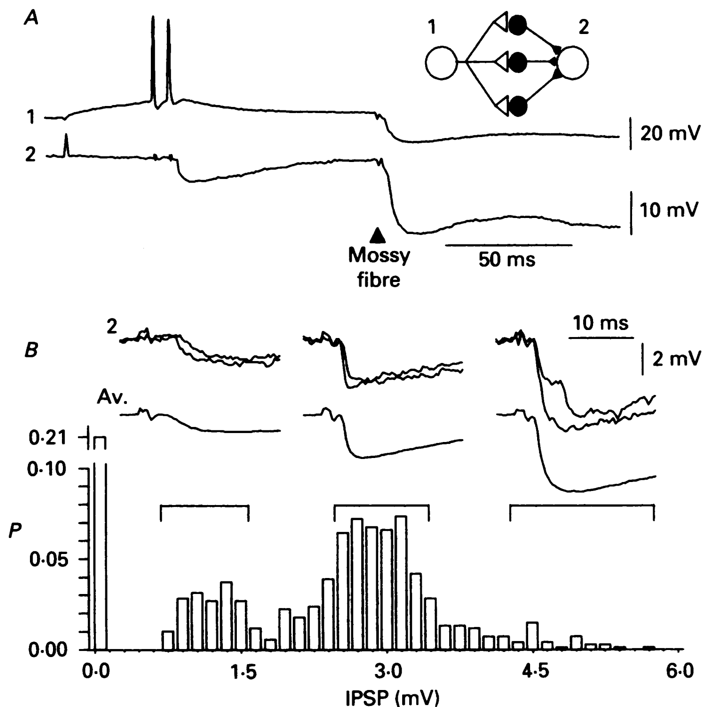

Fig. 10. Divergence of recurrent inhibitory pathways. *A*, a disynaptic IPSP elicited in cell 2 by cell 1 compared with the maximal response to mossy fibre stimulation. It had a similar time course and reversal potential (not shown) to the first phase of the afferent inhibition. The disynaptic IPSP generated up to 30% of the hyperpolarization evoked by a maximal afferent stimulus. *B*, amplitude distribution of disynaptic IPSPs elicited by single presynaptic spikes. 133 of 662 action potentials had no effect. Small (0·7–1·5 mV, $n = 108$), mid (2·5–3·3 mV, $n = 257$) and large amplitude (4·2–6·0 mV, $n = 33$) IPSPs, indicated by the lines above the histogram, were separated. Averages and two selected traces from each group are shown above the amplitude histogram.

DISCUSSION

This report sought to uncover the basis for powerful inhibitory interactions between presumed CA3 pyramidal cells (Fig. 1). Since these interactions were uncoupled by an excitatory amino acid antagonist (CNQX), chemically operated excitatory synapses seemed to be involved (Fig. 2). The timing of disynaptic inhibitory interactions (Fig. 1) suggested that unitary EPSPs should cause inhibitory cells to fire with latencies of 2–3 ms. Figure 7 shows that EPSPs evoked by single pyramidal cell spikes could elicit firing with this latency in inhibitory cells located close to the CA3 stratum pyramidale.

Firing is also transmitted from pre- to postsynaptic cell at excitatory synapses between CA3 pyramidal cells. However, transmission at synapses between pyramidal cells is most effective when the presynaptic cell fires a burst of action potentials and

spike-to-spike intervals are close to 12 ms (Miles & Wong, 1986, 1987). What factors underlie the differences in efficacy of these synapses formed by CA3 pyramidal cell axon collaterals?

Postsynaptic factors

One factor contributing to spike-to-spike transmission is the low firing threshold of inhibitory neurones. Furthermore unitary EPSPs elicited by pyramidal cells in inhibitory cells are larger than those they evoke in CA3 pyramidal cells (Miles & Wong, 1986). Both EPSPs are larger than the EPSPs (0·1–0·2 mV) evoked in CA1 pyramidal cells via the Schaffer collaterals (Turner, 1988; Sayer, Redman & Andersen, 1989). One important difference between the two synapses terminating on CA3 cells may be the faster membrane time constant and higher input resistance of inhibitory cells. Equivalent synaptic currents impinging at similar electrotonic locations should then initiate larger, faster synaptic potentials in inhibitory cells than in pyramidal cells.

The location of excitatory terminals may also differ. The time course of recurrent EPSPs made on pyramidal cells is slow suggesting they impinge on dendrites (Miles & Wong, 1986). In contrast EPSPs in inhibitory cells had a fast time to peak (2–3 ms) and decayed with a time constant similar to that of the postsynaptic membrane (Fig. 4). The rapid time course of EPSPs in inhibitory cells (see also Lacaille, Mueller, Kunkel & Schwartzkroin, 1987) may have an anatomical correlate. Presumed excitatory terminals face the soma of inhibitory cells (Frotscher, 1985; Kisvarday, Martin, Whitteridge & Somogyi, 1985; Schwartzkroin & Kunkel, 1985; Seress & Ribak, 1985) whereas such terminals are absent from the soma of pyramidal cells. Another difference is that excitatory synapses often terminate on pyramidal cell spines (Hamlyn, 1963), which may attenuate synaptic events, while inhibitory cells in guinea-pig hippocampus do not possess spines (Frotscher, 1985; Schwartzkroin & Kunkel, 1985; Seress & Ribak, 1985).

CA3 pyramidal cells presumably release the same transmitter at synapses with other pyramidal cells and with inhibitory cells. The effect of membrane potential on EPSP amplitude and shape may help identify the postsynaptic receptors (Dale & Roberts, 1985; Thomson, West & Lodge, 1985; Forsythe & Westbrook, 1988). At recurrent synapses onto pyramidal cells, EPSP amplitude did not vary linearly with potential and EPSP duration decreased with membrane hyperpolarization (Miles & Wong, 1986). We suggested interactions of synaptic events with inward, dendritic currents might account for these non-linearities. Activation of postsynaptic NMDA receptors would have similar effects, although the involvement of NMDA receptors was not directly tested. In contrast, postsynaptic hyperpolarization increased the amplitude of EPSPs in inhibitory cells and did not change their shape appreciably (Fig. 5). These EPSPs decayed with time constants close to 10 ms (Fig. 4), whereas the NMDA component of synaptic currents recorded in cultured hippocampal cells may last for 100–200 ms (Forsythe & Westbrook, 1987). These observations suggest that transmitter released by single pyramidal cell spikes did not activate NMDA receptors on inhibitory cells. This possibility should be tested directly with NMDA receptor antagonists and does not preclude activation of NMDA receptors by other synapses which excite inhibitory cells. Alternatively recurrent EPSPs may be

mediated by a rapidly inactivating quisqualate channel of conductance 18–35 pS (Tang, Dichter & Morad, 1989; Trussell & Fischbach, 1989). If estimated synaptic conductance changes (1–2 nS) from somatic recordings approximate those at the synaptic site, then 30–110 of these quisqualate channels might open during a unitary EPSP and 8–28 channels might be activated by transmitter released from a single presynaptic site.

Presynaptic factors

Transmitter release depends on presynaptic properties. Failures of release appear to occur at excitatory synapses made by CA3 pyramidal cells on inhibitory cells (Fig. 6) and on pyramidal cells (Miles & Wong, 1986). Possibly action potentials do not propagate reliably along pyramidal cell axon collaterals. However, if release depends solely on events in presynaptic terminals, the frequency of transmission failure suggests that few transmitter quanta were released. Several assumptions were made to extract quantal parameters from EPSP amplitude fluctuations. At these synapses it is unclear whether the probability of release from different terminals is similar or if the postsynaptic actions of single quanta fluctuate. Furthermore non-linear summation (Martin, 1955), which might interfere to some extent with the even spacing assumed for peaks in amplitude histograms, was not considered. However, the analysis suggested that few transmitter release sites exist and that the quantal amplitude is larger than 0·5 mV. These results could be independently checked by attempting to record single excitatory quanta in the presence of tetrodotoxin and by morphological estimation of the number of release sites. In the cortex anatomical studies suggest pyramidal cell synapses onto nearby excitatory and inhibitory cells involve very few (one to five) terminations (Kisvarday, Martin, Freund, Magloczky, Whitteridge & Somogyi, 1986).

Facilitation of synaptic events during repetitive activation is also thought to depend on presynaptic factors (Magelby, 1987). At spike intervals of 8–20 ms EPSPs in inhibitory cells were facilitated (Fig. 7), as were EPSPs in pyramidal cells (Miles & Wong, 1986). The apparent reduction of EPSP facilitation during longer trains of presynaptic spikes requires further investigation.

Recurrent synapses onto inhibitory cells in CA3 circuitry

Mossy fibre stimulation appears to excite directly some CA3 inhibitory cells which also receive recurrent excitation from CA3 pyramidal cells (Fig. 9). An anatomical substrate for this feedforward excitation has been described (Frotscher, 1985). However, latency differences (about 2 ms) between the onset of feedforward synaptic events in inhibitory cells and in pyramidal cells are large if a single excitatory synapse and similar axon conduction times are involved.

Finally, the activity of the CA3 neuronal population depends on a balance between recurrent excitation and recurrent inhibition (Traub, Miles, Wong, Schulman & Schneiderman, 1987). When recurrent excitation is dominant, excitation can spread between pyramidal cells and cause pathologically synchronous firing. However, in several respects, recurrent inhibition is normally more powerful than recurrent excitation and thus ensures stability. Single inhibitory connections have a higher conductance than excitatory ones and often are strategically located on the soma of

pyramidal cells whereas excitatory synapses terminate on dendrites. Furthermore disynaptic IPSPs have shorter latencies than disynaptic excitation. Fast EPSPs and a low inhibitory cell threshold produce disynaptic IPSPs with latencies as short as 3–4 ms (Fig. 1) whereas disynaptic EPSPs typically have latencies closer to 12 ms (Miles & Wong, 1987). Also, the connectivity for disynaptic inhibition is higher. Inhibitory interactions between pyramidal cells were detected in about 30% of cell pairs, whereas disynaptic excitatory interactions were observed in about 15% of dual recordings, when $GABA_a$-mediated inhibition was blocked (Miles & Wong, 1987). The divergence of inhibitory cell contacts (Somogyi *et al.* 1983; Schwartzkroin & Kunkel, 1985) may be the major factor underlying the high disynaptic inhibitory cell connectivity. However, Fig. 10 suggests that pyramidal cells can also excite more than one inhibitory cell. A more limited divergence of excitatory synapses onto inhibitory cells may allow a fine control of CA3 population activity if these synapses possess plastic properties.

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