The Time Course and Amplitude of EPSPs Evoked at Synapses Between Pairs of CA3/CA1 Neurons in the Hippocampal Slice

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Unitary EPSPs were evoked in CA1 pyramidal neurons by activation of single CA3 pyramidal neurons. Seventy-one EPSPs were recorded. The peak amplitudes of these EPSPs ranged from 30 to 665 μ V with a mean of 131 μ V. Rise times and half-widths were measured, the means $\pm$ SD being 3.9 ± 1.8 and 19.5 ± 8.0 msec, respectively. The time courses of these EPSPs were consistent with a brief synaptic current at a localized electrotonic region of the dendritic tree followed by passive spread of current to the soma. EPSPs varied in amplitude from trial to trial. Sufficient records were collected for 12 EPSPs to demonstrate that this variation was greater than could be accounted for by baseline noise. The amplitude variations of one EPSP were reliably resolved from the background noise, and this EPSP fluctuated between 4 discrete amplitudes (including failures) separated by a quantal increment of 278 μ V.

The current interest in excitatory transmission at synapses on hippocampal neurons stems largely from excitement about the dramatic form of plasticity, known as long-term potentiation (LTP), which occurs at these synapses. LTP occurs at the monosynaptic connection formed by CA3 pyramidal neurons with CA1 pyramidal neurons, and much progress has been made towards understanding junctional mechanisms at this synapse while investigating LTP phenomena (see reviews by Teyler and Di Scenna, 1987; Gustafsson and Wigstrom, 1988). Transmission at this synapse has traditionally been studied by synchronously stimulating a large number of afferents in the stratum radiatum (SR), thereby generating a large compound EPSP in CA1 neurons. The fine details of transmission at CA3-CA1 synapses cannot be obtained from this technique, nor can details of convergence of CA3 pyramidal neurons onto single CA1 neurons or of divergence of CA3 neurons into the CA1 field.

A knowledge of the amplitudes and time courses of EPSPs evoked at connections formed between single CA3 and CA1 neurons is essential if we are to know how many CA3 pyramidal neurons must be simultaneously active to discharge a CA1 pyramidal cell and whether synaptic location is a factor in deter-

mining the size of an EPSP and its contribution to somatic depolarization. Single-fiber EPSPs can also be analyzed for quantal fluctuations, thereby separating presynaptic effects from postsynaptic changes during alterations in synaptic strength. These types of analyses have been pursued at the monosynaptic connection formed between a single group Ia axon and lumbar motoneurons and they have been very useful in determining the integrative response of motoneurons to peripheral inputs.

This report is the first to describe details of time course, amplitude, and variability in amplitude of EPSPs evoked at single CA3-CA1 pyramidal cell connections, as well as some details on divergence and convergence between these groups of neurons. The stimulus for this work was to study LTP at single CA3-CA1 connections, the results of which are presented in the following paper (Friedlander et al., 1990). A brief report of these results has been presented (Friedlander et al., 1988).

Materials and Methods

A general description of the surgical procedure and preparation and maintenance of slices, together with basic electrophysiological techniques, has been given recently (Sayer et al., 1989). Each guinea pig (600–900 gm) was deeply anesthetized with ether and after carotid section the brain was removed and chilled in artificial cerebrospinal fluid (ACSF) bubbled with 95% O₂/5% CO₂ at 2–4°C. The hippocampus was dissected out from one side and 450–500 μ m slices were cut with a tissue chopper. The hippocampus was aligned on the chopper so that the slices were cut parallel to the lamellae, 20°–30° from the transverse plane with the rostral end of the blade turned medially. The slices were transferred onto a net covered with lens tissue in a recording chamber where they were maintained at 32–33°C between ACSF and humidified gas (95% O₂/5% CO₂). The ACSF consisted of (in mM): NaCl, 124; KCl, 2; MgSO₄, 2; CaCl₂, 2; KH₂PO₄, 1.25; NaHCO₃, 26; and glucose, 11, saturated with 95% O₂/5% CO₂.

Simultaneous CA3-CA1 intracellular recordings. Recording electrodes were pulled from 1.5 mm thin-walled glass (AM Systems), were shielded with aluminum foil, and had resistances of 40–80 M Ω when filled with 2 M potassium methylsulfate. To improve the chances of finding a connecting CA3-CA1 pair, we first looked for a CA3 cell which could be antidromically activated by a stimulating electrode (25- μ m-diameter tungsten in glass with 20 μ m of tip exposed) placed in the CA1 stratum radiatum (Fig. 1). This ensured that the CA3 neuron had a collateral running in the plane of the slice through the CA1 apical dendritic field. Pyramidal cells were impaled in the CA1 stratum pyramidale 200–1000 μ m from the CA3/CA1 border. Once stable intracellular penetrations were achieved, the CA3 neuron was stimulated at 2 Hz by an intracellular depolarizing current pulse. The depolarizing pulse was adjusted (0.5–2.5 nA, 3–5 msec) to evoke a single action potential. Sometimes a small steady hyperpolarizing current (0.1–0.5 nA) was applied to the CA3 cell to prevent spontaneous firing or to the CA1 neuron to hold the membrane potential more negative than –60 mV. If on-line averaging showed any postsynaptic response, as many records (usually 100 msec in duration) as possible were digitized at 4 kHz and saved on disk while the membrane potentials in each cell remained satisfactory. After withdrawing the electrode from the CA1 cell, an extracellular average

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Figure 1. A pyramidal cell was impaled in the CA3 stratum pyramidale. The presence of a Schaffer collateral (*Sch.*) in the CA1 stratum radiatum was checked by antidromically activating the collateral with a stimulating electrode (*STIM*). This fiber could then be orthodromically discharged by injecting a current pulse into the CA3 neuron through the CA3 intracellular electrode. CA1 pyramidal cells were then impaled in the CA1 stratum pyramidale.

was taken while stimulating the CA3 neuron to check for any extracellular field that may have been generated by activation of the CA3 afferent. However, none was ever observed. Small surgical lesions were made at the recording sites, and after overnight fixation in 3% glutaraldehyde, the slices were stained with thionine for microscopic localization of the recording sites with respect to the CA3/CA1 border.

Presynaptic records were checked to ensure that CA3 cells discharged only once for each current pulse. If there was multiple firing or a failure to discharge, the corresponding CA1 record was not included in the analysis.

For each connected CA3-CA1 pair we attempted to record as many individual responses as possible (commonly more than 1000). These responses were averaged using the time of the peak of the CA3 action potential as $t = 0$ to minimize the effect of latency variations on the average time course. The procedures for calculating the peak amplitude of individual responses, for constructing histograms of EPSP peak amplitude and baseline noise amplitude, for deconvolving these histograms to reveal the underlying discrete amplitudes of the EPSP, and for calculating the time course of the standard deviation of the EPSP have all been described by Sayer et al. (1989).

In these experiments no attempt was made to block GABA_A-mediated synaptic inhibition for 2 reasons: (1) because in the absence of inhibition, polysynaptic excitatory connections between CA3 neurons are revealed (Miles and Wong, 1987b) and this might allow resolution of polysynaptic connections between CA3 and CA1 neurons, and (2) we wished to preserve any IPSPs which might be evoked in CA1 neurons.

Results

We made 1178 simultaneous recordings from CA3 and CA1 neurons in 55 experiments. On 74 occasions, postsynaptic potentials (PSPs) were seen on the averaged CA1 records, in response to activation of the CA3 neuron. The yield of unitary PSPs was therefore approximately 1 for every 16 pairs tested. Seventy-one of the PSPs were EPSPs (depolarizing only), but 3 had prominent hyperpolarizing components and will be presented separately. The 74 PSPs were obtained from 72 neurons in the CA1 stratum pyramidale. For each of 2 CA1 cells, synaptic connections were obtained from 2 CA3 cells (i.e., convergent inputs). Only 63 CA3 neurons were activated to obtain the 74

Figure 2. Simultaneous recordings from a CA3 neuron and a CA1 neuron with a synaptic connection. *A*, Approximate locations of the electrodes. *S.R.*, stratum radiatum stimulating electrode. *B*, Action potential evoked in the CA1 cell by a 20 μ A, 0.1 msec cathodal stimulus delivered through S.R. (the stimulus artifact is cut off to the left of the figure). *C*, Response of the CA1 neuron to a 0.1 nA, 100 msec hyperpolarizing current pulse (switched current clamp, 4 kHz). *D*, Responses of both cells to a 10 μ A S.R. stimulus. *E*, An action potential evoked in the CA3 neuron by a short depolarizing current pulse (4 msec duration) adjusted to produce a single action potential. *F*, Examples of individual responses in the CA1 neuron. *G*, Average of 1780 CA1 responses, after adjustment for latency variations of the CA3 action potential. The capacitive coupling artifact has been masked. The bar indicates the duration of the CA3 current pulses (4 msec). *H*, Average of 150 records taken on exit from the CA1 cell, while continuing to activate the CA3 neuron.

unitary PSPs. On 7 occasions, 2 postsynaptic CA1 cells were found for single CA3 neurons; and 3 postsynaptic targets were found on 2 occasions (i.e., divergence).

Figure 2 illustrates features of simultaneous CA3-CA1 recordings. The recording sites are indicated in Figure 2*A*, as is the position of the stimulating electrode in the CA1 stratum radiatum. The CA1 action potential in Figure 2*B* was evoked by stimulating the SR. The response of the CA1 neuron to a 0.1 nA, 100 msec hyperpolarizing current pulse (switched current clamp) is shown in Figure 2*C*, indicating an input resistance of 31 M Ω . Figure 2*D* shows the responses in both cells to a 10 μ A stimulus to the SR. At this stimulus strength, the CA3 cell was activated antidromically, indicating that it had an intact axon collateral in the CA1 SR while the EPSP in the CA1 neuron

was subthreshold. The direct distance between the SR electrode and the CA3 recording electrode was 1600 μm , and the latency from stimulus to onset of the antidromic action potential was 1.25 msec. This implied a minimum conduction velocity of 1.3 m/sec for the axon collateral, and a greater conduction velocity depending on the extent to which it deviated from a direct line between the electrodes.

The right-hand column of Figure 2 shows records obtained while evoking unitary EPSPs in the same CA1 cell. The CA3 neuron was activated by a depolarizing current pulse (Fig. 2E). Examples of individual responses in the CA1 neuron are shown in Figure 2, F1–F4, and the average of 1780 individual records is shown in Figure 2G. The averaging procedure included adjustments for latency variations of the CA3 action potential. An extracellular average, taken from immediately outside the cell while continuing to activate the CA3 cell (Fig. 2H) indicates that there was no extracellular field potential.

Stable intracellular penetrations of CA3–CA1 pairs with synaptic connections were commonly maintained for 10–60 min, and sometimes for longer (maximum 3 hr). For the CA1 cells, the average action potential amplitude was 82 ± 9 mV (means $\pm$ SD). The mean resting membrane potential, with no current applied and determined on exit from these neurons was 64 ± 8 mV ($n = 42$, as this could not be obtained for penetrations which were lost suddenly in the presence of steady current). The average input resistance was 20.2 ± 10.8 M Ω ($n = 54$). These values are comparable to other reports for guinea pig CA1 pyramidal cells *in vitro* (e.g., Turner, 1988).

The CA3 pyramidal cells were generally more robust than CA1 neurons, and stable intracellular recordings were often maintained for several hours while searching for postsynaptic (CA1) cells. CA3 neurons were discarded if their action potential amplitudes declined to 60 mV or less, if they could not be reliably activated by the depolarizing current pulse, or if more than about 15 pairs were tested without any synaptic connections being found. The input resistances and membrane potentials of the CA3 neurons were not routinely recorded.

Locations of the CA3 and CA1 neurons

CA3 neurons which were activated antidromically from the CA1 stratum radiatum were mostly found in the two thirds of the CA3 region nearest to the CA1 region. As the stimulating electrode in the CA1 stratum radiatum was moved towards the subicular end of CA1, fewer antidromically activated CA3 neurons were found. This implied that fewer intact Schaffer collaterals remained within the slice as the distance from CA3 was increased. For this reason, we preferentially impaled CA1 neurons in the stratum pyramidale within a few hundred microns from the CA3/CA1 border, believing this would provide the highest yield of synaptically connected pairs. The recording sites were identified by thionine staining (see Materials and Methods) for 41 CA3–CA1 pairs. Shrinkage was determined for each slice by comparing the distances between the lesions measured *in vitro* with those after processing and was on average 19%. For this group of CA3–CA1 pairs, the distance between the CA3 and CA1 recording sites (measured around the stratum pyramidale, not the straight line distance) ranged from 500 to 1835 μm (mean, 1030 μm). The CA3 electrode to CA3/CA1 border distance ranged from 135 to 1075 μm (mean, 675 μm), and the distance from the CA1 electrode to the CA3/CA1 border ranged from 130 to 970 μm (mean, 355 μm).

Schaffer collateral conduction velocities and latency of onset of CA1 EPSPs

The delay from the SR stimulus to the onset of the antidromic action potential in CA3 neurons and the distance between the SR and CA3 electrodes were both recorded for 27 CA3 neurons. The conduction velocities determined for the Schaffer collaterals from these parameters ranged from 0.33 to 1.70 m/sec, with a mean of 1.01 m/sec. However these values are likely to be underestimates, depending on how far the axons deviate from the direct line between the CA3 somata and the stimulating electrode in the SR. In addition, as CA3 axons emerge from the basal dendritic side of the soma, a variable amount of extra axon length must be involved before crossing the stratum pyramidale and reaching the stratum radiatum.

For all 71 unitary EPSPs, the latency was measured from the peak of the CA3 action potential to the time at which the averaged CA1 EPSP reached 10% of its peak amplitude. The mean latency was 3.4 ± 1.2 msec, with a range from 0.7 to 5.8 msec. Any errors in these measures, due to encroachment of the capacitive coupling artifact into the rising phase of the EPSP, would affect the shortest latency EPSPs most and would tend to prolong them. The value for the mean latency given above should therefore be regarded as an approximation, and the true mean may be slightly less.

Amplitudes of the averaged unitary EPSPs

When unitary EPSPs were present on the CA1 records, the largest responses could be detected in individual records (Fig. 2F). However, the baseline noise levels often required 100–200 records to be averaged before the presence of a unitary PSP could be established. We estimated that under typical recording conditions, the noise levels on averages of 100–200 records allowed us to detect EPSPs with mean peak amplitudes greater than 20 μV .

Measurements of the mean peak amplitude of the EPSPs, evoked at 2 Hz, were made from averages of 230–3000 records. These were taken after stable penetrations of the cells had been achieved, and before any further experimental manipulations (e.g., tetanic stimuli). The inset in Figure 3 shows a sample of 6 averaged unitary EPSPs, illustrating a range of time courses and amplitudes. The EPSPs are arranged in order of decreasing rise time. The histogram of mean peak amplitudes for the 71 unitary EPSPs is also shown in Figure 3. The amplitudes ranged from 30 to 665 μV , with an overall mean of 131 μV . The variability of the unitary EPSPs from trial to trial is apparent from the single records in Figure 2F. However, when the EPSPs were averaged over sequential 1 min intervals (120 records/average, at 2 Hz), their mean peak amplitudes rarely showed any consistent drift from the overall mean. Of 23 unitary EPSPs held for more than 10 min and not manipulated experimentally in any way, 20 showed no trend towards a decline or increase in mean peak amplitude (see Fig. 4 in Friedlander et al., 1990).

The time courses of unitary EPSPs

The rise time, half-width, and decay time constant were measured for all 71 unitary EPSPs. Four EPSPs are plotted on linear and log voltage scales in Figure 4, and the 10–90% rise time, half-width, and decay time constant are indicated for each EPSP. The rise times and half-widths are plotted in Figure 5A. The average rise time was 3.9 ± 1.8 msec, and the average half-

Figure 3. Distribution of the mean peak amplitudes of the 71 unitary EPSPs. Bin size, 25 μ V. Inset, Six examples of averaged unitary EPSPs. They are arranged in order of slowest (A) to fastest (F) rise times. The numbers of individual records comprising each average were 579, 900, 1049, 1879, 581, and 673 for A–F, respectively. The capacitive coupling artifacts have been masked, and the bars indicate the duration of the depolarizing current pulses in the CA3 neurons.

width was 19.5 ± 8.0 msec. To ensure that these values had not been biased by encroachment of the capacitive coupling artifact onto the rising phases of the EPSPs, 11 EPSPs were selected which had a short segment of flat baseline immediately before the rising phase. Examples of such EPSPs can be seen in Figures 2G and 3D. The mean rise time and half-widths for this group were then compared with those of the other 60 EPSPs. There was little difference between the groups; their mean rise times were 3.87 and 3.94 msec ($n = 11$ and 60, respectively) and their mean half-widths were 21.5 and 19.2 msec. These differences were not significant ($p = 0.90$ for rise time and $p = 0.35$ for half-width), and it was considered appropriate to combine the 2 data groups.

The linear fit to the log plot of the final decay of the EPSPs was judged to be adequate for 66 of the 71 EPSPs. In general, the fits were very good, as illustrated by the 4 examples in Figure 4. The average time constant of decay for the 66 EPSPs was 22.6 ± 11.0 msec. Figure 5B shows the rise times plotted against the half-widths for the 66 EPSPs, after both have been normalized by the time constant of decay for the EPSP (normalized shape-index plot; Jack et al., 1971). Most of the results are clustered between normalized rise times of 0.1 to 0.5 and normalized half-widths of 0.5 to 2.0.

As shape indices can be used to predict synaptic location, it was of interest to compare shape indices with peak amplitude. Mean peak amplitude is plotted against normalized rise time in Figure 5C. Small amplitudes are associated with the full range of normalized rise times, and short rise times are associated with a wide range of EPSP amplitudes. However, there are no EPSPs with longer rise times and large amplitudes.

The scatter of mean amplitude against normalized half-width is more widely spread (not illustrated), with only a weak indication that the longest normalized half-widths are associated with only small-amplitude EPSPs.

Hyperpolarizing responses

On 3 occasions PSPs were seen which were predominantly hyperpolarizing (Fig. 6). In 2 cases, the responses consisted of a smaller depolarization followed by a larger hyperpolarization (Fig. 6, A, B). Latencies from the CA3 action potential to the time at which the hyperpolarizing phase reached 10% of its maximum, were 8.5, 9.8, and 5.7 msec for the PSPs in Figure 6, A, B, and C1, respectively. The duration of the hyperpolarization was about 30–35 msec in each case.

These CA3-CA1 recordings were not held for long enough to allow a thorough examination of the hyperpolarizing PSPs. For one PSP, an average was obtained for 2 different steady hyperpolarizing currents (0.6 and 1.0 nA; Fig. 6, C1, C2). The input resistance of this CA1 neuron was 12 M Ω , so the additional 0.4 nA would have hyperpolarized the cell by about 5 mV (assuming a linear I - V response). This voltage change was sufficient to reduce the amplitude of the PSP, and the response in Figure 6C2 suggests that the membrane potential was close to the reversal potential for the synaptic current. With no current applied, the membrane potential was -56 mV, and assuming a linear I - V relationship for the neuron, the membrane potential when Figure 6C2 was obtained would have been -68 mV.

EPSP variability from trial to trial

The amplitude of individual EPSPs varied from trial to trial. This variation was usually obscured by baseline noise, but occasionally an EPSP was sufficiently large compared with the noise to make it obvious that the EPSP amplitude had its own intrinsic variability. One such EPSP is illustrated in Figure 7. Four individual responses have been selected with clearly different amplitudes (Fig. 7B). The average of these responses (and many more) is shown in Figure 7C, and an action potential evoked in the CA3 neuron appears in Figure 7A. A measure of

Figure 4. The 4 EPSPs *A–D* illustrate a range of time courses, with rise times and half-widths as shown. *E–H*, Corresponding log plots for EPSPs for EPSPs *A–D*. The vertical scale bar (100 μ V) does not apply to these plots. The filled squares indicate the beginning and end of the log-linear regions used for measurement of the time constant (τ).

the variability of an EPSP can be obtained from the standard deviation of the EPSP (about its mean) after removing the contribution of the baseline noise (Edwards et al., 1976). The time course of the average and standard deviation for 6 EPSPs are shown in Figure 8. All the standard deviation records show increases in variability during the EPSP. The maxima of the standard deviation records occur at almost the same time as the peaks of the corresponding averaged EPSPs, with the exception of one EPSP (no. 3), where the peak in the standard deviation record occurs during the rising phase of the averaged EPSP.

One way of quantifying the degree of fluctuation in these EPSPs is to compare the standard deviation of the noise-contaminated EPSP amplitude with the standard deviation of the noise alone. The standard deviations for the 6 EPSPs illustrated in Figure 8, and for 6 other EPSPs, are listed in Table 1. These 12 EPSPs were selected from the 71 unitary EPSPs on the basis

that more than 2000 individual responses were recorded for each of them. The standard deviation of the EPSP amplitude is greater than that of the noise in every case. The significance of these differences has been estimated by Fisher's *z*-approximation to the *F*-distribution, and the *z* and *p* values are listed in Table 1. The null hypothesis, which is that both the noise distribution and the noise-contaminated EPSP distribution have been sampled from distributions with the same standard deviation, can be rejected at the 0.0001 level of confidence for 9 of the 12 EPSPs. The *p* values for the other three EPSPs (nos. 2 and 4 in Fig. 8 illustrate 2 of them) indicate that if the null hypothesis were correct, then differences of this magnitude in the standard deviations would commonly occur by chance. The data for these 3 EPSPs are therefore consistent with either the presence or absence of intrinsic variability in the EPSP amplitude.

Figure 5. Shape indices for the unitary EPSPs. *A*, Half-widths and rise times. *B*, Half-widths and rise times normalized by the time constant of decay for each EPSP. *C*, Mean peak amplitude of the unitary EPSPs plotted against normalized rise time.

Histograms of peak amplitude were constructed for all 12 EPSPs, as well as for the baseline noise associated with each EPSP, as described by Sayer et al. (1989). The noise and EPSP histograms for the 2 EPSPs illustrated in Figure 10, *A*, *B*, are

Table 1. Unitary EPSP variability from trial to trial

EPSP no.	$\bar{v}$ (μ V)	σ_E (μ V)	σ_N (μ V)	CV	z	p
1	451	446	349	0.62	7.55	<0.0001
2	205	268	257	0.37	0.97	0.33
3	170	222	147	0.98	8.32	<0.0001
4	67	237	233	0.65	0.48	0.63
5	471	402	254	0.66	9.76	<0.0001
6	377	309	145	0.72	16.69	<0.0001
7	274	416	308	1.02	8.35	<0.0001
8	324	236	127	0.61	17.72	<0.0001
9	159	211	158	0.88	7.49	<0.0001
10	380	375	169	0.88	20.17	<0.0001
11	155	210	169	0.80	6.32	<0.0001
12	69	255	246	0.97	0.92	0.36

Parameters derived from the standard deviations of the histograms of the noise-contaminated EPSP amplitudes and the histograms of the noise amplitudes.

$\bar{v}$, mean amplitude of EPSP. σ_E , standard deviation of the noise-contaminated EPSP amplitude histogram. σ_N , standard deviation of the noise amplitude histogram. CV, $\sqrt{(\sigma_E^2 - \sigma_N^2)/\bar{v}}$, the coefficient of variation for the noise-free EPSP. This measure assumes that σ_E and σ_N are accurate (i.e., not subject to sampling error) and that their difference is caused by the variability of the underlying noise-free EPSP. z , $\sqrt{N} \log(\sigma_E/\sigma_N)$, Fisher's z -approximation to the F -distribution. z is normally distributed with variance = 1. N is the number of records used to make the histograms.

If the following is true:

there is no intrinsic variability in the EPSPs, so that σ_E and σ_N would be equal except for the effects of finite sampling,

then p is the expected probability of obtaining a z value equal to or greater than that given (and therefore an equal or greater difference between σ_E and σ_N than obtained in the experiment), purely due to random sampling error.

shown in Figure 9, *A*, *D*. The noise and EPSP distributions were deconvolved using a maximum likelihood criterion for goodness-of-fit (Sayer et al., 1989). The result shown in Figure 9C is one where the EPSP has 4 discrete amplitudes, each occurring with a different probability. The amplitude (μ V)/probability combinations in Figure 9C are 17/0.19; 267/0.38; 523/0.30; 850/0.10, and the way in which these amplitudes combine with the noise to form the measured histogram is shown in Figure 9B. The average quantal size for this EPSP (V_1) is 278 μ V, and the noise standard deviation (σ_N) is 145 μ V. These figures give $V_1/\sigma_N = 1.92$, and based on the results of Monte Carlo simulations (Sayer et al., 1989), the result in Figure 9C can be accepted as reliable. The same conclusion cannot be reached about the result in Figure 9F, where the quantal size is insufficient to give a reliable resolution of the underlying discrete amplitudes and probabilities ($V_1/\sigma_N = 225/147 = 1.53$). This same problem occurred for the remaining 10 EPSPs; the noise level was too high compared with the quantal size for the discrete amplitudes to be reliably resolved.

Convergent inputs to CA1

In 2 experiments, a CA1 penetration was held long enough for 2 presynaptic CA3 cells to be found. Figure 10, *A*, *B*, shows the 2 averaged EPSPs from the CA1 neuron for one of these examples. The 2 CA3 neurons involved were found in nearby tracks (<100 μ m apart). The rise times for these EPSPs were different (0.8 and 1.2 msec), but the half-widths were similar (5.0 and 4.9 msec). The most marked difference between the 2 EPSPs was in their peak amplitudes, which were 170 and 377 μ V.

Figure 6. Averaged PSPs with hyperpolarizing components evoked in CA1 neurons by activation of single CA3 cells. *A–C* are from 3 different CA3–CA1 pairs. *C1*, 0.6 nA, and *C2*, 1.0 nA continuous hyperpolarizing current was applied through the CA1 intracellular electrode.

In the other example of convergent input to a CA1 neuron, the amplitudes of the 2 EPSPs were also quite different, being 446 and 193 μ V (not illustrated). Their rise times were 2.5 and 1.5 msec, and their half-widths were 19.5 and 15.7 msec, respectively. Again, the 2 CA3 cells were very close to each other.

Figure 7. *A*, Action potential evoked in the CA3 neuron by a brief depolarizing current pulse. *B*, Examples of individual records from the CA1 neuron, showing the responses to activation of the CA3 neuron. *C*, Average of 2953 CA1 records, after adjustment for latency variations of the CA3 action potential. The capacitive coupling artifact has been masked. The bar indicates the duration of the CA3 current pulse (4 msec).

Divergence from CA3

It was more common to find multiple postsynaptic CA1 neurons for a single CA3 cell, as CA3 neurons could often be held without deterioration for several hours. This happened on 9 occasions; in 7 experiments, 2 postsynaptic CA1 neurons were found for single CA3 cells, and in 2 experiments, 3 postsynaptic cells were found. One result with 2 postsynaptic neurons for a single CA3 cell is illustrated in Figure 10, *C*, *D*. The 2 EPSPs differed in time course and amplitude. In general, the CA1 neurons postsynaptic to a common CA3 cell were found close to each other in the CA1 stratum pyramidale. Having found one connection in a given area, and therefore knowing that there was an axon collateral with terminals nearby, we tended to search for others in the same region.

Discussion

The amplitude of unitary EPSPs

This paper provides the first report of direct measurements of the synaptic strength of a single CA3–CA1 connection. The only previous estimate of the unitary EPSP amplitude in CA1 neurons was 100 μ V (Andersen, 1982); a calculation based on the number of SR fibers which needed to be activated to discharge a CA1 pyramidal cell and close to the measured average amplitude of 131 μ V. The pooled amplitudes were obtained over a range of membrane potentials in the CA1 neurons, and no investigation was made of any voltage dependence of these amplitudes. The small amplitudes of the CA3–CA1 unitary EPSPs contrast with the amplitudes of unitary EPSPs in CA3 neurons. Those evoked via the recurrent excitatory pathway between CA3 neurons are about 10 times larger (1–2 mV; Miles and Wong, 1986), and presumed unitary EPSPs obtained in CA3 pyramidal cells from iontophoretic application of excitatory amino acids to dentate granule cells are greater than 1 mV (Yamamoto, 1982; Higashima et al., 1986).

The EPSP amplitude was not constant from trial to trial. The standard deviation records for 6 EPSPs illustrated in Figure 8, and for another 6 described in Table 1, indicate that these EPSPs fluctuate in amplitude more than can be attributed to background noise. The peak of the standard deviation record occurred at a similar time to the peak of the averaged EPSP for 11 of the 12 EPSPs, indicating that the variability in the EPSP did not result from latency variations. [Variations in latency will cause the peak of the standard deviation record to occur in the rising phase of the average response. This was only seen in one EPSP: Fig. 8(3).]

The histograms of EPSP amplitude also indicated that genuine variations in peak amplitude occurred. A comparison of the standard deviations of the noise amplitude histograms with those

Figure 8. Comparison of the average and standard deviation time courses for 6 unitary EPSPs. The *solid lines* are the averages, and the *dotted lines* are the standard deviation records. These 6 EPSPs correspond to entries 1–6 in Table 1.

of the noise-contaminated EPSP histograms showed the differences in these standard deviations to be highly significant ($p < 0.0001$ using Fisher's z -approximation) for 9 of the 12 unitary EPSPs for which a sufficiently large number of records (>2000) were obtained. It is possible that the other 3 EPSPs had no intrinsic variability and that the standard deviations of their amplitude histograms exceeded those of the noise because of finite sampling errors. However, in view of the time course of these standard deviation records, this seems unlikely. Thus, there were no EPSPs which could be shown with certainty not to fluctuate in amplitude.

Only one EPSP (of 12 for which sufficient records were obtained) gave a reliable deconvolution result, not because the noise level was particularly small, but rather because the quantal size of $278 \mu\text{V}$ was large. In a previous analysis (Sayer et al., 1989), 2 minimal EPSPs evoked by SR stimulation gave quantal sizes of 224 and $193 \mu\text{V}$, and it was argued that these quantal

sizes also represented the upper end of the quantal size range. The probabilities associated with the 4 discrete amplitudes in Figure 9C translate into approximately equal probabilities of release (p) at 5 different active sites with $p = 0.27$; i.e., uniform binomial statistics govern the release of transmitter from this synapse. Assuming that p can be increased by conditioning procedures, there is considerable reserve capacity to increase the strength of this synapse.

Time course of unitary EPSPs

Are the time courses of unitary EPSPs consistent with the passive spread of synaptic current to the soma? If dendritic membrane properties remain passive during synaptic current spread, the time course of an EPSP can be used as a guide to the dendritic location of the activated synapse. However, if the EPSP activates voltage-dependent conductances, the synaptic location becomes less important in shaping the time course of an EPSP. While it

Figure 9. Deconvolution analysis for EPSPs illustrated in Figure 10, *A*, *B*. Selected single records are illustrated in Figure 7*B*. *A*, The noise amplitude histogram (shaded) superimposed on the noise-contaminated EPSP amplitude histogram, for EPSP in Figure 10*A*. The filled circles indicate the 2-Gaussian distribution fitted to the noise amplitude histogram, used as the noise distribution by the deconvolution procedure. Note that the vertical scale is different for the noise histograms (parentheses). *B*, Filled circles indicate the reconvolved deconvolution result and were obtained by summing the 4 underlying curves. These points have been superimposed on the noise-contaminated EPSP amplitude histogram. The 4 underlying distributions have the same standard deviation (σ_n) as the measured noise distribution, their mean values are equal to each of the discrete amplitudes, and their areas are equal to the probabilities associated with the discrete amplitudes. *C*, Deconvolution results for the EPSP, shown as a sequence of discrete amplitudes and associated probabilities. *D–F* correspond to *A–C* but are for the EPSP in Figure 10*B*.

is known that the dendrites of CA1 pyramidal neurons contain voltage-dependent conductances (Kandel and Spencer, 1961; Andersen and Lomo, 1966; Wong and Prince, 1979; Schwartzkroin and Prince, 1980; Benardo et al., 1982; Masukawa and

Prince, 1984; Miyakawa and Kato, 1986), it is not known if these conductances are activated by the voltage excursions associated with unitary EPSPs. A comparison of the time courses of unitary EPSPs with those of minimal EPSPs evoked by stim-

Figure 10. *A* and *B*, EPSPs evoked in the same CA1 neuron by stimulating 2 different CA3 neurons (labeled *A* and *B*). *C* and *D*, EPSPs evoked in 2 different CA1 neurons (labeled *C* and *D*) by stimulating the same CA3 neuron.

ulation of proximal and distal SR (Turner, 1988), and with the time courses predicted from passive cable models of CA1 pyramidal cells (Turner, 1984a, b, 1988) suggests that active membrane currents do not contribute to the unitary EPSPs.

Minimal EPSPs evoked by proximal and distal stimulation of the SR had average amplitudes of 470 and 400 μ V, respectively, and average rise time/half-width values of 3.2/17.8 and 6.7/27.2 msec, respectively (Turner, 1988). Reference to Figure 5A indicates that the shape indices for proximal minimal EPSPs are located among the smallest shape indices for unitary EPSPs, while the shape indices for distal minimal EPSPs are located among the largest shape indices of unitary EPSPs.

These comparisons are explored further in Figure 11, where the normalized shape indices shown in Figure 5B have been rescaled and replotted, and where the broken line is the boundary of the shape indices of minimal EPSPs obtained from Turner (1988; Fig. 7B). The shape indices of minimal EPSPs would be expected to have a greater variation than those obtained for unitary EPSPs, as the amplitude of the minimal EPSPs suggests that on average, 3 Schaffer collaterals are excited by graded SR stimulation. These axons will make synaptic connections at a variety of dendritic locations, with the rise time of the composite EPSP being determined by the most proximal synapses, while the half-width will be dominated by the most distal connections. For normalized rise times <0.5 and normalized half-widths <1.8 , the range of shape indices obtained for unitary EPSPs would predict the shape indices of the minimal EPSPs. Normalized rise times and half-widths exceeding these values were found infrequently for unitary EPSPs, and relatively frequently for minimal EPSPs, almost all resulting from distal SR stimulation. Either the method of finding CA3-CA1 pairs discriminated against connections which give rise to shape indices in this range, or the distal minimal EPSPs activate membrane currents which slow the rise and decay of the EPSP.

Cable modeling of CA1 neurons (Turner, 1984a, b, 1988) based on reconstruction of these neurons following HRP injection supports the proposition that minimal EPSPs evoked in distal dendrites activate membrane currents. The continuous line in Figure 11 is the locus of normalized shape indices predicted by passive cable models of CA1 neurons when the synaptic location is progressively moved from the soma to the distal apical dendrites. The shape indices of the unitary EPSPs are well predicted by modeling the dendrites with passive membrane properties, while those of the distally evoked minimal EPSPs are not.

Further evidence in support of passive electrical spread of unitary EPSPs to the soma comes from the observation that no EPSPs were observed with a combination of a large amplitude and a slow rise time (Fig. 5C) and that small, delayed hyperpolarizing potentials which follow EPSPs in some neurons (the "undershoot"; Ito and Oshima, 1965; Miles and Wong, 1986) were never observed in the unitary EPSPs. We stress, however, that during normal integrative activity in CA1 neurons when the membrane potential in the dendrites is likely to vary widely, passive membrane properties are unlikely to determine the amplitude and time course of unitary EPSPs.

Are the unitary EPSPs monosynaptic?

While we have assumed that the EPSPs evoked in CA1 neurons by activation of single CA3 neurons are monosynaptic, there are alternative possibilities. A disynaptic linkage via recurrent excitatory connections between CA3 pyramidal neurons

Figure 11. Comparison of the shape indices of unitary EPSPs with those of minimal EPSPs reported by Turner (1988; Fig. 7). The data from Figure 5B have been replotted on a different scale. The dashed line encloses the shape indices of minimal EPSPs reported by Turner (1988). The solid line encloses the predictions of shape indices of EPSP generated at single spine inputs on a cable model of a CA1 neuron, with the synaptic input at various locations between the soma and the most distal regions of the apical dendrite (Turner, 1988, 1984a, b).

(MacVicar and Dudek, 1980; Miles and Wong, 1986) can be dismissed from latency measurements. Latencies of 8 msec or more were observed in the polysynaptic potentials evoked between pairs of CA3 neurons (Miles and Wong, 1987a), whereas the latencies measured for the EPSPs in this study did not exceed 5.8 msec.

Another possibility is that the EPSP contains a component arising from recurrent excitatory pathways within CA1. While simultaneous recordings from pairs of CA1 pyramidal cells failed to find excitatory interactions (Knowles and Schwartzkroin, 1981), EPSPs evoked in CA1 neurons by local applications of glutamate (Christian and Dudek, 1988) suggest that they may exist. However, these EPSPs were all clearly subthreshold, and there is no evidence for unitary inputs to CA1 pyramidal cells which would be powerful enough to activate recurrent excitatory circuits.

Although IPSPs are commonly associated with small EPSPs (500 μ V or less) elicited by stimulation of the SR (Turner, 1985, 1988), it was a surprise to find what appeared to be IPSPs or EPSP/IPSP sequences in CA1 cells in response to activation of single CA3 neurons. It seems unlikely that these could be mediated by recurrent inhibitory circuits within CA1 (Andersen et al., 1963, 1964 a, b; Knowles and Schwartzkroin, 1981), because the unitary EPSPs were well below threshold. A more plausible mechanism would be via feedforward inhibition (Buzsaki and Eidelberg, 1982; Alger and Nicoll, 1982; Ashwood et al., 1984). This would require discharge of an inhibitory interneuron in CA1 by a single afferent from CA3. We have no results which would confirm this possibility, as we have not recorded unitary EPSPs in identified CA1 interneurons. The latencies to the hyperpolarizing phases of the PSPs were, at 5.7–9.8 msec, longer than those for the unitary EPSPs (mean, 3.4 msec) and would be consistent with a disynaptic connection from CA3.

Two of the responses appeared to have an early depolarizing

phase (Fig. 6, *A, B*). These presumably arise from the coexistence of monosynaptic excitatory connections from CA3, with the disynaptic inhibitory inputs. The question then arises whether the other responses obtained in this study, initially assumed to be purely excitatory, could have also been contaminated by IPSPs. We rarely tested for IPSPs by changing the membrane potential because the first priority was to use the limited recording time (while simultaneous recordings were held) for experiments on LTP (Friedlander et al., 1990). However, clear IPSPs (or EPSP/IPSP sequences) were seen in only 3 out of 1178 paired recordings. Probably the 2 requirements for the disynaptic IPSP, namely (1) a synaptic connection between the impaled CA3 pyramidal cell and a CA1 inhibitory interneuron in that slice and (2) for that connection to discharge the interneuron following a single presynaptic action potential, were both satisfied only rarely.

Need for morphological data on the synaptic connections between CA3 and CA1

The results presented in this report are a first step in developing an understanding of the integrative action of CA1 pyramidal neurons on the monosynaptic input from CA3 neurons. These results now need to be supplemented by a detailed morphological study of the patterns of connectivity between CA3 and CA1: The number of CA1 pyramidal neurons contacted by a CA3 neuron; where these CA1 neurons are located; how many synaptic contacts are made on any one CA1 neuron by a single CA3 neuron; and where these contacts are located on the dendrites are all questions awaiting such a study.

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