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EXCITATORY SYNAPTIC INTERACTIONS BETWEEN CA3 NEURONES IN THE GUINEA-PIG HIPPOCAMPUS

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SUMMARY

1. Excitatory synaptic interactions between CA3 neurones in slices from guinea-pig hippocampus were examined. Recurrent excitatory post-synaptic potentials (e.p.s.p.s) were evoked by action potentials in a single presynaptic neurone or by the antidromic activation of part of the CA3 pyramidal cell population.

2. The peak amplitude of unitary e.p.s.p.s was 1–2 mV at potentials between −64 and −70 mV. Their time to peak was 7–12 ms and the initial phase of their decay was slower than that of a somatically injected voltage pulse. Recurrent e.p.s.p.s were often followed by a small (0·3 mV) hyperpolarization, or undershoot.

3. Recurrent e.p.s.p.s were compared with e.p.s.p.s evoked by stimulating mossy fibres, which terminate proximally on apical dendrites of CA3 pyramidal cells. They were of slower time course and reversed at a more positive potential than mossy fibre e.p.s.p.s. Some synaptic terminals made by recurrent axon collaterals apparently terminate at distant locations on apical dendrites.

4. The decay of both recurrent e.p.s.p.s and dendritic voltage pulses was prolonged by membrane depolarization within a 10–15 mV subthreshold potential range. Voltage-dependent inward currents activated by the synaptic depolarization may contribute to the slow initial decay of these synaptic events.

5. The undershoot did not occur when transmission of a unitary e.p.s.p. failed and was of slower time course than the hyperpolarization due to an inhibitory post-synaptic potential (i.p.s.p.). It was suppressed by intracellular application of K^+ channel blockers and probably reflects an intrinsic outward current activated as a consequence of the synaptic depolarization.

6. Considerable temporal summation of synaptic potentials occurred when recurrent synapses were activated twice at an interval of 5–10 ms, typical of the spontaneous burst firing pattern of CA3 neurones. The mean facilitation of a second e.p.s.p. at this interval was about 0·6.

7. The efficacy of a third and subsequent e.p.s.p.s at similar interval was reduced. Presynaptic bursts of three to five action potentials evoked summed e.p.s.p.s of amplitude 2–4 mV, with time to peak 20–40 ms and decaying phase of similar duration. Their rising phase was relatively smooth and summed events were succeeded by an undershoot. Presynaptic bursts could cause a post-synaptic neurone to discharge.

INTRODUCTION

Excitatory synaptic connexions exist between pyramidal cells, the principal neurones in the mammalian cortex (Stefanis & Jasper, 1964; Takahashi, Kubota & Uno, 1967; Haberly & Bower, 1984). Excitatory interactions are mediated by synaptic terminals made by local axon collaterals (Winfield, Brooke, Sloper & Powell, 1981). The axons of hippocampal pyramidal cells also have extensive local ramifications (Ramón y Cajal, 1911; Lorente de No, 1934; Finch, Nowlin & Babb, 1982) and recordings from pairs of connected cells suggest that these axon collaterals make excitatory synapses (MacVicar & Dudek, 1980; Knowles & Schwartzkroin, 1981). Some synapses made by pyramidal cell collaterals excite inhibitory neurones (Andersen, Eccles & Loyning, 1963; Miles & Wong, 1984). Other excitatory synapses may terminate on neighbouring pyramidal cells, since antidromic stimulation evokes excitatory synaptic events in these neurones (Lebovitz, Dichter & Spencer, 1971).

Such local excitatory circuits could augment the activity of small groups of cells by recruiting additional neurones. The spread of activity between members of a neuronal population via recurrent excitatory synapses may be associated with the generation of synchronous discharges including the extreme forms seen in the epilepsies (Dichter & Spencer, 1969; Traub & Wong, 1982). The association is supported by the demonstration that the activation of a single cell can influence the simultaneous discharge of a large population of hippocampal neurones *in vitro* (Miles & Wong, 1983). This observation suggests that local excitatory connexions must be divergent for one cell to recruit other neurones to the population discharge. Further it appears that recurrent synapses are sufficiently powerful that activity in one cell can lead to the firing of coupled neurones.

This study was undertaken to examine factors which could underlie a strong excitatory coupling between neurones in the CA3 region of the hippocampus. One approach was to look for mono-synaptic excitatory interactions in simultaneous recordings from pairs of cells in guinea-pig hippocampal slices. Since such connexions were rarely found, additional data were obtained by antidromically activating some CA3 pyramidal cells in order to evoke recurrent synaptic actions in other neurones. Our results suggest that although local excitatory synapses terminate at distant dendritic sites the resulting unitary events may activate intrinsic currents which act to increase synaptic efficacy. Stereotyped bursts of presynaptic action potentials further increase the functional strength of recurrent excitatory synapses between CA3 neurones.

METHODS

The methods used in these experiments were similar to those described in a previous report (Miles & Wong, 1984). Transverse hippocampal slices, of thickness 400 μm were prepared with a vibratome from guinea-pigs weighing 200–300 g. In some experiments slices were microdissected so that axon collaterals of CA3 pyramidal cells could be selectively stimulated. Dissection was performed under microscopic observation using small scissors. Slices were then transferred to a recording chamber where they were supported on nylon mesh and exposed to a warmed, moistened 5% CO_2 in O_2 atmosphere. Slices were maintained at 37 °C, at a pH of 7·4, with their lower surface in contact with a perfusing solution of normal composition (in mM): NaCl, 124; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1·6; $NaHCO_3$, 26 and D-glucose, 10. Picrotoxin (5×10^{-6}–10^{-4} M) was added to this solution in some experiments to suppress synaptic inhibition mediated by γ-aminobutyric acid (GABA).

Recording electrodes were pulled from fibre-filled glass capillaries. Electrodes containing 2 M-NaCl, of resistance 3–6 MΩ, were used for recording extracellular field potentials. Intracellular recording electrodes were bevelled to a resistance of 40–90 MΩ and usually filled with 3 M-K acetate. Electrodes containing 2 M-Cs acetate and 0·5 M-tetraethylammonium chloride (TEA) were used to suppress K^+ currents. In other experiments electrodes filled with 0·3 M-Cs fluoride (CsF) allowed stable membrane potentials in the range −50 to +50 mV to be maintained by intracellular current injection (cf. Kostyuk & Krishtal, 1977). Recordings with these electrodes were improved by the addition of 0·01 M-ethyleneglycol-bis-(β-aminoethylether)*N*,*N*′-tetraacetic acid (EGTA), which may prevent CaF_2 precipitation at the electrode tip. We relied on diffusion occurring during long-lasting penetrations to ensure some intracellular exchange of electrode solutions. The pH of the solutions was adjusted to 7·2.

Signals were amplified by high input impedance amplifiers, with facilities for current injection through the recording electrode using an active bridge circuit and for capacitance compensation (WPI M707). Bipolar tungsten electrodes of tip diameter 20 μm and separation 200 μm were used to stimulate fibre pathways with electrical pulses of duration 50–100 μs. During an experiment signals were displayed on a digital oscilloscope (Nicolet 4562) and recorded on FM tape (Vetter). Subsequently some synaptic responses were averaged using the oscilloscope. Permanent records were made with a chart recorder (Gould 2400).

Simultaneous penetrations were made with electrodes controlled by separate manipulators (Zeiss, Jena) and entering the stratum pyramidale of the CA3 region less than 200 μm apart. When two stable intracellular recordings were obtained, steady current was applied to one cell to maintain a spontaneous discharge at a slow frequency. The record of the putative post-synaptic neurone, hyperpolarized to prevent discharge, was examined for synaptic events initiated by action potentials in the first cell. The procedure was then reversed. The possibility that cells were connected electrotonically was examined by injecting a current pulse of duration 100 ms to evoke an intracellular hyperpolarization of at least 20 mV. Potential changes in an electrically coupled cell of about 1 mV, corresponding to a somatic coupling ratio of 0·05, could be resolved on averaging. Electrotonic interactions were occasionally observed but will not be described here. Electrode coupling artifacts were sometimes observed. A signal similar to an attenuated and differentiated version of action potentials recorded with one electrode was apparent in the other recording. These artifacts were suppressed after an experiment by subtracting differentiated, scaled presynaptic records, from post-synaptic traces using the digital oscilloscope. Membrane potentials were measured from the potential change on withdrawal of the electrode from a cell. Neuronal input resistances and time constants were measured from responses to hyperpolarizing current injections of intensity less than 0·5 nA and duration 80–120 ms.

RESULTS

Antidromic activation of excitatory synapses between CA3 neurones

Pyramidal cells in the CA3 field possess axon collaterals which project to the stratum oriens of the CA1 region (Lorente de No, 1934; Swanson, Wyss & Cowan, 1978). Slices were prepared (Fig. 1*A*) so that these axon collaterals could be stimulated to directly activate pyramidal cells in the CA2–3a region. In addition a cut was made orthogonal to the alveus and extending beyond the layer of mossy fibres in the CA3a field. Neurones located beyond the cut in CA3b,c could not be activated antidromically or by afferent fibres projecting in stratum oriens. Instead these neurones might receive recurrent synaptic inputs from CA2–3a cells if, as anatomical evidence suggests, local axon collaterals ramify widely in distal apical dendritic fields (Ramon y Cajal, 1911; Finch *et al.* 1983).

An extracellular field potential consisting of one or more spikes was elicited in the CA2–3a region, with latency 1–4 ms, by CA1 stratum oriens stimulation. Field potential spikes were correlated with action potentials in simultaneous intracellular recordings (Fig. 1*B*). These action potentials appeared to be generated antidromically

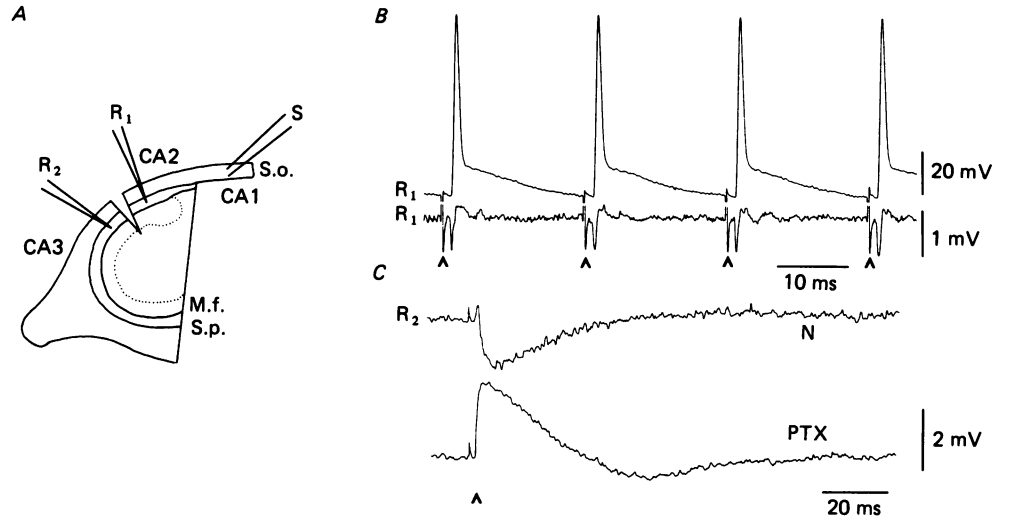

Fig. 1. E.p.s.p.s elicited by antidromic activation of part of the CA2–3 pyramidal cell population. *A*, location of recording and stimulating electrodes. Stimulation, S, in CA1 stratum oriens, s.o. The remainder of the CA1 area and the dentate gyrus were removed in slice preparation. Recordings were made at sites R_1 in the stratum pyramidale, s.p., of CA2–3a, and R_2 in s.p. of CA3b,c. A cut orthogonal to s.p. and extending to the distal edge of the mossy fibre, m.f., layer, was made in CA3a during slice preparation. *B*, intracellular (upper) and extracellular (lower trace) recordings from site R_1. CA1 stimulation at 50 Hz elicited action potentials in this neurone and no e.p.s.p. was apparent on hyperpolarization by about 20 mV. The field response shows a simultaneous antidromic spike. *C*, synaptic potentials in a CA3b cell at site R_2, evoked by CA1 s.o. stimulation before, N, and 10 min after adding 10^{-5} M-picrotoxin (PTX) to the perfusing solution. Notations for recording sites and arrowheads showing stimulus timing are also used in subsequent Figures.

since they followed stimulation at frequencies up to 100 Hz and since membrane hyperpolarization revealed that they arose from subthreshold potentials.

Stimulation of CA1 stratum oriens did not cause antidromic firing of CA3b,c neurones located beyond the cut. Instead synaptic events consisting of an excitatory post-synaptic potential (e.p.s.p.) followed by an inhibitory post-synaptic potential (i.p.s.p.) were evoked (Fig. 1*C*). E.p.s.p.s were examined in the presence of picrotoxin (5×10^{-6}–10^{-4} M), an antagonist of synaptic inhibition mediated by GABA. In these experiments extracellular divalent cation concentrations were elevated (from Mg^{2+}, 1·6 mM: Ca^{2+}, 2 mM to Mg^{2+}, 6 mM: Ca^{2+}, 4 mM) to suppress synchronous firing. Fig. 1*C* shows that picrotoxin suppressed the inhibitory component of the synaptic response and revealed a prolonged e.p.s.p.

We examined the relation between the antidromic field potential in CA2 and e.p.s.p.s recorded simultaneously from CA3b neurones as the intensity of CA1 stratum oriens stimulation was varied. Fig. 2 shows traces from one experiment, with the peak amplitude of the antidromic spike plotted against that of the e.p.s.p. in Fig. 2*F*. At low stimulus intensities an antidromic spike was generated in CA2 but no synaptic event was observed intracellularly (Fig. 2*A*). The intensity needed to evoke an e.p.s.p. was always greater than that at which the antidromic field potential first

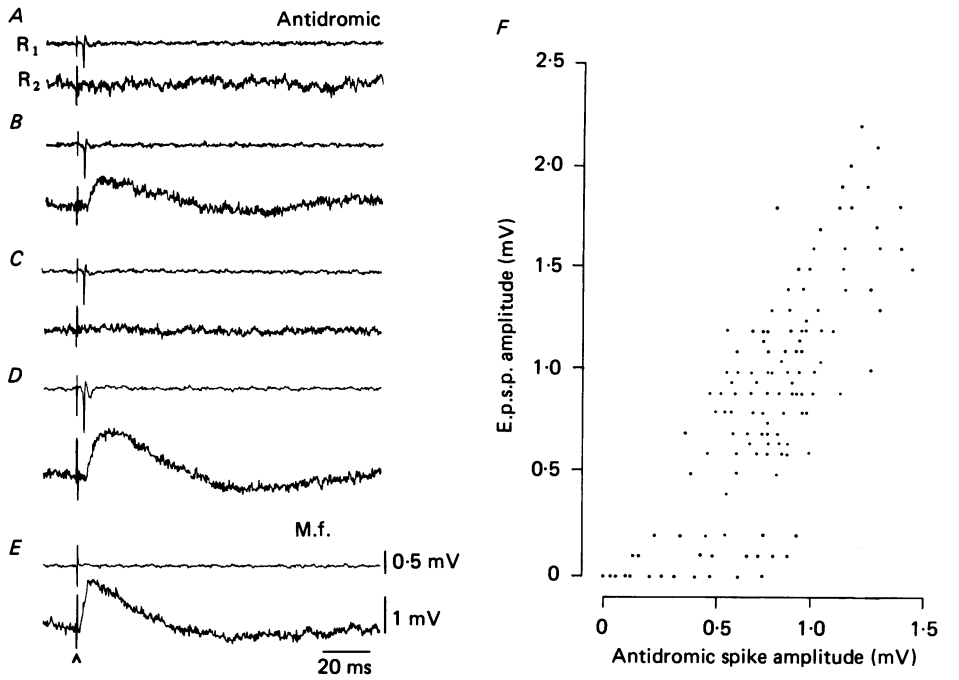

Fig. 2. Correlation of antidromic field potential in CA2, R_1, with an e.p.s.p. in a CA3b neurone, R_2. Slice prepared as in Fig. 1. *A–D*, responses obtained as the intensity of CA1 s.o. stimulation was increased. *F*, e.p.s.p. amplitudes plotted against those of field responses. E.p.s.p.s were evoked with longer latency and at higher threshold than extracellular antidromic spikes (*A*,*B*). At low stimulus intensities e.p.s.p.s and apparent failures of transmission were observed in successive trials (*B*, *C*). Both field potential and e.p.s.p. amplitude increased at higher stimulus intensities (*D*). *E* shows an e.p.s.p. but no field response elicited by mossy fibre stimulation suggesting that the cut separating CA3a and CA3b was effective. Recordings made in the presence of 5×10^{-6} M-picrotoxin, 6 mM-Mg^{2+} and 4 mM-Ca^{2+}.

appeared. When an e.p.s.p. was elicited (Fig. 2*B*) its latency was always longer than that of the antidromic spike. E.p.s.p.s could appear in an all-or-none fashion, within a range of stimulus intensities which invariably evoked an antidromic spike (Fig. 2*B*,*C*,*F*). Such e.p.s.p.s could be unitary synaptic events and will be referred to as minimal e.p.s.p.s. As stimulus intensity was increased further the amplitudes of both field responses and e.p.s.p.s increased (Fig. 2*D*,*F*). These data suggest that e.p.s.p.s recorded in neurones beyond the cut arose as a consequence of activity in the CA2–3a cell population.

The latency of minimal e.p.s.p.s from the peak of the antidromic spike in CA2, was $1{\cdot}1 \pm 0{\cdot}4$ ms (mean $\pm$ S.D., $n = 19$ cells), consistent with one synaptic delay and some time for conduction along an axon collateral. The mean time to peak of averaged minimal events was $9{\cdot}5 \pm 1{\cdot}9$ ms ($n = 19$). In the presence of 6 mM-Mg^{2+} and 4 mM-Ca^{2+}, the mean amplitude of averaged e.p.s.p.s was $0{\cdot}9 \pm 0{\cdot}4$ mV ($n = 19$), at membrane potentials between -67 and -72 mV.

Most minimal e.p.s.p.s were followed by a small hyperpolarization of amplitude

0·2–0·4 mV which reached a peak at 50–80 ms after their onset (sixteen out of nineteen cells). The hyperpolarization persisted in 10^{-4} M-picrotoxin. E.p.s.p.s followed by a hyperpolarization could also be evoked in the presence of 10 mM-Mg^{2+} ($n = 4$ cells) which blocks di-synaptic events such as the fast i.p.s.p. elicited by mossy fibre stimulation (unpublished observation) and a slower K^+ dependent i.p.s.p. (Alger, 1984). The hyperpolarization will be termed an ' undershoot ', after the name given to a similar event sometimes observed following e.p.s.p.s evoked in spinal motoneurones by Ia afferent stimulation (Jack, Miller, Porter & Redman, 1971).

Excitatory synaptic interactions in simultaneous recordings

Seven excitatory interactions, seen in about 400 paired recordings, were thought to be mediated mono-synaptically. One presynaptic cell also elicited di-synaptic i.p.s.p.s. Fig. 3 shows unitary e.p.s.p.s, initiated by single presynaptic action potentials, characteristic of the excitatory coupling seen in the remaining six pairs of neurones. The wave form of unitary events was slow and variable (Fig. 3*A*). Their peak could be prolonged and e.p.s.p.s were sometimes followed by a hyperpolarization of amplitude and time course similar to that of the undershoot following antidromically evoked synaptic events.

These e.p.s.p.s were considered to be mono-synaptic on the basis of their latency and the proportion of transmission failures. Mean latency, measured from the onset of the presynaptic action potential, ranged between 0·8 and 1·2 ms ($n = 6$). The latency distribution in one case is shown in Fig. 3*B*. The latency includes a time for conduction along the presynaptic axon collateral. Recording sites were less than 200 μm apart in the CA3 stratum pyramidale but the length of collaterals and their conduction velocity are not known. Failures of transmission (third trace of Fig. 3*A*) were observed at all synapses. The greatest proportion of failures observed was 23 %. In comparison di-synaptic events mediated via axon collaterals of CA3 pyramidal cells showed at least 68 % failures (Miles & Wong, 1984). The shortest latency of a di-synaptic i.p.s.p. recorded in our previous study was 2·4 ms.

At membrane potentials between -64 and -70 mV the mean amplitude of averaged unitary e.p.s.p.s was $1{\cdot}4 \pm 0{\cdot}3$ mV ($n = 6$). The distribution of amplitudes in one case is shown in Fig. 3*C*. All distributions had a single peak. The occurrence of failures allowed statistical parameters of transmitter release to be estimated in two ways. Using the variance technique (Hubbard, Llinás & Quastel, 1969), a value of 1·8 was derived for m, the mean number of quanta of transmitter released at the synapse of Fig. 3. The value estimated for q, the mean quantal amplitude was 0·6 mV. Poisson statistics were assumed and e.p.s.p. amplitudes were corrected for non-linear summation (Martin, 1955). With an analysis based on the number of failures (del Castillo & Katz, 1954) values of 2·6 for m and 0·4 mV for q were obtained for this synapse. The assumption of Poisson statistics is justified since both methods gave values for m that were less than 3 (McLachlan, 1978). We will show that the amplitude of recurrent e.p.s.p.s recorded in the soma probably differed from that at the post-synaptic site. Thus, values of m obtained by counting failures may be more accurate than those derived by measuring the variance of amplitude distributions.

If recurrent excitatory synapses terminate on the soma and elicit post-synaptic currents of short duration, the resulting e.p.s.p.s should decay with time constant

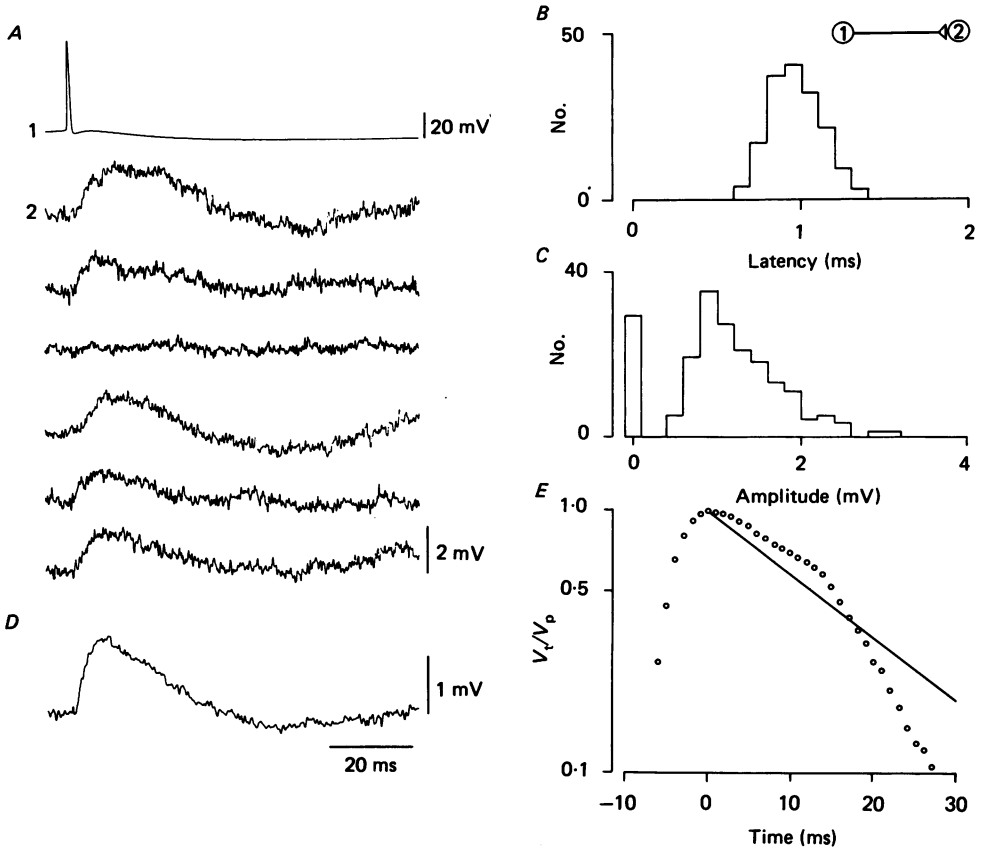

Fig. 3. Monosynaptic e.p.s.p. *A*, six responses in cell 2 to single spontaneous action potentials in cell 1. The third trace shows a transmission failure. Some e.p.s.p.s had a prolonged peak and some were followed by a hyperpolarization. *B*, e.p.s.p. latency distribution. The mean latency from the start of the upstroke of the presynaptic action potential, was $1{\cdot}0 \pm 0{\cdot}2$ ms ($n = 163$). *C*, distribution of peak e.p.s.p. amplitudes at potentials of -66 to -70 mV in cell 2. The mean amplitude of 163 e.p.s.p.s elicited by 192 action potentials was $1{\cdot}3 \pm 0{\cdot}6$ mV. Twenty-nine responses could not be resolved above the recording system noise (0·4 mV) and are plotted at 0 mV. *D*, average of twenty e.p.s.p.s. Note the undershoot. *E*, semilogarithmic plot of the averaged e.p.s.p. Time $t = 0$ corresponds to the peak of the synaptic event and potential at time t, V_t, is normalized with respect to the peak potential V_p. The straight line is the final time course of potential decay in response to a step hyperpolarizing current injection into cell 2 (not shown). Initially the e.p.s.p. decayed more slowly than the somatic time constant, but the rate of e.p.s.p. decay increased at about 15 ms from its peak. Recordings made in the presence of 10^{-5} M-picrotoxin.

similar to that of the post-synaptic membrane (Rall, 1967; Jack, Noble & Tsien, 1975). None of the unitary events we recorded decayed in this way. Fig. 3*E* is a semilogarithmic plot of the time course of an averaged e.p.s.p. For about 15 ms following its peak the e.p.s.p. decayed more slowly than the final decay of membrane potential in response to a step hyperpolarizing current. Two factors which might underlie this slow decay, a distant synaptic location and a contribution from intrin-

sic membrane currents, are considered below. The later phase of decay was faster than passive. An undershoot of amplitude up to 0·4 mV and time to peak 45–70 ms was resolved following the e.p.s.p. in five out of six averaged unitary events (Figs. 3*D* and 5*A–D*).

The undershoot could be distinguished from an i.p.s.p. elicited via an inhibitory cell. Fig. 4 shows recordings from a neurone which could initiate e.p.s.p.s and di-synaptic i.p.s.p.s in a coupled cell. I.p.s.p.s could occur in isolation when the e.p.s.p.

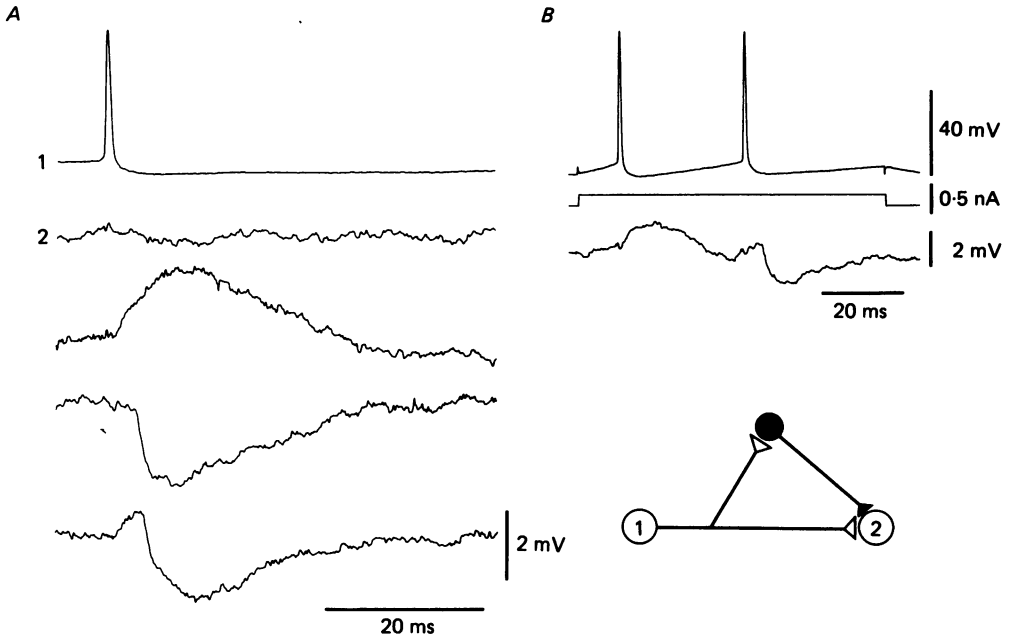

Fig. 4. E.p.s.p.s and di-synaptic i.p.s.p.s initiated by the same neurone. *A*, four responses in cell 2 evoked by single action potentials in cell 1. Traces are from above: an apparent failure, an e.p.s.p., a longer latency i.p.s.p. and an e.p.s.p.–i.p.s.p. sequence. The probability of occurrence of these events was: 0·12, 0·54, 0·10, 0·21 respectively ($n = 160$ presynaptic spikes). The mean e.p.s.p. latency ws $1·5 \pm 0·4$ ms ($n = 87$) and the mean i.p.s.p. latency was $4·9 \pm 2·6$ ms ($n = 16$). *B*, a step depolarization of cell 1 elicited two action potentials with prominent after-hyperpolarizations, unlike the usual pyramidal cell discharge pattern. The first action potential evoked an e.p.s.p., the second elicited an e.p.s.p. followed by an i.p.s.p. The inset shows a possible circuit arrangement.

failed (third trace of Fig. 4*A*), whereas an undershoot never occurred in isolation. The wave form of a composite synaptic event consisting of an e.p.s.p.–i.p.s.p. sequence (lowest trace of Fig. 4*A*) also differed in appearance to that of an e.p.s.p. succeeded by an undershoot. The termination of the e.p.s.p. by the i.p.s.p. was more abrupt, possibly due to a higher conductance change of the i.p.s.p.

Electrotonic location of recurrent excitatory synapses

The averaged wave forms of four mono-synaptic e.p.s.p.s are shown in Fig. 5*A–D*. Apart from the absence of an undershoot in one case (Fig. 5*B*) the events were of

similar time course with an initial phase of decay which was slower than the passive time constant of the post-synaptic membrane. A slow decay could result if recurrent synaptic terminals were located at electrotonically remote sites.

The electrotonic distance between a synapse and the soma may be derived from the time to peak and half-width of the resulting synaptic potential when the passive cable properties of the post-synaptic cell and the duration of the synaptic current

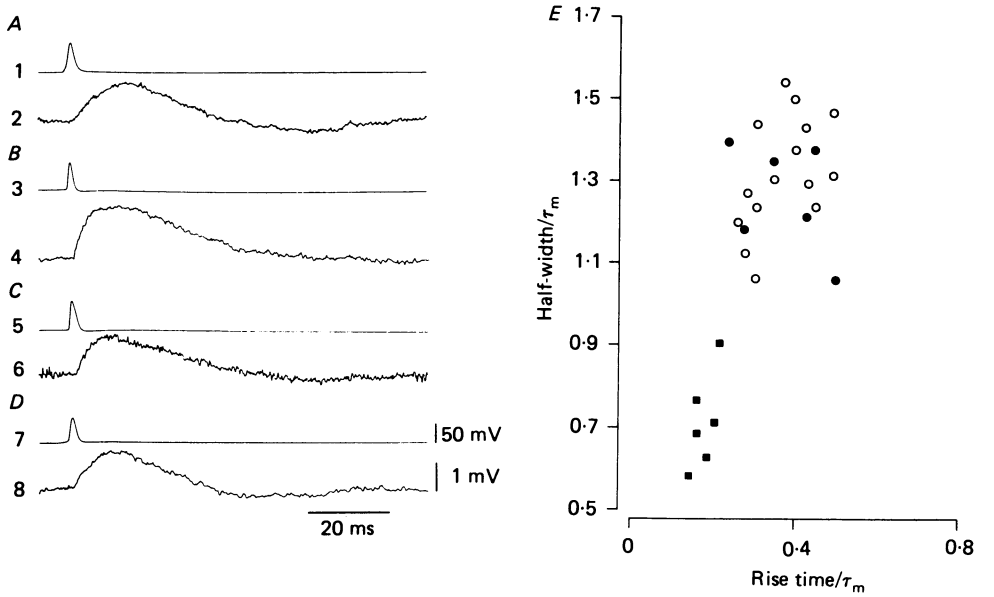

Fig. 5. Shape indices of synaptic events. *A–D*, averaged unitary e.p.s.p.s (lower traces) elicited by single action potentials (upper traces) at four mono-synaptic connexions. At least twenty events were averaged at post-synaptic membrane potentials between −64 and −70 mV. All e.p.s.p.s except that shown in *B* were followed by an undershoot. *E*, time to peak of synaptic potentials plotted against half-width (normalized by the time constant of the post-synaptic neurone, τ_m). Filled circles correspond to unitary e.p.s.p.s from paired recordings. Open circles represent minimal excitatory synaptic events elicited by antidromic stimulation. Filled squares correspond to identified unitary i.p.s.p.s from a previous study (Miles & Wong, 1984).

are known (Rall, 1967; Jack *et al.* 1975). The time to peak of unitary e.p.s.p.s was 8·4±2·1 ms (mean ± S.D., $n = 6$) and their mean duration at half amplitude was 27·2±6·2 ms. These shape indices, normalized with respect to the time constant of the post-synaptic membrane, are plotted for each event in Fig. 5*E*. Values for six unitary i.p.s.p.s recorded from CA3 pyramidal cells in a previous study (Miles & Wong, 1984) are also plotted. I.p.s.p.s occupied a different region of the plot with shorter values of time to peak and half-width. The post-synaptic cells were from the same neuronal population so their electronic properties should be comparable. The faster time course of unitary i.p.s.p.s appears not to result from a shorter duration of the underlying currents since the evidence available suggests that inhibitory synaptic currents in hippocampal pyramidal cells (Collingridge, Gage & Robertson,

1984) decay more slowly than excitatory synaptic currents (Brown & Johnston, 1983). The difference seems most likely to reflect a more distant location of recurrent excitatory synapses and is consistent with evidence that many inhibitory synapses terminate somatically on pyramidal cells (Andersen *et al.* 1963; Ribak, Vaughn & Saito, 1978).

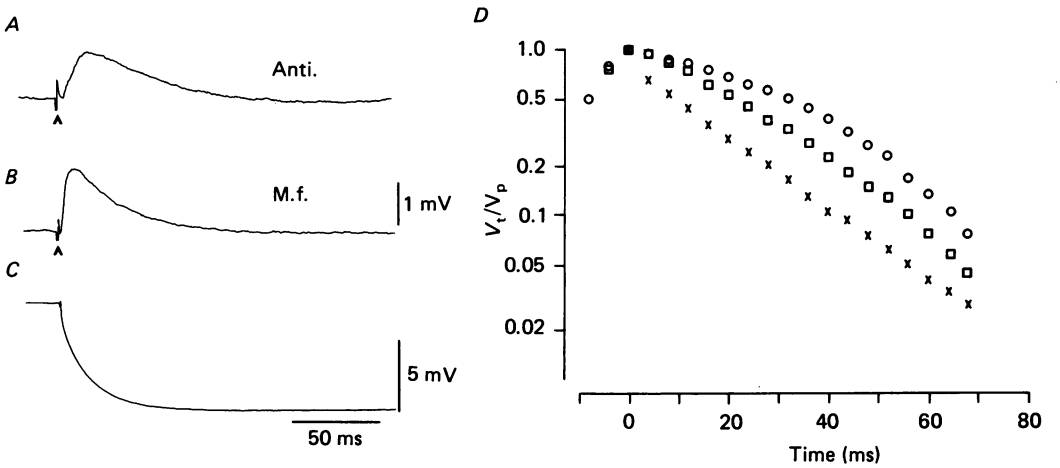

Fig. 6. Comparison of the time course of recurrent (anti.) and mossy fibre (m.f.) e.p.s.p.s. *A*, minimal recurrent e.p.s.p. elicited by stimulation of CA1 s.o. in a slice prepared as in Fig. 1. *B*, e.p.s.p. evoked in the same CA3b neurone by mossy fibre stimulation. *C*, onset of response to somatic hyperpolarizing current step injected through the recording electrode. All traces are averaged from fifteen responses at membrane potentials between −78 and −81 mV. Extracellular solution contained 5×10^{-6} M-picrotoxin, 6 mM-Mg^{2+} and 4 mM-Ca^{2+}. *D*, semilogarithmic plots of responses shown in *A–C*. Time $t = 0$ corresponds to the peak of recurrent e.p.s.p. (open circles), the peak of the mossy fibre e.p.s.p. (open squares) and the onset of the hyperpolarization due to current injection (crosses). In each case the potential at time t, V_t, is normalized with respect to potential at time $t = 0$, V_p.

The location of these synapses was further explored by comparing recurrent e.p.s.p.s with those elicited by mossy fibre stimulation. Mossy fibres terminate proximally on the apical dendrites of CA3 pyramidal cells (Ramón y Cajal, 1911; Brown & Johnston, 1983). Since minimal e.p.s.p.s elicited by antidromic stimulation occupied a similar region of the shape index plot to unitary e.p.s.p.s from paired recordings (Fig. 5*E*), it seemed appropriate to compare these events with mossy fibre e.p.s.p.s.

The time to peak of a recurrent e.p.s.p. was always longer than that of a mossy fibre e.p.s.p. of comparable amplitude recorded in the same neurone (Fig. 6). The mean time to peak of recurrent e.p.s.p.s was $10{\cdot}9 \pm 2{\cdot}1$ ms (mean ± S.D., $n = 9$ cells) while the value for mossy fibre e.p.s.p.s was $5{\cdot}7 \pm 1{\cdot}2$ ms ($n = 9$). Recurrent e.p.s.p.s initially decayed more slowly than did mossy fibre e.p.s.p.s as shown in the semilogarithmic plot of Fig. 6*D*. The initial decay of both e.p.s.p.s was convex upwards (Fig. 6*D*), whereas the initial decay of the membrane response to current injection (Fig. 6*C*) was often convex downwards. The later phase of the mossy fibre e.p.s.p. was of similar slope to the final membrane decay and the decay of the recurrent e.p.s.p. became

increasingly rapid, presumably due to the onset of the undershoot. These observations are consistent with a more distal location on apical dendrites for recurrent synapses, activated in this way, than for mossy fibre synaptic terminals.

The reversal potentials of recurrent and mossy fibre e.p.s.p.s were also compared. This parameter might provide further information on the location of recurrent synapses since the somatic potential at which an e.p.s.p. reverses is predicted to increase with the electrotonic distance to the synapse (Calvin, 1969; Jack *et al.* 1975). In order to measure synaptic events over a wide range of post-synaptic potentials,

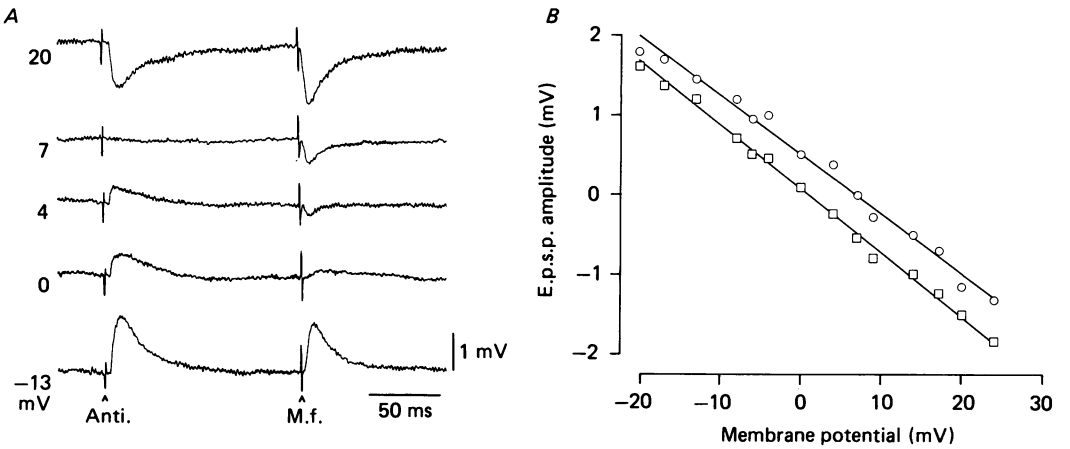

Fig. 7. Recurrent (anti.) e.p.s.p. reverses at a more positive membrane potential than the mossy fibre (m.f.) e.p.s.p. *A*, averages of five responses at different membrane potentials. Somatic membrane potential was changed by maintained current injection with the amplifier bridge adjusted to balance responses to hyperpolarizing current pulses. The recording electrode contained 0·3 M-CsF and 0·01 M-EGTA and e.p.s.p.s were recorded at times from 25 to 50 min after penetration. Stimulus intensities were adjusted to evoke synaptic events of a similar size at membrane potentials in the range shown. The extracellular solution contained 5×10^{-6} M-picrotoxin, 6 mM-Mg^{2+} and 4 mM-Ca^{2+}. *B*, mean e.p.s.p. amplitude, measured from five or more responses, plotted against membrane potential. Open circles correspond to the recurrent e.p.s.p. and open squares to the mossy fibre e.p.s.p. Linear regression analysis gave a reversal potential of +7 mV for the recurrent e.p.s.p. ($r = 0{\cdot}99$). The mossy fibre e.p.s.p. reversed at +1 mV ($r = 0{\cdot}99$).

an attempt was made to suppress voltage-dependent conductances using recording electrodes filled with 0·3 M-CsF and 0·01 M-EGTA. Steady potentials up to levels as depolarized as +50 mV could be maintained by direct current injection applied 10–15 min after penetration. In contrast prolonged action potentials often occur, in the range −60 to −10 mV, when electrodes filled with 2 M-Cs acetate are used. The difference may result from suppression of a Ca^{2+} current by intracellular fluoride (Kostyuk & Krishtal, 1977; Kay, Miles & Wong, 1985).

Fig. 7 *A* shows an antidromically evoked recurrent e.p.s.p. and a mossy fibre e.p.s.p. recorded in the same neurone at several membrane potentials close to their reversal. Recurrent e.p.s.p.s recorded with CsF-filled electrodes were not followed by an undershoot and both synaptic events reversed uniformly. In six cells tested, the recurrent e.p.s.p. reversed at a more positive somatic potential than the mossy fibre

e.p.s.p. (Fig. 7*B*). Straight lines fitted to averaged responses evoked at various membrane potentials yielded mean reversal potentials of $+7 \pm 3$ mV for the recurrent e.p.s.p. and -1 ± 2 mV for the mossy fibre e.p.s.p.

Activation of voltage-dependent post-synaptic conductances

Apical dendrites of hippocampal pyramidal cells possess voltage-dependent conductances (Wong, Prince & Basbaum, 1979; Masakawa & Prince, 1984). Since some

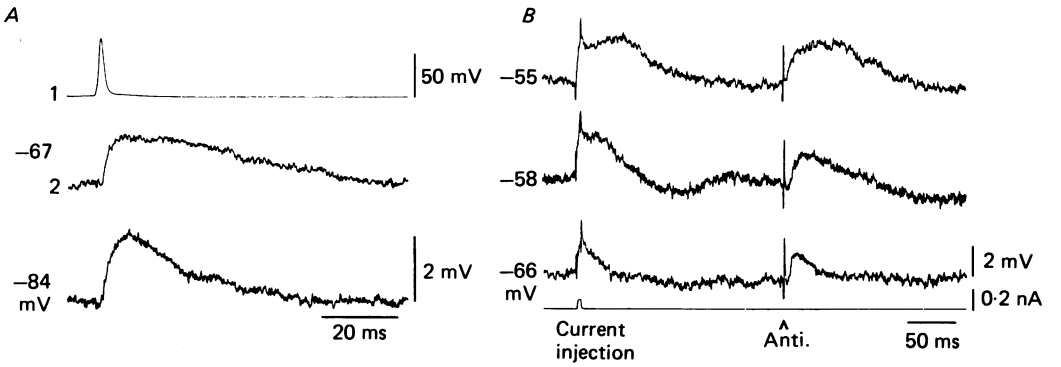

Fig. 8. Voltage dependence of recurrent e.p.s.p.s. Recordings in *A* are from monosynaptically connected cells 1 and 2. Averages of ten e.p.s.p.s, at post-synaptic membrane potentials of −67 mV (upper trace) and −84 mV (lower trace). The duration at half amplitude increased from 20 to 37 ms, and peak amplitude fell from 2·1 to 1·7 mV with this depolarization. E.p.s.p.s in *B* were elicited by antidromic stimulation. Their potential dependence was compared with that of the response to a small, short depolarizing current injection. Recording from a neuronal element located in the distal third of the mossy fibre layer, which was presumed to be a pyramidal cell dendrite. Its threshold for action potential generation was −52 mV. At hyperpolarized potentials both the e.p.s.p. and the voltage pulse appeared to decay exponentially. The wave form of both events varied at more depolarized potentials. Responses were of increased amplitude, often prolonged and could be followed by an undershoot. The extracellular solution contained 5×10^{-6} M-picrotoxin, 6 mM-Mg^{2+} and 4 mM-Ca^{2+}.

recurrent synapses apparently terminate on apical dendrites we asked whether active dendritic responses might contribute to the slow decay of these synaptic events. Fig. 8*A* shows that the time course of a unitary recurrent e.p.s.p. was affected by membrane potential. Synaptic events were averaged to reduce the effect of fluctuations in responses to single presynaptic action potentials. Post-synaptic depolarization significantly increased the e.p.s.p. half-width and reduced the peak amplitude. Unitary events followed by an undershoot were also prolonged on depolarization.

Two factors could underlie a voltage dependence of e.p.s.p. duration. The kinetics of excitatory transmitter channels might depend on potential (Nowak, Bregestovski, Ascher, Herbet & Prochiantz, 1984). Alternatively, intrinsic membrane currents might be activated at subthreshold potentials (Masakawa & Prince, 1984; Stafstrom, Schwindt, Chubb & Crill, 1985). To discriminate between these possibilities, the voltage dependence of antidromically evoked e.p.s.p.s was compared with that of voltage pulses evoked by current injection. Attempts were made to record from

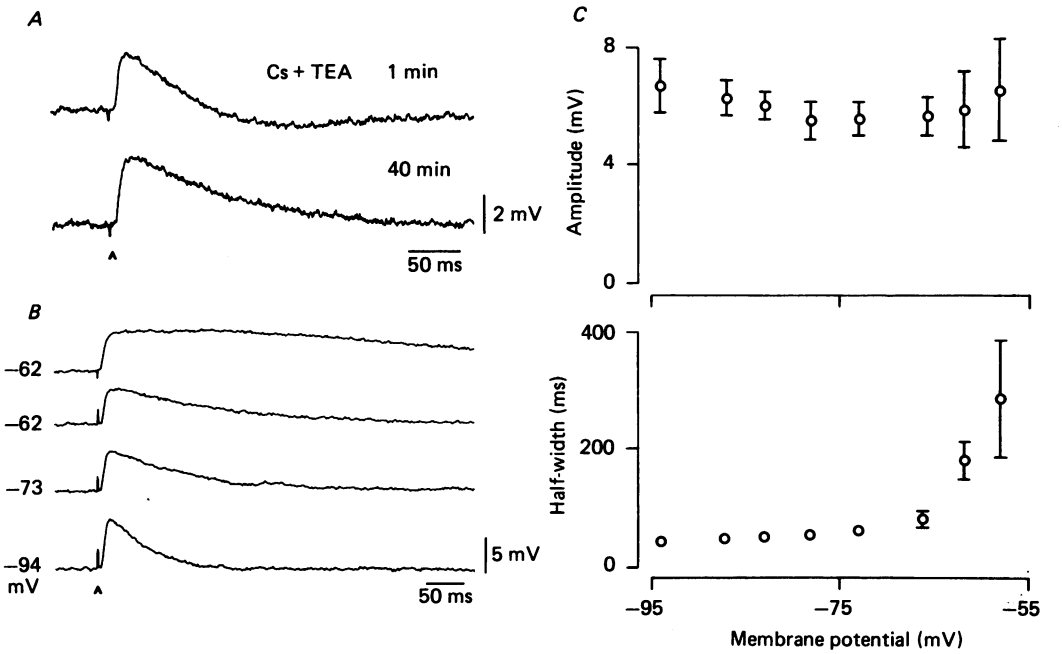

Fig. 9. Intrinsic currents shape recurrent e.p.s.p. wave form. *A*, effects of intracellular Cs^+ and TEA^+ on the synaptic event. An undershoot followed the recurrent e.p.s.p. recorded 1 min after penetrating a CA3 neurone with an electrode containing 2 M-CsAc and 0·5 M-TEA Cl (upper trace). The lower trace was recorded 40 min later at similar membrane potential and stimulus intensity and no undershoot was apparent. *B*, shows the voltage dependence of the recurrent e.p.s.p. in another neurone with the undershoot suppressed by Cs^+ and TEA^+ injection. At a threshold potential of −53 mV prolonged action potentials were generated. E.p.s.p. duration increased as somatic potential was raised from −94 to −62 mV (lower three traces). In a potential range of about 12 mV below threshold, synaptic events could be greatly prolonged in an all-or-none fashion (upper two traces). *C*, e.p.s.p. amplitude and duration plotted against membrane potential. Each point corresponds to the mean measured from at least eighteen responses obtained within a ±2 mV range of the potential shown. Bars indicate standard deviation where appropriate. All records obtained in the presence of 5×10^{-6} M-picrotoxin, 6 mM-Mg^{2+} and 4 mM-Ca^{2+}.

pyramidal cell dendrites in the stratum lucidum and radiatum of CA3 since our results suggest that recurrent synapses may terminate on apical dendrites. The properties of the elements impaled ($n = 8$) resembled those observed in previous intradendritic recordings (Wong *et al.* 1979). Current injection was adjusted to elicit a voltage pulse of amplitude (2–4 mV) and duration (5–10 ms) resembling the rising phase of the e.p.s.p.

The wave forms of dendritically recorded recurrent e.p.s.p.s and short, small voltage pulses were influenced in a similar way by membrane potential. The time course of decay of intradendritic voltage pulses fluctuated considerably at resting potential suggesting that an active process was involved. In the recording shown in Fig. 8*B* both the voltage pulse and the recurrent e.p.s.p. appeared to decay passively at potentials hyperpolarized beyond −65 mV. Both responses were prolonged and were often followed by an undershoot at more depolarized potentials. Responses consisting

of a plateau of duration 20–50 ms followed voltage pulses at immediately subthreshold potentials (note that threshold was elevated in these experiments, presumably due to the increase in divalent cation concentrations). E.p.s.p.s were also larger and more prolonged at subthreshold potentials. The similarity of these responses suggests that a recurrent e.p.s.p. generates a dendritic depolarization which may be large enough to activate a voltage-dependent inward current. The observation that a small short depolarization could initiate a depolarizing–hyperpolarizing wave form (Fig. 8*B*) suggests that an intrinsic outward current is responsible for the undershoot.

These results imply that the shape of these synaptic events partly depends on a balance between inward and outward currents which varies with time and membrane potential. The contribution of the inward current to the recurrent e.p.s.p. wave form could be examined in isolation if the outward current could be suppressed. Accordingly, recordings were made using electrodes containing TEA Cl (0·5 M) and CsAc (2 M). These agents act intracellularly to reduce most voltage-dependent K^+ conductances (Armstrong & Binstock, 1965; Tillotson, 1979). After recording for 10–25 min with such electrodes, neuronal input resistance was increased and action potential duration increased to 70–120 ms. In addition the undershoot following recurrent e.p.s.p.s was completely suppressed (Fig. 9*A*). In the absence of an undershoot the effect of membrane potential on e.p.s.p. duration was accentuated. Depolarization in the range −95 to −65 mV caused a small increase in e.p.s.p. half-width, and a small reduction in peak amplitude (Fig. 9*B* and *C*). As potential was shifted into a range of 10–15 mV below threshold e.p.s.p.s could be dramatically prolonged in an all-or-none fashion (upper two traces of Fig. 9*B*). The amplitude of synaptic events could be increased in this potential range but their rate of rise appeared to be unchanged.

Synaptic response to a presynaptic burst of action potentials

CA3 pyramidal cells often discharge in bursts of three to six action potentials of separation 5–15 ms. Considerable temporal summation occurs when recurrent synapses are activated at these intervals (Fig. 10). In addition both unitary e.p.s.p.s (Fig. 10*A*) and minimally evoked e.p.s.p.s (Fig. 10*C*) were facilitated when activated twice at intervals less than about 200 ms. Synaptic events evoked by spontaneously occurring pairs of presynaptic spikes of interval 5–10 ms were selected for analysis from the recordings shown in Fig. 10*A*. The amplitude distributions of the first and second e.p.s.p.s evoked by two action potentials are shown in Fig. 10*B*. The number of transmission failures of the second event was similar to that for the first. The mean amplitude of the first e.p.s.p. was $1{\cdot}3 \pm 0{\cdot}4$ mV (mean ± S.D., $n = 86$) and it was $1{\cdot}8 \pm 0{\cdot}5$ mV) ($n = 86$) for the second e.p.s.p. In this case facilitation, calculated as the increase in amplitude of the second over the first synaptic event, divided by the amplitude of the first e.p.s.p., was 0·38.

The facilitation of minimally evoked e.p.s.p.s could be examined over a wider range of intervals. Field responses in CA2 were also monitored to ensure that the second stimulus did not evoke increased antidromic firing (Fig. 10*C*). The mean facilitation observed in six experiments was about 0·2 at an interval of 100 ms. At shorter intervals facilitation increased, reaching a plateau value close to 0·6 at intervals between 7 and 15 ms (Fig. 10*D*). This interval is comparable to that between action potentials in spontaneous bursts discharged by pyramidal cells.

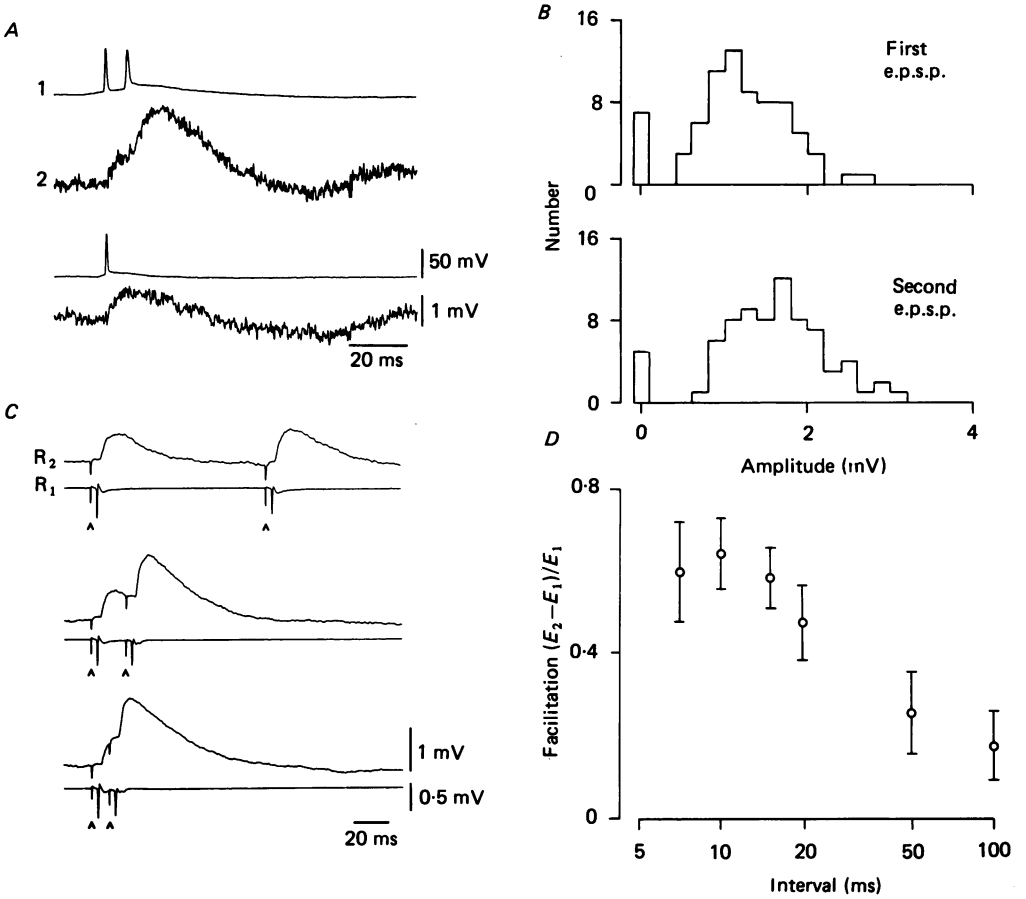

Fig. 10. Facilitation of recurrent e.p.s.p.s. *A*, mono-synaptically coupled cells 1 and 2. Synaptic events elicited by one action potential (lower traces) and two action potentials at short interval (upper trace). *B*, amplitude distributions for the first and second e.p.s.p.s evoked in cell 2 by two spikes occurring spontaneously in cell 1 at interval 5–10 ms. The amplitude of the second e.p.s.p. was estimated by subtracting an extrapolated wave form for the first e.p.s.p. based on comparison with single synaptic events. The mean amplitude of the first e.p.s.p. was $1{\cdot}3 \pm 0{\cdot}4$ mV (mean $\pm$S.D., $n = 103$) and seven transmission failures occurred. The second e.p.s.p. failed five times and its mean amplitude was $1{\cdot}8 \pm 0{\cdot}5$ mV. Post-synaptic membrane potential was -68 ± 3 mV. *C*, averages ($n = 30$) of minimal e.p.s.p.s evoked by two antidromic stimuli at various intervals. Field responses in CA2, R_1, are shown below synaptic events in a CA3 neurone, R_2. The cell was held hyperpolarized close to -80 mV to suppress the undershoot. *D*, mean facilitation, from six cells, plotted against interval. The bars indicate standard deviation. At short intervals temporal summation obscured the time course of second e.p.s.p.s. In this case amplitude was measured from a wave form obtained by digitally subtracting the response evoked by one stimulus from the response to two stimuli. Facilitation was calculated as $(E_2 - E_1)/E_1$ where E_1 was the amplitude of the first e.p.s.p. and E_2 was the amplitude of the second e.p.s.p.

Synaptic responses to short bursts of presynaptic action potentials were also examined. For the pair of cells shown in Fig. 11*A* the time to peak of the unitary e.p.s.p. was 10–12 ms, and the interval between action potentials during a presynaptic burst was 5–8 ms. With this timing it was difficult to discern the contribution

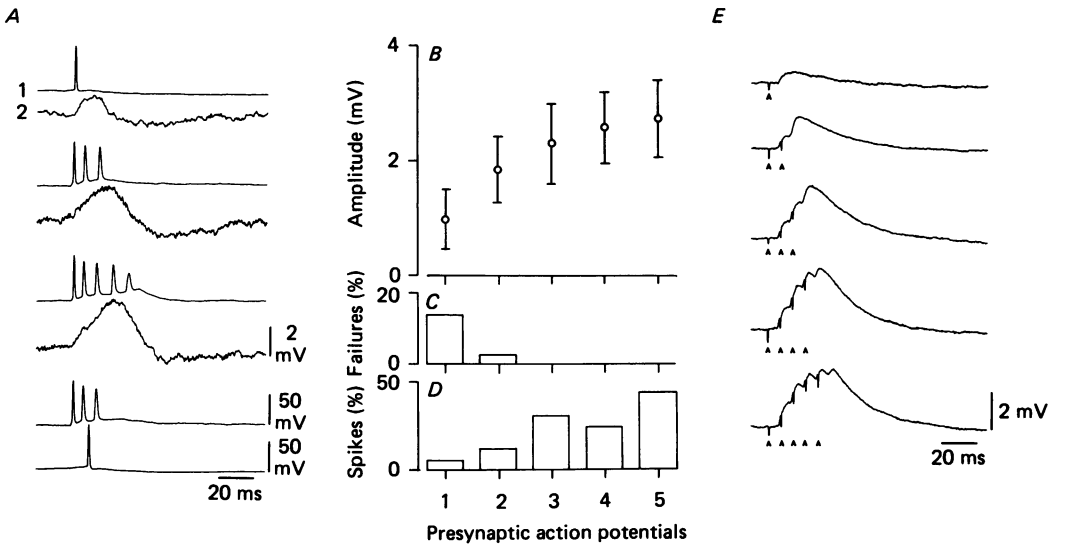

Fig. 11. Post-synaptic responses to a presynaptic burst. *A*, synaptic events evoked in cell 2 by varying numbers of action potentials in cell 1. In the upper traces an e.p.s.p. of rise time about 10 ms was evoked by a single action potential. Second and third traces show summed e.p.s.p.s evoked by three and five presynaptic action potentials. Lower traces show a single post-synaptic action potential evoked with latency 12 ms by three action potentials. *B*, mean and standard deviation of the amplitude of synaptic events plotted against the number of presynaptic action potentials. The amplitude of summed events did not increase linearly with the number of action potentials. In *C* the proportion of occasions when no post-synaptic response was evoked is plotted against the number of action potentials. *D*, shows proportion of times when presynaptic activity led to post-synaptic firing with latency less than 20 ms. The number of events analysed for *B–D* was: one spike, $n = 104$; two spikes, 68; three spikes, 74; four spikes, 54; five spikes, 98. *E*, averaged minimal responses to up to five antidromic stimuli at interval 7 ms. Synaptic efficacy was progressively reduced for the third and subsequent stimuli. The post-synaptic cell was hyperpolarized to suppress the undershoot.

made by single e.p.s.p.s to the rather smoothly rising summed post-synaptic response evoked by a presynaptic burst. An accurate analysis of the facilitation of single e.p.s.p.s at this synapse was thus not possible. Therefore peak post-synaptic responses evoked by differing numbers, from one to five, of presynaptic action potentials were analysed. The peak amplitude of summed e.p.s.p.s grew markedly as the number of spikes increased from one to three (Fig. 11*A* and *B*). However, additional action potentials led to smaller increases in the post-synaptic response (Fig. 11*A* and *B*). Summed e.p.s.p.s also decayed more rapidly and were followed by a more pronounced undershoot as the number of presynaptic action potentials increased.

The contributions of later e.p.s.p.s to summed responses resulting from repeated activation of minimal recurrent e.p.s.p.s were resolved on averaging. The time to peak of the averaged minimal event shown in Fig. 11*E* was 9 ms and trains of up to five stimuli were delivered at intervals of 7 ms. The undershoot was suppressed by hyperpolarizing the post-synaptic neurone. The third, fourth and fifth events were clearly reduced in amplitude suggesting that facilitation due to each e.p.s.p. did not

sum linearly. The decay of the summed synaptic event also became more rapid as additional stimuli were added.

In simultaneous recordings the probability that no post-synaptic response was evoked fell as the number of action potentials in a presynaptic burst increased. Transmission failures resulted from 14 % of single presynaptic spikes at the connexion shown in Fig. 11*A*. Pairs of spikes evoked no response on 2 % of occasions and a summed e.p.s.p. was always elicited by bursts of three or more action potentials (Fig. 11*C*). Discharge of the post-synaptic neurone resulted from unitary e.p.s.p.s evoked by 4 % of single action potentials (Fig. 11*A* and *D*). The probability of reaching threshold was increased to about 35 % for bursts of three or more action potentials. Thus burst firing significantly enhances the probability that action potentials are transmitted between pyramidal cells.

DISCUSSION

These results show that local excitatory synapses exist between CA3 hippocampal neurones. Recurrent excitatory synapses were activated in two ways. Simultaneous intracellular recordings from coupled neurones provided information on the properties of the unitary event. This approach was limited by the low probability of success: 1–2 % of simultaneously recorded neurone pairs were connected mono-synaptically. The antidromic stimulation technique was therefore valuable in allowing us to reliably and selectively activate recurrent e.p.s.p.s. Our results suggest that the same synapses were activated with both methods. The resulting e.p.s.p.s had similar time courses (Figs. 2 and 3), shape indices (Fig. 5) and were usually followed by an undershoot. Their duration increased with membrane depolarization (Figs. 8 and 9). Unitary e.p.s.p.s and those evoked by antidromic stimulation also exhibited comparable responses to repetitive activation (Figs. 10 and 11).

The similarities between e.p.s.p.s evoked with the two methods may be revealing. The cut made in the CA3 area of slices prepared for antidromic stimulation, probably favoured the activation of synapses on distal apical dendrites whereas in paired recordings the location of synaptic terminals which could be activated was not restricted. The finding that e.p.s.p.s of similar wave form were elicited suggests that these synapses predominantly terminate at electrotonically remote dendritic sites. A distal dendritic location of recurrent synapses may contribute to their slower rise time in comparison with e.p.s.p.s evoked by a mossy fibre stimulation (Fig. 6). Such a location might also underlie the difference in somatic reversal potentials of recurrent and mossy fibre e.p.s.p.s. Alternatively a difference could arise if different post-synaptic receptors were activated by the excitatory transmitter (Crunelli, Forda & Kelly, 1984). A slow rise time for recurrent e.p.s.p.s might also result if a somatic action potential led to a slightly asynchronous activation of presynaptic collateral terminals (Redman & Walmsley, 1983). Transmitter release statistics suggest that several recurrent terminals are made on to a post-synaptic cell, the axon collaterals may follow tortuous paths (Finch *et al.* 1983) and e.p.s.p. latencies indicate that conduction along these processes may be slow.

Synaptic activation of intrinsic voltage-dependent currents

In spinal motoneurones (Iansek & Redman, 1973) and in CA1 pyramidal cells (Andersen, Silfvenius, Sundberg & Sveen, 1980) distal dendritic e.p.s.p.s are not much less effective than those generated at proximal sites. If dendritic conduction to the soma was passive in CA3 pyramidal cells recurrent e.p.s.p.s impinging on distal dendrites would be attenuated. However, the present results suggest that unitary synaptic events generated sufficient depolarization to activate intrinsic currents (Fig. 8). The activation of an inward current could effectively amplify and prolong recurrent e.p.s.p.s compensating for decrement due to passive spread. Such an influence of intrinsic post-synaptic properties was initially described for e.p.s.p.s in a snail neurone (Kandel & Tauc, 1966). Active subthreshold conductances have been invoked to explain increases in e.p.s.p. amplitude and duration with membrane depolarization in hippocampal (Masukawa & Prince, 1984) and cortical neurones (Takahashi *et al.* 1967). We did not explore the ionic basis of the inward current elicited by recurrent e.p.s.p.s. In several mammalian neurones subthreshold depolarizations activate currents carried by Na^+ and Ca^{2+} (inferior olivary cells: Llinás & Yarom, 1981; dorsal root ganglion cells: Carbone & Lux, 1984; cerebellar Purkinje cells: Llinás & Sugimori, 1984; sensorimotor cortex cells: Stafstrom *et al.* 1985; hippocampal pyramidal cells: Hotson, Prince & Schwartzkroin, 1979; Halliwell, 1983). The voltage dependency and kinetics of these currents may be such that they can modify the time course of synaptic events (spinal motoneurones: Lux & Schubert, 1975; cortical pyramidal cells: Stafstrom *et al.* 1985).

It is difficult to assess precisely when inward currents were activated during these synaptic events. However, the rising phase of unitary and antidromically elicited e.p.s.p.s fluctuated much less than did their falling phase (Figs. 3, 8 and 9). Under conditions when the contribution of an inward current was likely to be enhanced (Fig. 9*B* and *C*) membrane depolarization caused a large increase in the half-width of compound e.p.s.p.s together with a small increase in their amplitude. The intrinsic component of the event may thus be activated rather smoothly at a time close to their peak and act primarily to cause a prolongation of their decay. Variation in the extent of this contribution at different connexions may partly underlie the variability of e.p.s.p. shape indices shown in Fig. 5.

Our results suggest that an intrinsic outward current was responsible for the undershoot. Since the undershoot was blocked by intracellular Cs^+ and TEA^+ this current was probably mediated by K^+. The activation of K^+ conductances by e.p.s.p.s has been observed in other neurones. Dendritic Ia e.p.s.p.s in spinal motoneurones are also prolonged by intracellular TEA^+ injection (Clements, Nelson & Redman, 1985). An undershoot which follows e.p.s.p.s in sympathetic neurones has been attributed to the activation of a K^+ current suppressed by muscarine but not by TEA^+ (Tosaka, Tasaka, Miyazaki & Libet, 1983). Further, a K^+ current dependent on intracellular Ca^{2+} may underlie the hyperpolarization which follows depolarizations evoked by glutamate application on to CA1 pyramidal cell dendrites (Nicoll & Alger, 1981).

A functional consequence of the activation of a potassium current by excitatory events may be to limit the post-synaptic effects of repetitive discharge. Although a

second e.p.s.p. at short interval was facilitated, subsequent e.p.s.p.s, with timing coincident with the undershoot following a single event, were reduced in amplitude (Fig. 11). A lower security of impulse conduction to axon collateral terminations at high frequency might also account for this observation. A contribution from non-linear e.p.s.p. summation seems less likely due to the large difference between the e.p.s.p. peak and its reversal potential. Potassium currents, acting to control the efficacy of synaptic communication between pyramidal cells may represent an interesting site for modulatory actions of transmitters. Both acetylcholine (Ben-Ari, Krnjevic, Reinhardt & Ropert, 1981; Halliwell & Adams, 1982) and noradrenaline (Madison & Nicoll, 1982) suppress K^+ currents in hippocampal neurones.

Functional significance of local excitatory synapses

The function of recurrent excitatory synapses between CA3 neurones is illuminated by a comparison with the local inhibitory synapses (Miles & Wong, 1984). These excitatory synapses terminate on dendrites. In contrast inhibitory synapses located close to pyramidal cell bodies will influence somatic potential strongly and will dominate a simultaneous recurrent excitatory input. Local excitatory synapses are weaker than inhibitory ones in other ways. Whereas action potentials in an inhibitory cell invariably evoke an i.p.s.p., the transmitter release statistics at excitatory synapses are such that significant numbers of failures occur. Transmission failures reflect a low quantal number, pointing to either a small number of axon collateral terminations on a coupled cell or a low probability of release, if somatic activity propagates faithfully to each terminal. The importance of single transmission failures may be minimized by repeated synaptic activation consequent on the burst firing pattern of these cells. Finally the peak somatic conductance change evoked by a unitary recurrent e.p.s.p. seems to be rather smaller than that due to an i.p.s.p. An approximate value of about 1 nS for the peak somatic conductance change, $G_{e.p.s.p.}$, was derived from the relationship (Ginsborg, 1973):

$$\frac{1}{G_{e.p.s.p.}} = R_m\left(\frac{(V_m - V_{rev})}{V_{e.p.s.p.}} - 1\right)$$

using values of 25 MΩ for the neuronal input resistance, R_m, +5 mV for, V_{rev}, the e.p.s.p. reversal potential, and 1·5 mV for, $V_{e.p.s.p.}$, the peak e.p.s.p. amplitude, at a potential, V_m, of −65 mV. Values of 6–9 nS were obtained for identified unitary i.p.s.p.s in a previous study. The value for the e.p.s.p. may be subject to significant error since the effects of intrinsic currents and possible voltage dependence of the transmitter channels are ignored. The somatic conductance change is likely to underestimate that occurring at the post-synaptic site.

It is also interesting to compare recurrent excitatory synapses between CA3 neurones with synapses made by Ia afferent fibres on spinal motoneurones. Although our experimental procedure may have favoured observation of the most prominent synaptic events, unitary hippocampal e.p.s.p.s appear to be significantly larger and longer in duration than unitary Ia e.p.s.p.s. Recurrent e.p.s.p. amplitudes were 1–2 mV compared with 0·1–0·4 mV for spinal motoneurone e.p.s.p.s, and half-widths were 20–30 ms compared with 4–8 ms (Jack *et al.* 1971; Iansek & Redman, 1973). These differences probably result from a higher input resistance and longer time

constant of CA3 pyramidal cells as well as the contribution from intrinsic currents. Differences in the firing thresholds of the two cell types may have functional implications. Whereas the threshold of spinal motoneurones is 10–15 mV from rest, CA3 pyramidal cells are spontaneously active pace-maker cells. Motoneurones, which represent the final common pathway for the control of skeletal muscle, must receive many synchronous synaptic inputs in order to fire (Kirkwood, Sears & Westgaard, 1984). In contrast burst firing in one pyramidal cell may cause the discharge of other coupled CA3 neurones. The effectiveness of a recurrent synapse in synchronizing the activity of two coupled cells is likely to vary during post-synaptic bursting activity (cf. Hermann, 1979). Both synaptic efficacy and the probability of discharge should increase during the decay of the hyperpolarization following a post-synaptic burst.

Activity in one CA3 pyramidal cell may initiate the synchronous discharge of a large neuronal population when synaptic inhibition is suppressed (Miles & Wong, 1983). It has been suggested that local excitatory connexions may underlie this neuronal recruitment process (Traub & Wong, 1982). The low mono-synaptic connectivity observed in paired recordings seems to argue against such a powerful role. However, nearly all CA3b neurones received some recurrent excitatory input when the CA2–3a neuronal population was antidromically activated. Furthermore neuronal firing may spread between two cells across a local excitatory synapse. If a single synapse has these properties, activity initiated by one cell will grow if the synaptic network is divergent. When synaptic inhibition is suppressed the probability of recording from two cells which are connected multi-synaptically appears to be significantly higher than that reported for mono-synaptic connexions in the present study. We will describe the properties of multi-synaptic excitatory circuits in a subsequent report.

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