Transient potentiation of spontaneous EPSPs in rat mossy cells induced by depolarization of a single neurone

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1. The amplitude and frequency of spontaneously occurring EPSPs recorded intracellularly in rat mossy cells was estimated by measuring membrane potential variance in short segments of a continuous voltage record. Changes in variance reflected changes in the amplitude and/or the frequency of spontaneous EPSPs.

2. Short trains of depolarizing current pulses evoked a delayed increase in membrane potential variance in 55% of trials. Variance increased by 487% during these responses and remained elevated for 124 ± 16 s. Increases in variance were not associated with large changes in the intrinsic properties of the mossy cell such as resting membrane potential and input resistance. We termed this phenomenon depolarization-related potentiation (DRP).

3. Epochs of elevated variance were associated with an increase in both the average amplitude and frequency of spontaneous EPSPs. During the peak of the response, the mean interval between spontaneous EPSPs decreased by 36·8%. Computer-generated voltage records with randomly distributed EPSP amplitudes and inter-EPSP intervals suggested that this decrease in inter-EPSP intervals was not sufficient to account for the magnitude of the variance increase observed. Based on this model, we estimated that a 90% increase in the average amplitude of spontaneous EPSPs, in addition to the experimentally measured decrease in the average inter-EPSP interval, was required to reproduce the magnitude of the change in variance observed. In the potentiated state, the amplitude of spontaneous EPSPs often exceeded 10 mV.

4. We also observed epochs of increased variance that occurred spontaneously. These spontaneous epochs closely resembled epochs evoked by depolarizing stimuli, suggesting that the stimulus was acting as a trigger for a spontaneously occurring behaviour. Additional evidence supporting this hypothesis was provided by the observation that stereotyped patterns of increased variance could be evoked by brief stimuli, such as a single 5 s depolarizing step. Dual intracellular recordings from two mossy cells demonstrated that spontaneous epochs of increased variance occurred independently in different neurones. This result makes it unlikely that these variance increases were due to a global change in the slice environment such as a propagating wave of potassium ions.

5. Bath application of the Na^+ channel blocker TTX eliminated most, but not all, of the normal on-going spontaneous EPSPs in mossy cells. Treatment with depolarizing current pulses was effective in potentiating TTX-resistant spontaneous EPSPs in three of seven trials. Potentiation also decreased the mean interval between TTX-resistant miniature EPSPs (by an average of 66·9%) in two trials examined.

6. These results suggest that DRP results from the activation of an intrinsic phenomenon within the dentate gyrus by strong depolarization of a single mossy cell. Our data suggest that several mechanisms are involved in the expression of DRP since changes in EPSP amplitude and frequency can occur with varying delays from the stimulus. The ability of depolarizing current pulses to potentiate TTX-resistant miniature EPSPs suggests that at least one component of this plasticity occurs at the granule cell–mossy cell synapse.

The mossy cell is the major cell type in the rat dentate hilus and is distinct from both CA3 pyramidal cells and dentate granule cells (Scharfman & Schwartzkroin, 1988; Buckmaster, Strowbridge & Schwartzkroin, 1993). These excitatory, multipolar neurones are characterized by large somata and extensive dendritic ramifications throughout the dentate hilus (Ribak, Seress & Amaral, 1985; Scharfman & Schwartzkroin, 1988; Frotscher, Seress, Schwerdtfeger & Buhl, 1991; Buckmaster, Strowbridge, Kunkel, Schmiege & Schwartzkroin, 1992; Buckmaster *et al.* 1993) where they receive massive excitatory input from granule cells axons *en route* to the CA3 subregion of the hippocampus. Synapses formed by granule cell axons can be identified morphologically and are distributed along the proximal regions of primary dendrites (Frotscher, Misgeld & Nitsch, 1981; Ribak *et al.* 1985; Claiborne, Xiang & Brown, 1993). Studies of monosynaptically coupled granule cell–mossy cell pairs (Scharfman, Kunkel & Schwartzkroin, 1990) have suggested that spontaneous spiking activity in granule cell axons is responsible for the high frequency of large-amplitude spontaneous EPSPs that are routinely recorded in mossy cells *in vitro* (Scharfman & Schwartzkroin, 1988; Strowbridge, Buckmaster & Schwartzkroin, 1992; Buckmaster *et al.* 1993) and *in vivo* (Soltesz & Deschênes, 1992; Buckmaster & Schwartzkroin, 1995). However, it is not clear whether these spontaneous EPSPs are associated with action potentials in granule cell somata. Nearly all spontaneous EPSPs are blocked by the non-NMDA receptor antagonist CNQX (Scharfman, 1992; Strowbridge & Schwartzkroin, 1992).

We recently discovered an unusual form of plasticity involving these spontaneous EPSPs. Short trains of depolarizing current pulses injected into a mossy cell induced a dramatic, transient potentiation of the spontaneous synaptic potentials recorded in that neurone (Strowbridge *et al.* 1992). The mechanisms underlying this plasticity are not understood, although overt changes in the intrinsic properties (e.g. input resistance and resting membrane potential) were ruled out. More recently, Kullman and colleagues (Kullman, Perkel, Manabe & Nicoll, 1992) have reported that a similar transient potentiation of evoked synaptic responses can be observed following injection of a train of depolarizing pulses into a single hippocampal CA1 pyramidal cell. This form of potentiation decayed within 30 min and resembled the short-term potentiation (STP) observed following focal application of NMDA to CA1 pyramidal cells (Collingridge, Kehl & McLennan, 1983; Kauer, Malenka & Nicoll, 1988).

In the present report we apply a novel method – examining changes in membrane potential variance over time – to quantify depolarization-related potentiation (DRP) and estimate the time course of potentiation of spontaneously occurring synaptic potentials in mossy cells. We have found that depolarization of a mossy cell can trigger a stereotyped pattern of changes in variance that corresponds to a 1–3 min epoch of potentiated EPSPs. This study addresses three major questions concerning DRP. (1) What is the time course of potentiation? (2) Is DRP due to a direct effect of the depolarizing stimuli or does the stimulus act as trigger for an intrinsic response at the granule cell–mossy cell synapse? (3) Is the expression of DRP due solely to postsynaptic modifications?

Some of these results have been presented previously in abstract form (Strowbridge & Schwartzkroin, 1992).

METHODS

Brain slice preparation

Adult female Sprague–Dawley rats were killed by decapitation. Horizontal brain slices (400 μm thick) containing transverse sections through the ventral hippocampus were prepared using a refrigerated vibrotome. Slices were maintained in a standard interface chamber at 35 °C and superfused with oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF) at 0·5–1·0 ml min^{-1}. The composition of the ACSF was (mM): 124 NaCl, 5 KCl, 1·25 NaH_2PO_4, 1·3 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$, and 10 dextrose. Intracellular recordings in the dentate hilus were made through 45–80 MΩ sharp microelectrodes filled with 4 M potassium acetate (pH 7·4). An Axoclamp-2A amplifier (Axon Instruments) was used in bridge mode to record membrane potentials and inject current. Membrane potential and the current monitor were low-pass filtered at 10 kHz and continuously digitized at 1 kHz per channel by a microcomputer using the Axotape software (Axon Instruments). Action potentials presented in figures may be clipped due to the slow digitization rate; all action potentials (full and truncated) were automatically removed by digital filtering before analysing changes in variance (see below).

Identification of mossy cells

Mossy cells were readily distinguished from CA3 pyramidal cells and hilar interneurones on the basis of action potential width (> 2 ms), small-amplitude or absent spike- and burst-related after-hyperpolarizations, minimal spike frequency adaptation, and the large-amplitude spontaneous EPSPs (Buckmaster *et al.* 1993). In previous studies from this laboratory (Buckmaster *et al.* 1992, 1993; Strowbridge *et al.* 1992), these electrophysiological characteristics have proved to be reliable identifiers of histologically confirmed mossy cells.

Variance analysis

Potentiation typically was induced by injecting a train of depolarizing current pulses (0·5–1·5 nA, 300 ms duration, 50% duty cycle) through the recording electrode. Epochs of potentiated EPSPs were often observed following 30–90 s of treatment with these pulses. Variation in the on-going spontaneous synaptic activity was detected by analysing membrane potential variance in 1 s blocks of the digitized voltage record. Data blocks that contained current steps were ignored for this analysis. Each data block was processed with a moving median filter to remove action potentials. In this process, each data point was replaced with the median value of it and its neighbours (using sets of 3 or 5 data points). While this digital filter removed most spike-related data points, noise in the basal variance measurements often could be decreased further by eliminating the few outlier (> 3–4 times the local S.D.) data points that remained. Visual inspection of the voltage record before and after filtering confirmed that EPSP shapes were not distorted by this protocol.

After filtering, the mean membrane potential was calculated for each 1 s block. Variance (σ^2) was determined by summing the square of the deviation of the membrane potential from this local mean. This sum was normalized to produce one average variance estimate for each 1 s block. In several figures, variance plots were smoothed by a moving average filter (width, 3 or 5 s). Since nearly all spontaneous potentials are eliminated by the non-NMDA receptor antagonist CNQX (Strowbridge & Schwartzkroin, 1992; Scharfman, 1992), and alteration of holding currents consistently failed to reveal spontaneous IPSPs, we assume that the fluctuations in membrane potential represent an index to the amplitude and frequency of spontaneously occurring EPSPs. We estimated the time course of potentiation by quantifying the duration of the epoch and the slopes of the rising and falling phases of the variance response. The rate of change of variance was estimated using linear regression.

Factors affecting variance measurements

Several factors influence the relationship between variance estimates and EPSP potentiation. Action potentials, evoked by either current pulses or large EPSPs, can affect variance measurements. We attempted to limit the number of suprathreshold EPSPs by holding the membrane at relatively hyperpolarized potentials (generally −80 to −75 mV), which also increased the amplitude of spontaneous EPSPs. Even at these potentials, 1 s windows occasionally contained several action potentials. A single pass with a moving median filter often completely eliminated the spike-related data points without distorting the underlying EPSP. The effectiveness of variance measurements in representing EPSP activity also depends on choosing the appropriate window duration. In particular, a window that is too narrow and only includes a few EPSPs will be dominated by the variability of individual EPSPs and not longer-term variation in the average amplitude and frequency of spontaneous EPSPs. The typical rate of spontaneous EPSPs in resting mossy cells was approximately 15 s^{-1} (inter-EPSP interval, 65·0 ± 13·7 ms; mean ± S.D.). Thus the 1 s windows usually sampled between 10·8 and 26·6 spontaneous EPSPs (2 times S.D.) in resting mossy cells and more when the neurone was potentiated.

Resting membrane potential was determined for each block to rule out the possibility that EPSPs were potentiated because of changes in the intrinsic properties of the cell. Frequent overlapping EPSPs precluded direct measurement of the resting potential. Instead, membrane potential was estimated by the most hyperpolarized voltage reached during each 1 s data block. Averages of these resting membrane potential estimates from multiple 1 s blocks were compared to determine if the EPSP potentiation was associated with a change in membrane potential.

EPSP frequency analysis

While the high frequency of overlapping EPSPs greatly complicated the measurement of the amplitudes of individual EPSPs (which was not attempted in this study), we were able to measure the time interval between EPSPs accurately. A custom-written computer program displayed sections of the data file with several moveable cursors that could be used to mark the time of onset of each EPSP. Due to the limited signal-to-noise ratio of the sharp microelectrode recordings, only EPSPs with amplitudes greater than 1 mV (0·75 mV for EPSPs recorded in ACSF containing TTX) were identified for this analysis. Since the entire experiment was recorded as one continuous data file, EPSP timing marks could be converted into a list of inter-EPSP intervals to allow analysis of the distribution of inter-EPSP intervals. In most experiments, the mean interval between EPSPs was compared at rest and in the 10–30 s period in which the variance increase was maximal. A minimum of 100 consecutive intervals were measured in each state (basal and potentiated).

Model of overlapping EPSPs

The effects of varying EPSP amplitude and frequency on membrane potential variance were tested using a simple computer model. Four 50 ms traces, each containing one prominent EPSP, were extracted from one mossy cell in the resting (non-potentiated) state. A computer model was created to determine the membrane potential variance associated with long periods of overlapping EPSPs in which the statistical properties of the constituent EPSPs (amplitude and frequency) were defined. An event list was created that listed the timing of each simulated EPSP in a long data block (typically 20–25 s) with a time resolution of 1 data point ms^{-1}, as well as the peak amplitude and the EPSP template. The inter-EPSP intervals and peak amplitudes were chosen randomly from a Gaussian distribution defined by a value for σ of 0·25 times the mean of the distribution. Each event was associated with one of the four normalized EPSP templates at random. A simulated voltage record was then created by superimposing each EPSP template in the blank data record using computer-generated inter-EPSP intervals and amplitudes. Overlapping EPSPs summated linearly and did not trigger action potentials. Finally, variance was calculated for 1 s windows swept along the simulated voltage record using the same method described above. Data are presented from an average of twenty-five 1 s variance measurements for each distribution of EPSP amplitudes and inter-EPSP intervals. The model was run on an IBM PC clone (Zeos Corp).

Drugs and statistics

All drugs were obtained from Sigma. Student's t test was used to test for statistically significant differences ($P < 0·05$ was used for threshold for significance). Data are expressed as means ± S.E.M, except where indicated.

RESULTS

Analysis of simulated spontaneous EPSPs

Before analysing the experimental results, we characterized the sensitivity of the membrane potential variance measure to alterations in EPSP properties (e.g. changes in amplitude and frequency). Within the estimated physiological range of EPSP amplitudes and inter-EPSP frequencies (mean amplitudes from 2 to 20 mV, mean intervals from 25 to 100 ms; obtained from the results described below), variance ranged from a minimum of 0·38 mV^2 (in simulations in which the mean EPSP amplitude was 2 mV and the mean interval was 100 ms) to a maximum of 63·3 mV^2 (mean amplitude, 20 mV; mean interval, 25 ms). The dependence of variance on EPSP amplitude was well fitted by the second-power relation: $\sigma^2 = a(\text{mean amplitude})^2$, as shown in Fig. 1*A*. The relationship between variance and the mean EPSP interval was more complex. As shown in Fig. 1*B*, this relationship was linear for inter-EPSP intervals greater than 40 ms. As the mean interval becomes smaller than 40 ms, EPSPs begin to summate more frequently and the variance increased rapidly. It is apparent from the graphs in Fig. 1*A* and *B* that membrane potential variance is far

more sensitive to changes in EPSP amplitude than to changes in the frequency of spontaneous EPSPs throughout most of the physiological range of these parameters (except for data containing very short EPSP intervals). For example, increasing the mean EPSP amplitude by 50% (from a basal state of 5 mV mean amplitude and 70 ms mean inter-EPSP interval) resulted in a large (132%) increase in variance. In contrast, decreasing the mean inter-EPSP interval by 50% from the same initial conditions resulted in only a 31% increase in variance. The effect of changing EPSP shapes was relatively small. We typically observed a 10–20% change in variance when simulations were repeated using a single template instead of a random mixture of four templates. We also tested the procedure used to estimate resting membrane potential, measuring the most hyperpolarized potential reached during each 1 s window. Within the physiological range of EPSP amplitudes and intervals described above, the resting membrane potential estimated by this procedure always was within 2 mV of the actual resting potential.

Intracellular depolarization triggers potentiation of spontaneous EPSPs

The effect of injecting trains of depolarizing current pulses on normal spontaneous excitatory postsynaptic potentials (EPSPs) was tested 43 times in eighteen mossy cells. The amplitude (0·99 ± 0·01 nA) and width (300 ms) of the current pulses were similar to those used in our previous study (Strowbridge *et al.* 1992). In the present study, the duration of the pulse train was reduced from the durations used in that study (10 min) to 84·8 ± 13 s (range, 25–225 s; 50% duty cycle) to permit analysis of the onset of potentiation. Potentiation of spontaneous EPSPs was estimated by analysing the change in variance in 1 s windows swept along a continuous digital record of the experiment (see Methods). Three trials were rejected because of unstable baseline variances. In 55% of the remaining trials (22 of 40), treatment with depolarizing current pulses resulted in dramatic, but transient elevation of variance to a mean of 5·9 times basal levels (see Table 1). The other eighteen trials resulted in no significant change in variance. No trials resulted in a decrease in variance.

In successful trials we often found that the change in variance followed a stereotyped time course, illustrated in Fig. 2, consisting of a delay following the offset of the pulse train (17·2 ± 4·0 s; see Table 1), a rapid increase in variance (to 32·7 ± 6·1 mV^2, representing a 487 ± 110% increase in variance) followed by a slower decay back to resting variance levels. The rate of change in variance during the rising and falling phases was constant in six responses

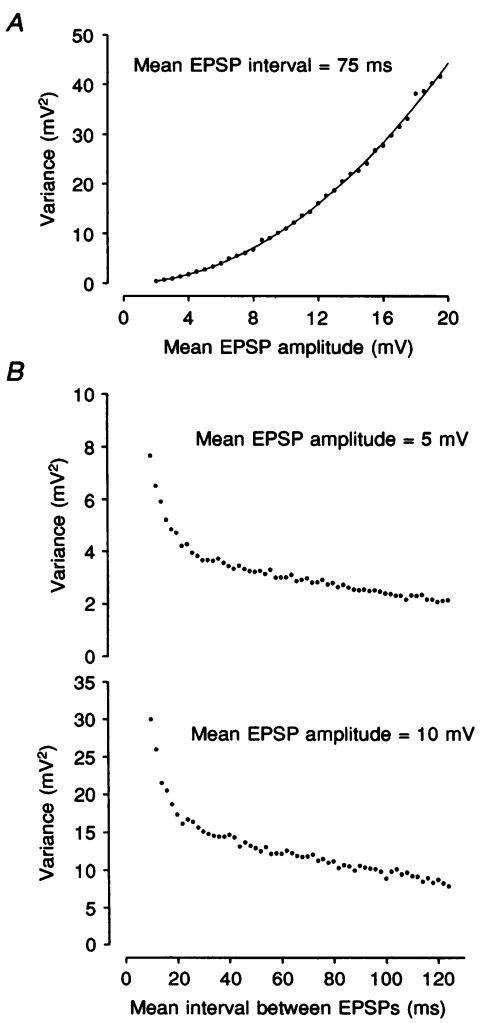

Figure 1. Computer simulation of variance measurements of overlapping mossy cell EPSPs

A, relationship between the variance and the mean EPSP amplitude for simulated random sequences of EPSPs. Each point represents the average of 25 s of simulated data. See text for details. Data were fitted with a second-power equation ($\sigma^2 = a(\text{mean amplitude})^2$; continuous line). *B*, plots of relationship between variance and EPSP frequency (expressed as mean interval between successive EPSPs) for two distributions of EPSP amplitudes (mean amplitude indicated above graph). There was a linear relation between variance and EPSP frequency for frequencies greater than 25 Hz (mean interval, >40 ms).

Table 1. Characteristics of epochs of depolarization-related potentiation

Event	n	Current injected (nA)	Time of pulses (s)	Onset latency (s)	Response duration (s)	Basal variance (mV^2)	Peak variance (mV^2)	Increase in variance (%)
Intracellular pulses								
Successes	22	1·0 ± 0·2	91 ± 15	17·2 ± 4·0	124 ± 16	8·7 ± 2·1	32·7 ± 6·1	487 ± 110
Failures	18	0·9 ± 0·1	75 ± 24	—	—	15·2 ± 5·3	—	—
Spontaneous epochs	7	—	—	—	92·5 ± 9·2	4·2 ± 0·9	29·5 ± 14·0	688 ± 350
Intracellular pulses in TTX								
Successes	3	2·2 ± 0·6	40 ± 0·06	17·5	56·7 ± 32·0	0·12 ± 0·06	0·87 ± 0·4	711 ± 310
Failures	4	1·8 ± 0·2	35 ± 0·05	—	—	0·08 ± 0·04	—	—

Results are presented from five groups of data: (1) trials of depolarizing current pulses that resulted in increases in variance, (2) similar trials that did not result in an increase in variance, (3) spontaneously occurring epochs of increased variance, (4) trials of depolarizing current pulses in the presence of 1 μM TTX which resulted in an increase in variance, and (5) similar trials in TTX which did not result in an increase in variance. There were no statistically significant differences in stimulus properties between either 'Success' or 'Failure' groups. 'Time of pulses' refers to the duration of the train of current pulses; the duration of each pulse was 300 ms. The onset latency was measured only in groups in which the change in variance occurred following a delay from the offset of the current pulse train (approximately 50% of trials). Values listed are means ± S.E.M.

analysed (rising phase, 0·33 ± 0·08 mV^2 s^{-1}; falling phase, 0·21 ± 0·04 mV^2 s^{-1}; falling phase significantly different from rising phase, $P < 0·05$; $n = 5$ cells). In direct comparisons, the rate of change in the rising phase was always faster than during the decay phase of the response (mean ratio, 2·32 ± 0·57; $n = 6$). The average duration of the DRP epoch was 124 ± 16 s. These parameters, which describe the time course of DRP epochs, were similar from cell to cell and trial to trial in spite of a wide range of stimulus train durations (30–180 s), consistent with the hypothesis that the depolarizing stimulus acts as a trigger for an intrinsic behaviour. It was not possible to measure the duration of variance increase in trials in which the potentiation began during the delivery of current pulses (50% of successful trials).

We examined the relationship between the stimulus strength and the properties of the DRP epoch. Stereotyped epochs of potentiated EPSPs were evoked by pulse train durations ranging from 25 to 225 s. Stimulus durations of successful trials were arbitrarily grouped into two categories: short trains (25–60 s; mean, 42 s; $n = 12$) and long trains (130–225 s; mean, 166 s; $n = 8$). Two trials were not digitized continuously and were excluded from this analysis. No significant differences were observed between the increase in variance in the two groups (short trains, 414%; long trains, 587%) or in the duration of variance increase (short trains, 104 s; long trains, 120 s). These results suggest that the properties of the depolarizing stimulus do not have a large effect on the magnitude or time course of the resulting variance increase. However, we did note a tendency for long trains to evoke larger, longer increases in variance, raising the possibility of some degree of stimulus–response coupling.

We next examined whether DRP epochs were associated with a change in the resting membrane potential. Since these experiments were performed with a moderate hyperpolarizing holding current (typically −0·5 nA, used to enhance EPSP amplitudes and prevent spiking), hyperpolarization of the membrane potential might indicate an increase in the input resistance of the neurone. Such an increase in input resistance could provide an explanation for the apparent potentiation of synaptic potentials. We measured the average resting membrane potential (see Methods) in 20 s periods when the DRP-related variance increase was maximal and when variance levels had returned to basal levels in eight successful trials. We observed an average hyperpolarization of 2·1 mV during the peak response, compared with the recovery period. This estimate of DRP-related change in resting membrane potential was not large enough to account for a significant component of the variance increase and confirms the results of our previous DRP study (Strowbridge *et al.* 1992).

Responses to repeated trials

The pattern of variance changes was reproducible in recordings from the same neurone. Figure 3*A* illustrates three trials recorded from the same neurone shown in Fig. 2; the peak variance and duration of the response were similar in all three trials (each treatment was a 30–45 s pulse train); current pulse amplitude ranged from 0·6 nA (Trial 1) to 0·9 nA (Trial 5). Even more striking were the similar rates of change in variance during the rising phase of the first two trials: 0·39 and 0·40 mV^2 s^{-1}, respectively. Note that the onset latency decreased for each trial, so that the potentiation was initiated during the train of current pulses in the last trial. Trials 3 and 4 failed to produce potentiation (not shown). The stimulus parameters of these

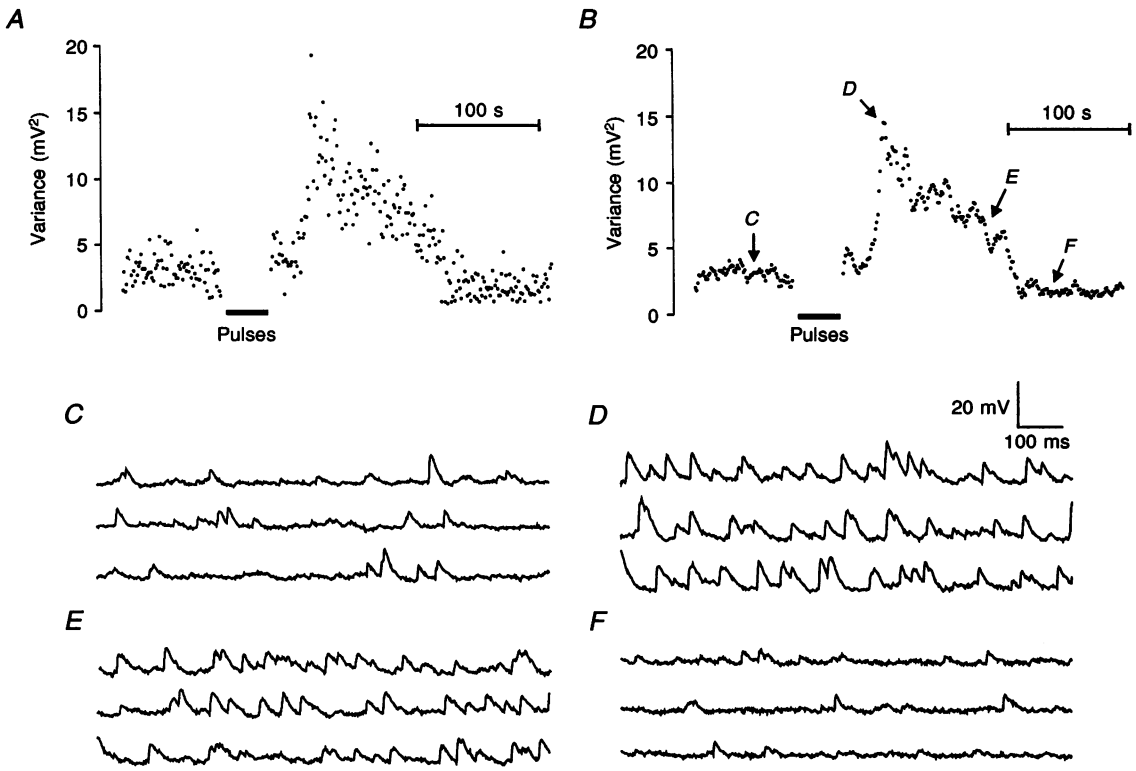

Figure 2. Time course of depolarization-related potentiation

Plots of variance measured in 1 s windows of a continuous record of the intracellular voltage *versus* time. After a baseline period, a 30 s train of depolarizing current pulses (300 ms on, 400 ms off, 1–2 nA) was injected. The bar under the variance plot indicates the period of the pulse train. After a slight delay following the offset of the pulse train, variance increased dramatically and then returned to baseline levels within approximately 150 s. The plot in *B* shows the same variance data after smoothing with a 3-point moving average filter. The points labelled *C–F* correspond to periods in which raw 1 s blocks are presented in the lower panels. *C*, before treatment; *D* and *E*, during the period of potentiated EPSPs; *F*, following return to baseline variances.

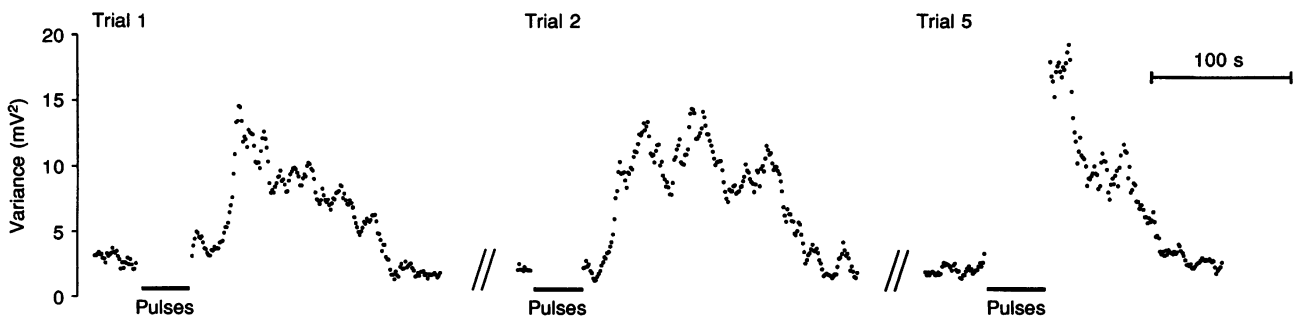

Figure 3. Stereotyped time course of EPSP potentiation

Smoothed variance plots from the mossy cell in Fig. 2, showing three periods of DRP. Separate depolarizing trains (30–45 s in duration) evoked similar elevations in variance (Trial 1 is the same trial presented in Fig. 2); Trials 3 and 4 in this cell did not trigger an increase in variance (not shown). Note that latency between the offset of the current pulses and the onset of the change in variance decreased with each successful trial.

unsuccessful trials were similar to those used in successful trials (duration, 30 s; pulse amplitude, 0·7 or 0·9 nA).

The explanation for failures was not apparent in our data. As shown in Table 1, there was little difference in the amplitude of the current pulses or in the duration of the pulse train between trials that resulted in successful potentiation and trials that failed. One possible explanation for failure of potentiation was that the neurone was partially potentiated before the test and therefore closer to its maximal variance level. We noted a trend for initial variance to be higher in failures (Table 1), but this difference was not statistically significant. There was also a moderate correlation ($R = 0{\cdot}40$) between the initial variance and the percentage increase in variance in the successful trials.

While the onset and decay of DRP epochs were relatively stereotyped, there was considerable variability in the plateau phase and some epochs appeared multiphasic. For example, the DRP epoch illustrated in Fig. 4 shows an initial period of potentiated EPSPs but no change in EPSP frequency (1·1% decrease in mean interval between spontaneous EPSPs). The very high variance recorded in this period (>50 mV^2) was due largely to the presence of giant (>20 mV) EPSPs which recurred at approximately 6 Hz. Even with hyperpolarizing holding current applied, these giant EPSPs often triggered action potentials, as shown in Fig. 4*C*. After approximately 20 s, the frequency of spontaneous EPSPs increased (36·4% decrease in mean interval between EPSPs) while the average EPSP amplitude and the membrane potential variance decreased. This second phase, which resembled the potentiation observed in Fig. 2, lasted about 50 s before the variance returned to basal levels. Other neurones often showed smaller variations or oscillations in the plateau component of the DRP epoch (see Figs 3 and 6). The appearance of multiple phases in the DRP variance plot was generally associated with different delays in the potentiation of EPSP amplitudes and the increase in EPSP frequency.

Spontaneous epochs of potentiated EPSPs

The variable onset latencies and characteristic time course of DRP epochs suggests that potentiation is not a direct consequence of the depolarizing stimulus itself, but rather an intrinsic behaviour of the dentate region which can be triggered by depolarization. A critical prediction of this hypothesis is that spontaneous epochs, if they occur, should have the same characteristics as evoked epochs. We

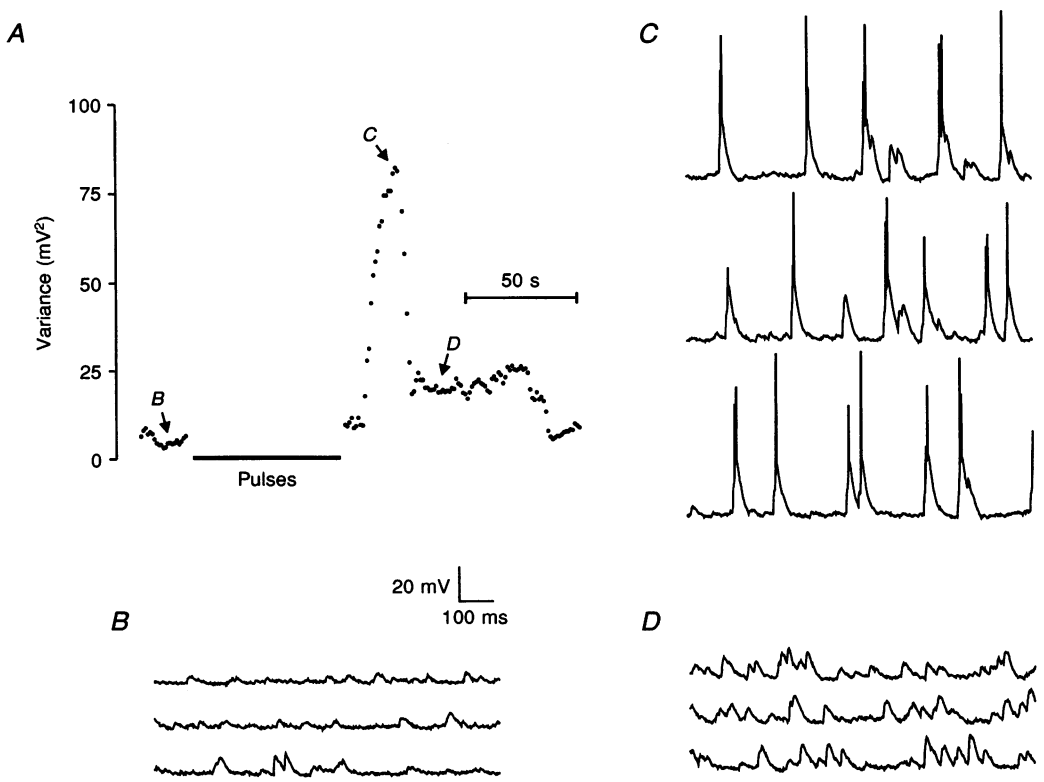

Figure 4. Two-phase time course of EPSP potentiation evoked by current pulses

A, variance increase evoked by a 45 s train of current pulses. Action potentials were removed digitally before calculating variance (see Methods); plot of variance *versus* time was smoothed with a 5-point moving average filter. *B–D*, representative traces extracted at the times indicated by arrows in *A*. The large initial increase in variance (*C*) was associated with extremely large spontaneous EPSPs, which often triggered 2 or 3 action potentials. The second phase of the response (*D*) was characterized by a moderate amplitude potentiation and an increased frequency of EPSPs.

observed seven spontaneous increases in variance in our population of mossy cells. As shown in Table 1, the duration of these epochs and the peak variance reached during the epoch were very similar to epochs of increased variance evoked by depolarizing stimuli (Fig. 5). Inspection of individual sweeps obtained during these potentiated periods (Fig. 5*B* and *C*) showed obvious potentiation of EPSP amplitude, much like that presented in Fig. 2. We also measured the rate of variance change during the rising and falling phases of five spontaneous epochs. These rates of change (rising phase, $0{\cdot}39 \pm 0{\cdot}10$ mV^2 s^{-1}; falling phase, $0{\cdot}20 \pm 0{\cdot}05$ mV^2 s^{-1}) were very similar to rates measured in evoked epochs (see above). The ratio of the rising and falling rates ($2{\cdot}40 \pm 0{\cdot}3$; $n = 4$) was also very similar to the ratio measured in epochs evoked by current pulses. In several neurones it was possible to compare the characteristics of spontaneous and evoked DRP epochs directly. We found both the time course and the variance changes to be similar in the two types of potentiation. This correspondence was particularly pronounced in one neurone in which depolarizing pulses evoked epochs with atypical, multiphasic time courses (Fig. 5*D*) similar to that seen during spontaneous DRP epochs in the same cell. We noted that most spontaneous epochs occurred during periods of relatively low variance (mean, 4·2 mV^2). This finding is consistent with the hypothesis that the probability of a DRP epoch occurring is inversely related to the basal variance.

Stereotyped epochs of potentiated EPSPs evoked by other stimuli

Epochs of potentiated EPSPs could be elicited by depolarizing stimuli other than trains of current pulses. Four epochs of potentiated EPSPs were observed following a single short current step. In the example presented in Fig. 6, the holding current on the mossy cell was released in stages. After several seconds with no holding current, a constant 0·5 nA depolarizing current was injected for five

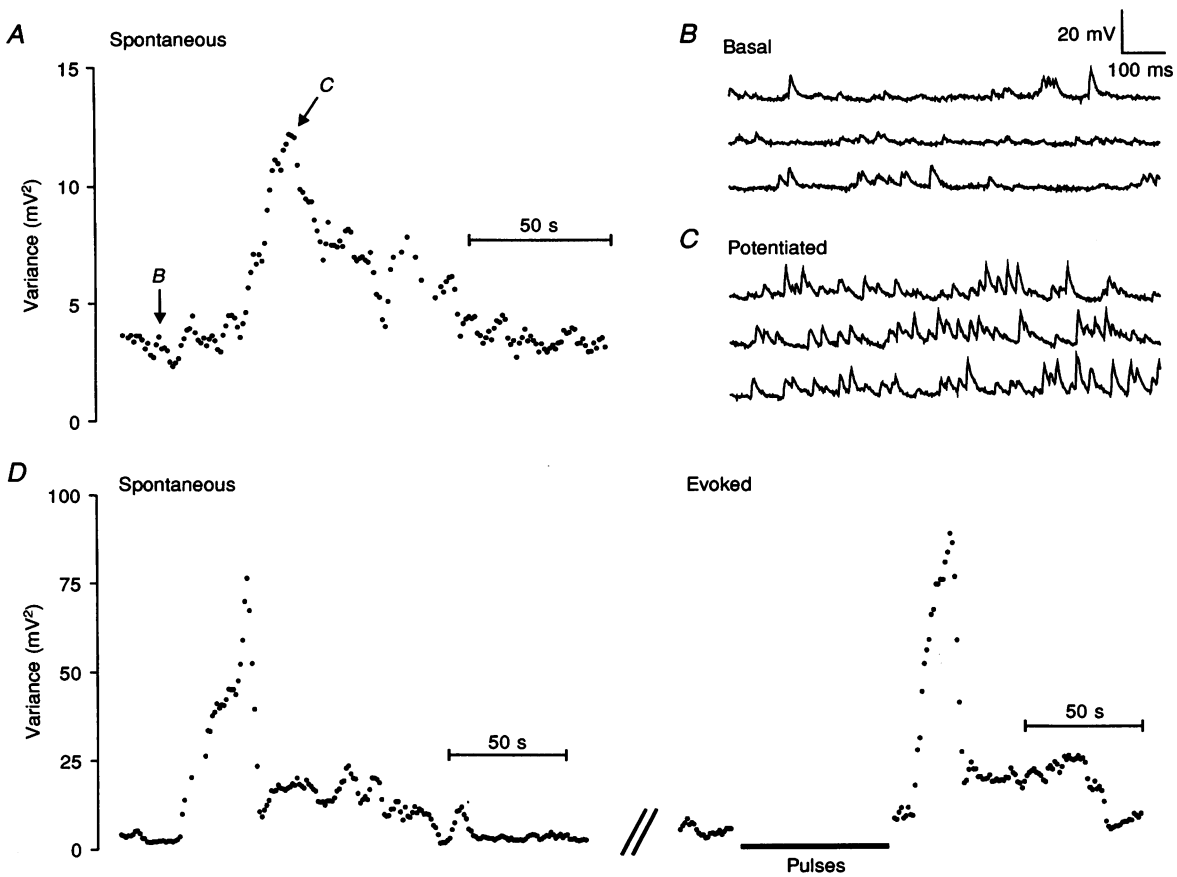

Figure 5. Time course of spontaneous epochs of potentiated EPSPs

A, typical time course of a spontaneously occurring variance increase. The duration of this epoch, the rates of change of variance during the rising and decaying phases of the epoch, and the percentage increase in variance were similar to epochs evoked by depolarizing current pulses. *B* and *C*, sample 1 s voltage traces obtained before the spontaneous epoch (*B*) and when the variance increase was maximal (*C*). *D*, in another mossy cell, both spontaneous and evoked epochs of increased variance followed an unusual, two-phase time course (same mossy cell as in Fig. 4). The initial period of extremely large variance measurements was characterized by giant (> 20 mV amplitude) EPSPs which recurred at approximately 6 Hz in both spontaneous and evoked epochs.

seconds. A large (650%) increase in membrane potential variance was evident immediately after the holding current was restored; variance decayed slowly over 2–3 min (Fig. 6*B–E*). The rapid response to a short stimulus suggests expression of DRP can be much faster than indicated by the average latencies we observed following injection of repetitive current pulses.

The response following a single current step in Fig. 6 also demonstrates a clear deviation between the variance measure – used to track EPSP potentiation – and the increase in frequency of spontaneous EPSPs. A prominent dip is evident in the DRP response in the variance plot in Fig. 6*A* (labelled *'D'*). This decrease coincided with a barrage of very frequent small-amplitude EPSPs in the voltage record (Fig. 6*D*). The variance increased again when the barrage of small-amplitude EPSPs subsided and the EPSP amplitude increased again (Fig. 6*E*). The non-parallel changes in EPSP amplitude and frequency in this neurone suggest that DRP is multifactorial; different mechanisms which regulate changes in the amplitude and frequency of spontaneous EPSPs recorded in mossy cells may contribute to DRP.

In two mossy cells, spontaneous synchronized synaptic input initiated an epoch of potentiated EPSPs that resembled those evoked by external stimuli. This behaviour, illustrated in Fig. 7, was remarkable because it demonstrated that a stimulus intrinsic to the slice – a burst of spontaneous synaptic input to the mossy cell – could trigger DRP. The mossy cell in this example was recorded in a slice which generated spontaneous synchronized discharges originating in the CA3 region of the hippocampus at approximately 0·4 Hz. (These responses were occasionally observed in slices incubated for prolonged periods in a holding chamber.) Synchronized discharges from CA3 evoked bursts in both CA1 pyramidal cells and hilar mossy cells through synaptic connections via Schaffer and hilar axon collaterals, respectively, from CA3 pyramidal cells (Ishuzuka, Weber & Amaral, 1990). In the mossy cell recordings, very high variances (>20 mV^2) were measured in data windows which overlapped with these synchronized synaptic bursts. An apparently spontaneous epoch is indicated by the increase in variance in the 1 s data windows not contaminated by spontaneous bursts in Fig. 7*E* (compare voltage records in *A* and *B*). Upon analysis

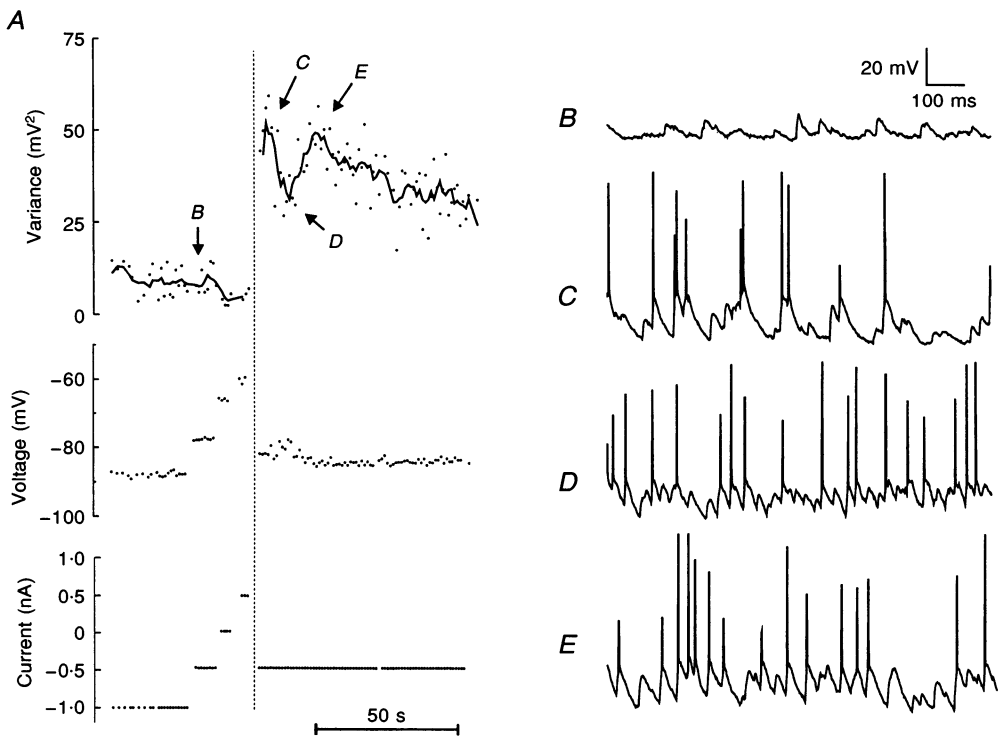

Figure 6. Potentiation of spontaneous EPSPs from a short current step

A, changes in variance, resting membrane potential (voltage), and injected current plotted during a series of current steps. A second plot of the same variance data after processing with a 5-point moving average filter (continuous line) also is shown in the top panel. Baseline variance (*B*) changes little as the cell is depolarized (from a holding level of −1·0 nA to +0·5 nA). However, a dramatic increase in variance is observed following the step back to the normal holding current (−0·5 nA). The variance plot suggests that the voltage step initiates a triphasic response. Inspection of raw data traces showed that the initial peak in variance was largely due to high-amplitude EPSPs (*C*). This period was followed by a sharp decrease in variance corresponding to a barrage of frequent, low-amplitude EPSPs (*D*). Variance again increased (*E*) during a slower barrage of moderately potentiated EPSPs, and then gradually returned towards baseline.

of the transition from the basal to the potentiated state (*C* and *D*), however, it was evident that individual CA3-driven summated EPSPs triggered short (several seconds) runs of potentiated EPSPs. Five consecutive CA3-driven responses in this transition period are shown in Fig. 7*D*. The train of spontaneous EPSPs initiated by the third CA3-driven response continued through the fourth CA3-driven response (2·2 s later). The fifth CA3-driven response initiated a stereotyped DRP epoch that persisted for more than 100 s (shown in *E*). The very short latency between the fifth response and the onset of the DRP epoch suggests that this epoch was triggered by the CA3-driven summated EPSPs and did not occur spontaneously. The DRP epoch observed in this neurone was especially clear because of the very low basal rate of spontaneous EPSPs; no other DRP epochs were observed in this neurone. These data, together with the fast response to single current steps shown in Fig. 6, suggest that the mechanisms underlying DRP can respond rapidly to depolarization evoked by either external or intrinsic stimuli.

Increased frequency of spontaneous EPSPs

Because the variance method used to determine the time course of potentiated EPSPs is biased towards measuring changes in EPSP amplitude rather than changes in the frequency of spontaneous EPSPs (see Fig. 1*C*), we directly measured the frequency of EPSPs before and after inducing DRP. This analysis focused on a subgroup of eight successful trials listed in Table 2. The average increase in variance in this subgroup of DRP trials (470%) was similar to the increase in variance for the entire group of successful DRP trials (487%). The average interval between EPSPs in the basal (non-potentiated) state ranged from 47 to 88 ms (mean, 65·0 ± 4·8 ms; see Table 2). The distribution of these intervals was skewed towards longer intervals in both the basal and potentiated states, as shown in the

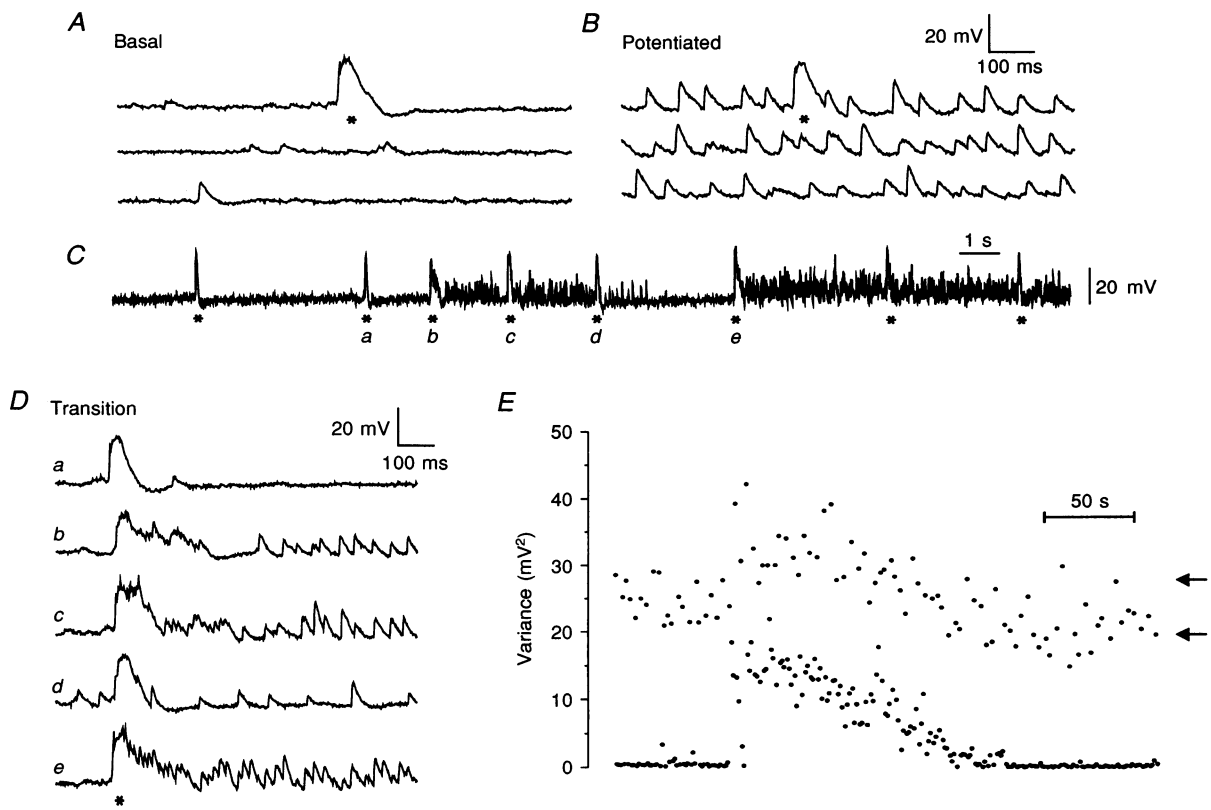

Figure 7. Stereotyped epoch of potentiation triggered by spontaneous synchronized bursts

This mossy cell had a very low rate of spontaneous EPSPs (*A*), but received input from a synchronized population of periodically bursting CA3 pyramidal cells ('summated EPSPs' marked by asterisks in *A–D*). As shown in *E*, baseline variance measurements were typically very small except when the 1 s sampling window contained a summated synaptic EPSP which caused the variance measurement to jump to 20–40 mV^2 (arrows). Synchronous bursts originating in the CA3 region of the hippocampus sometimes triggered short trains of spontaneous EPSPs in this mossy cell. A long segment of voltage record is shown in *C*; faster sweeps showing five spontaneous summated EPSPs that immediately preceded the DRP epoch (labelled *a–e* in *C*) are shown in *D*. While the first four of these EPSP trains decayed quickly, the fifth CA3-driven input initiated a prolonged (~2 min) epoch of potentiated EPSPs (cf. Basal in *A* and Potentiated in *B*). The time course of the variance changes in this epoch resembled the time course of epochs evoked by trains of current pulses. All spontaneous summated EPSPs triggered multiple action potentials which were truncated in this figure.

Table 2. Frequency of spontaneous EPSPs before and after potentiation

Trial	Basal EPSP interval (ms)	n	Potentiated EPSP interval (ms)	n	Decrease in interval (%)	Basal variance (mV^2)	Potentiated variance (mV^2)	Increase in variance (%)	Increase in est. EPSP amplitude* (%)
1	88·2 ± 6·5	105	52·1 ± 3·4	113	40·9	2·52 ± 0·33	10·5 ± 0·46	317	80
2	47·1 ± 2·2	170	39·8 ± 1·9	174	15·5	7·48 ± 0·33	10·4 ± 0·35	39	12
(3 initial)	(71·8 ± 6·5)	(102)	(71·0 ± 4·1)	(125)	(1·1)	(3·30 ± 0·50)	(43·3 ± 4·2)	(991)	(282)
3	71·8 ± 6·5	102	45·7 ± 2·7	150	36·4	3·30 ± 0·50	20·6 ± 1·2	524	160
(4 initial)	(65·7 ± 3·7)	(113)	(64·9 ± 4·2)	(138)	(1·2)	(5·07 ± 0·59)	(49·3 ± 5·3)	(906)	(212)
4	65·7 ± 3·7	113	40·4 ± 1·6	160	38·5	5·07 ± 0·59	20·1 ± 1·1	296	87
5	67·9 ± 5·0	108	41·3 ± 1·9	167	39·2	3·32 ± 0·19	11·4 ± 0·54	243	71
6	73·0 ± 4·8	112	42·7 ± 2·2	150	41·5	1·82 ± 0·14	10·9 ± 0·46	499	121
7	48·0 ± 3·1	113	26·2 ± 1·4	169	45·4	2·04 ± 0·15	14·5 ± 0·91	611	143
8	58·6 ± 4·5	126	36·9 ± 2·2	203	37·0	9·15 ± 0·68	22·9 ± 1·40	150	47
Mean	65·0 ± 4·8		40·6 ± 2·6		36·8	4·34 ± 0·95	15·2 ± 1·8	335	90·1

Analysis of a subgroup of eight successful DRP trials in normal ACSF. Intervals between spontaneous EPSPs greater than 1 mV in amplitude were measured immediately before treatment with depolarizing current pulses (Basal) and when membrane potential variance was maximal (Potentiated). n refers to the number of consecutive intervals measured in each state. * Increases in EPSP amplitudes were estimated using a computer model of EPSP summation to account for the measured increase in variance given the measured decrease in EPSP interval (see text for details). Trials 3 and 4 were multiphasic. The initial phases of both trials were dominated by very-large-amplitude EPSPs (cf. Fig. 4) and were analysed separately. The last line of the table (Mean) represents average values for each measure from this subgroup of successful trials. Values listed are means ± S.E.M.

representative histograms in Fig. 8*A* and *B*. In each of the eight trials examined, the increase in variance with DRP was also associated with a statistically significant decrease in the mean interval between spontaneous EPSPs (mean decrease in inter-EPSP interval, 36·8%; Fig. 8*C*). The mean interval between EPSPs during the peak of the change in variance was 40·6 ± 2·6 ms (range, 26·2–52·1 ms). Histograms of these intervals suggest that the increase in frequency of spontaneous EPSPs occurred in part by eliminating very long inter-EPSP intervals.

To verify that our EPSP analysis protocol was detecting most spontaneous events, we measured the mean interval between spontaneous EPSPs at two holding currents (−0·5 and −0·75 nA) in three non-potentiated mossy cells. If some EPSPs were too small to be detected at the normal holding current (−0·5 nA), further hyperpolarization of the membrane potential would be expected to increase the amplitude of these hidden EPSPs, raising some above the detection threshold, and thus artificially increase the estimate of EPSP frequency. In each comparison, hyperpolarization increased membrane potential variance (by an average of 17·8%) but failed to increase the mean interval between spontaneous EPSPs (mean increase, 1·4%; none of the interval comparisons showed statistically significant differences). These results suggest that most spontaneous EPSPs were detected at the normal holding current. The magnitude of the change in EPSP frequency was not correlated with the magnitude of the variance increase. As illustrated by the trials shown in Figs 4–6, the frequency of spontaneous EPSPs during the potentiated epoch was not always constant and did not always mirror the pattern of the change in variance (cf. Fig. 6).

We estimated the change in mean EPSP amplitude during DRP by combining EPSP frequency and variance measurements. Computer simulations were performed with inter-EPSP interval distributions constructed to have means that match the experimentally determined interval distributions in each neurone. The mean EPSP amplitude in these simulations was varied to determine the amplitude distribution required to match the measured membrane potential variance. In the basal state, these estimated EPSP amplitudes ranged from 4·0 to 8·7 mV (mean, 5·9 ± 0·59 mV), while during the 30 s periods containing potentiated EPSPs, the mean amplitudes ranged from 8·7 to 18·6 mV (mean, 11·8 ± 1·5 mV). As shown in Table 2, the computer simulations estimated a doubling of average EPSP amplitudes (mean increase, 90·3 ± 17%; range, 12–160%) to obtain the measured variance increases, in addition to the increase EPSP frequency.

Can potentiation of spontaneous EPSPs occur independently in different mossy cells?

We next addressed the issue of whether epochs of potentiated EPSPs occur simultaneously in multiple mossy cells. Three pairs of mossy cells (>150 μm separation) were recorded simultaneously and screened for spontaneous increases in variance in one or both neurones. One pair of mossy cells showed short-duration spontaneous epochs of potentiated EPSPs in each neurone. As illustrated in Fig. 9*A*, these epochs always occurred independently in

each neurone. Figure 9*B–D* illustrates individual traces showing spontaneous EPSP activity when variance levels are low for both cells (*B*), when Mossy cell 2 was potentiated (*C*), and when Mossy cell 1 was potentiated (*D*). No stimuli were applied to either neurone during this recording session other than the constant holding current. Note that Mossy cell 2 underwent a second epoch of spontaneously potentiated EPSPs after the peak of spontaneous variance increase in Mossy cell 1. These recordings demonstrate that the mechanisms responsible for these epochs of potentiated EPSPs can be localized to synapses (or synaptic circuits) innervating a subpopulation of mossy cells.

Does postsynaptic depolarization trigger potentiation of miniature EPSPs?

We investigated the origin of the normally occurring and potentiated spontaneous EPSPs by studying the effects of tetrodotoxin (TTX) on basal spontaneous activity and on the ability of depolarizing current pulses to evoke DRP epochs. Bath application of TTX (1 μM) eliminated most, but not all, spontaneous synaptic potentials (Fig. 10) and decreased basal variance levels by an average of 84·8 ± 3·8% (from 5·6 ± 2·2 to 0·54 ± 0·12 mV^2) in five mossy cells tested. In most mossy cells, large-amplitude EPSPs (>10 mV) were still observed in the presence of TTX (Fig. 10*D*), although much less frequently than in normal ACSF. It is unlikely that large-amplitude EPSPs persisted because of incomplete blockade of spike-related EPSPs, since this concentration of TTX completely eliminated fast action potentials evoked by direct current injection (not shown). Prolonged exposure to TTX (>1 h) also failed to block the occurrence of large-amplitude spontaneous EPSPs. These results suggest that TTX-sensitive Na^+ spiking, presumably in granule cell axons, is responsible for most of the normal spontaneous synaptic activity observed in mossy cells, but that there must be some TTX-resistant mechanisms that produce large-amplitude spontaneous EPSPs.

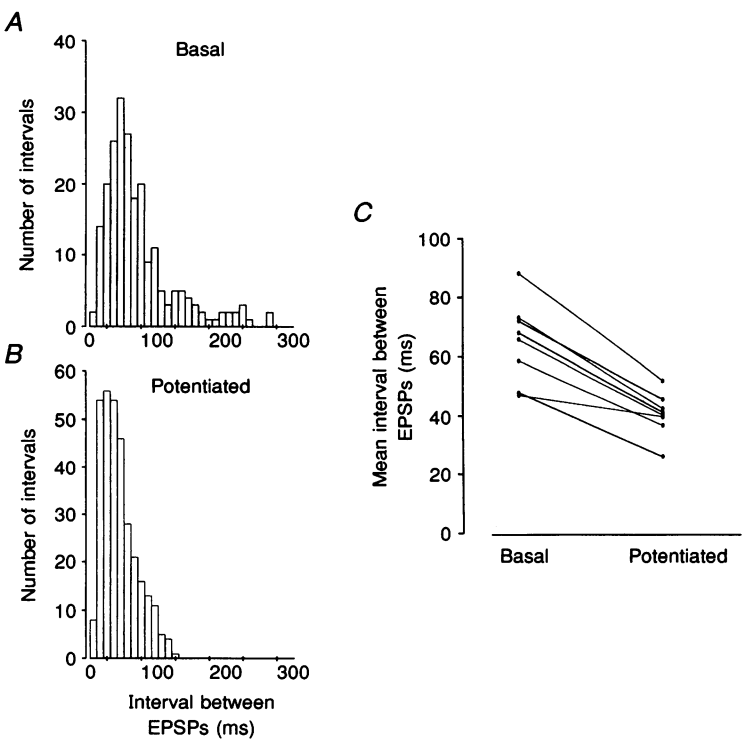

Figure 8. Depolarization-related potentiation is associated with increased frequency of EPSPs

A and *B*, frequency histograms from one mossy cell of intervals between spontaneous EPSPs of amplitude 1 mV or greater during basal activity (*A*) and following treatment with depolarizing current pulses to induce DRP (*B*). Intervals between potentiated EPSPs were obtained during the peak variance increase. Upon potentiation, the mean interval between EPSPs decreased from 70·5 ± 3·4 ms (Basal) to 41·9 ± 1·4 ms (Potentiated). The basal and potentiated histograms (bin width, 10 ms) contained 220 and 317 inter-EPSP intervals, respectively. The potentiated histogram contains data pooled from two successful trials in the same mossy cell (Trials 1 and 2 in Fig. 3). *C*, line graphs indicate the decrease in mean interval between EPSPs during an evoked DRP epoch in 8 successful DRP trials (same subgroup of trials as listed in Table 2). The decrease in mean interval between spontaneous EPSPs in each trial was statistically significant ($P < 0·05$).

We tested the ability of depolarizing current pulses to potentiate miniature EPSPs (mEPSPs) 7 times in mossy cells recorded in ACSF containing 1 μM TTX. Transient increases in membrane potential variance were observed following the pulse train in three of these seven trials – approximately the same proportion of successful potentiation as seen in normal ACSF (see above). Figure 11 illustrates an example of the transient potentiation of mEPSPs that followed a 90 s train of current pulses. There was often a delay between the offset of the current pulses and the onset of the variance increase (2 of 3 successful trials; mean, 17·5 s), as was also observed in many DRP epochs evoked in normal ACSF. However, the duration of epochs of potentiated mEPSPs was briefer than similar epochs observed normally (56·7 s in ACSF containing TTX, compared with 124 s in normal ACSF). Increases in variance ranged from 317 to 1320% in successful trials in TTX (mean, 711 ± 310%).

We assessed the effect of depolarizing current pulses on the frequency of mEPSPs in two trials which resulted in relatively long epochs of elevated variance. Fewer intervals were obtained in these epochs than in the analysis of DRP epochs evoked in normal ACSF described above (average of 61 *versus* 140 intervals). On average, the basal rate of spontaneous miniature EPSPs (greater than 0·75 mV in amplitude) was 2·6 s^{-1} (mean interval, 380 ms; $n = 5$). In two successful trials analysed, potentiation was associated with a dramatic decrease in the mean interval between mEPSPs (by 79·6 and 54·2%; see Fig. 11*E*); both differences were statistically significant ($P < 0·05$). During epochs of increased variance, the average rate of mEPSPs increased to 9·8 s^{-1} (mean interval, 102 ms; $n = 2$).

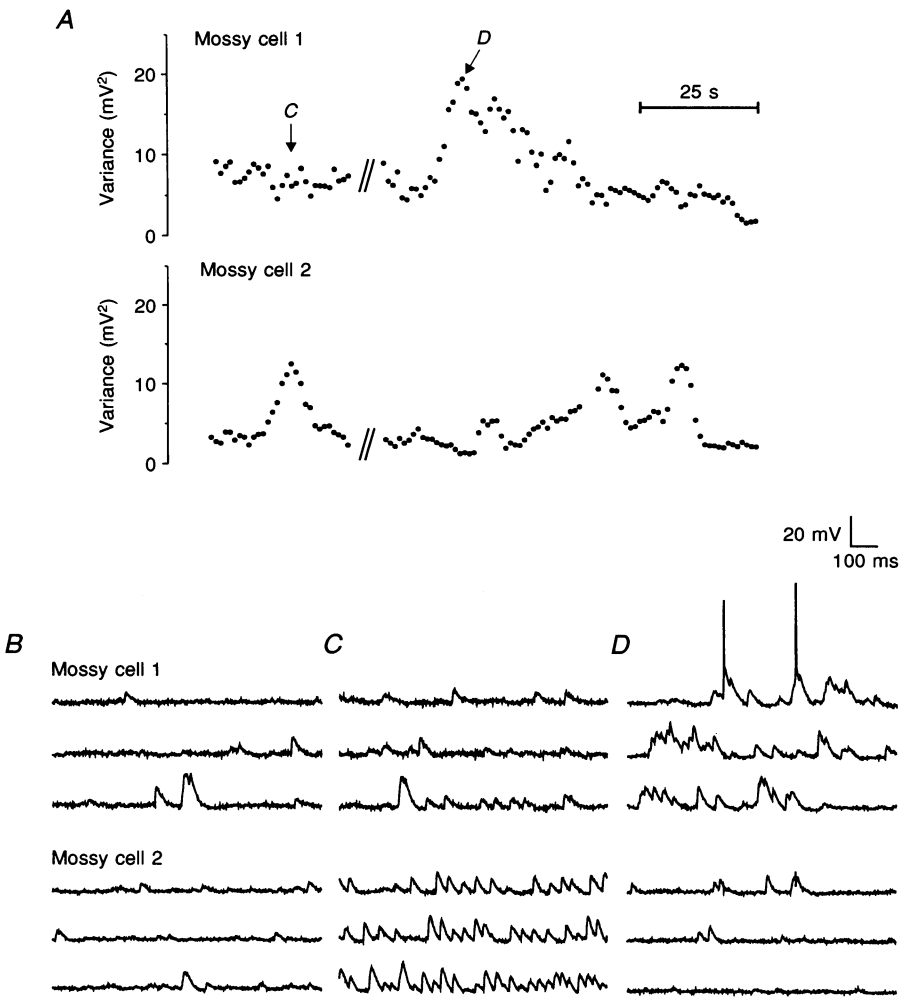

Figure 9. Epochs of potentiated EPSPs occur independently in different neurones

Simultaneous intracellular recording of spontaneous epochs of potentiated EPSPs in two mossy cells. *A*, smoothed variance plots for both recordings show alternating periods of elevated variance in each neurone. Records obtained during the peak of each variance increase for each neurone are shown in panels *C* and *D*. Epochs of potentiated EPSPs in Mossy cell 2 occurred when there was little change in EPSPs recorded in Mossy cell 1 (*C*). Selective potentiation of EPSPs in Mossy cell 1 also were observed (*D*). Records obtained when both neurones had basal EPSP variance levels are shown in *B*.

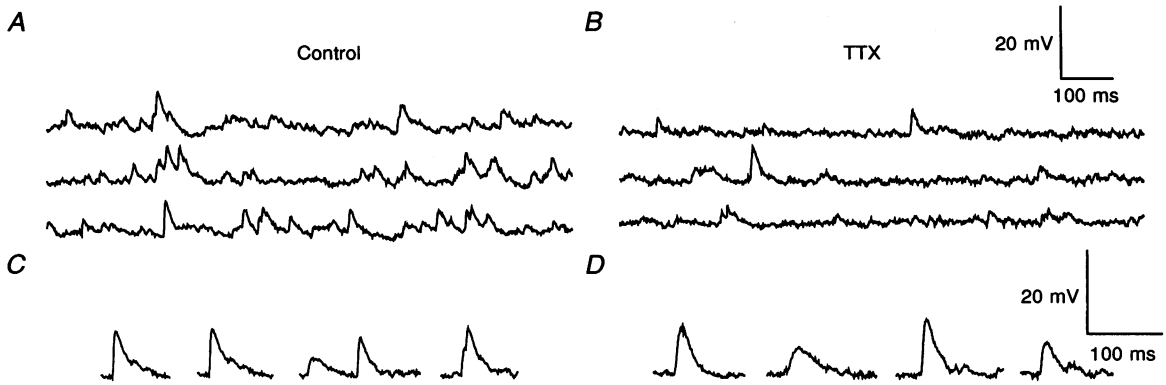

Figure 10. Most spontaneous EPSPs are due to spiking in presynaptic processes

A, consecutive 1 s sweeps showing normally occurring spontaneous EPSPs. After the addition of 1 μM TTX to the ACSF (sufficient to block fast action potentials in mossy cells), most spontaneous synaptic potentials were blocked (*B*). However, smaller-amplitude miniature EPSPs could still be recorded. Occasionally, these TTX-resistant potentials could be as large as 5–10 mV. *C* and *D*, examples of large-amplitude spontaneous EPSPs recorded in one mossy cell before (*C*) and after (*D*) blockade of action potentials with TTX. Note the TTX did not appear to alter the maximal amplitude of spontaneous EPSPs or the range of EPSP shapes observed.

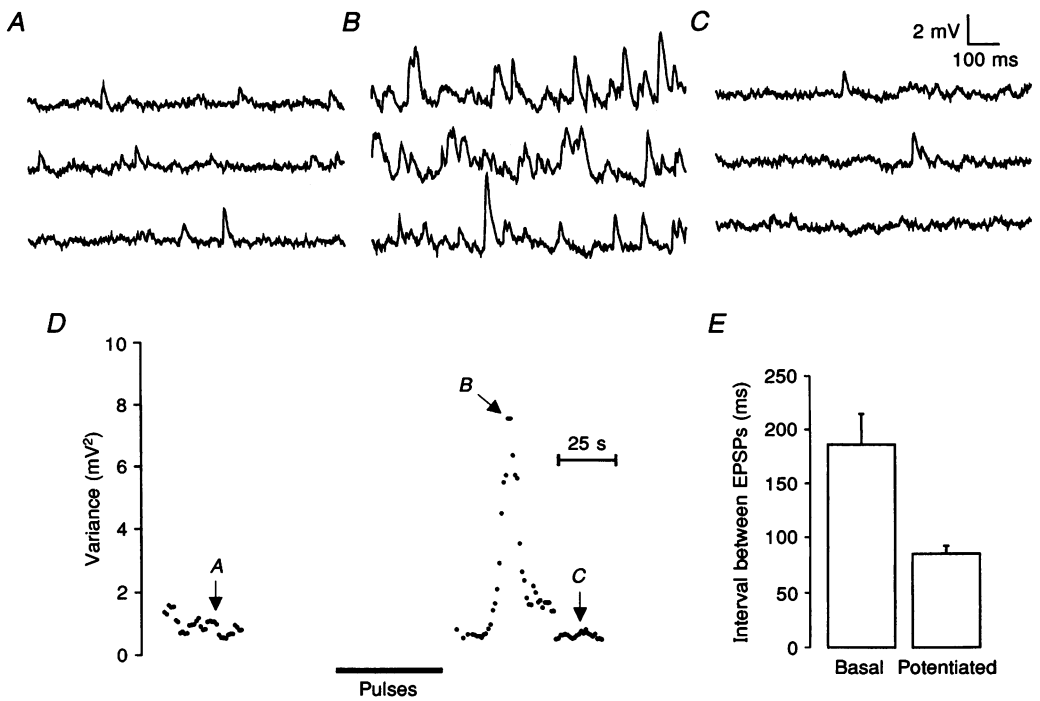

Figure 11. Potentiation of miniature EPSPs by depolarizing current pulses

A–C, intracellular recording from a mossy cell with 1 μM TTX added to the bathing solution to block fast action potentials. Note the increased gain on the raw records on this panel compared with previous figures (calibration bar represents 2 mV instead of 20 mV). Intracellular recordings in this experiment were passed through an AC-coupled ×10 amplifier and low-pass filtered at 1 kHz. TTX-resistant miniature EPSPs could be potentiated briefly by a 45 s train of depolarizing current pulses (300 ms on, 400 ms off, 1–2 nA) injected into the neurone. Sweeps are presented showing examples of EPSP activity immediately before treatment with depolarizing pulses (*A*), when the variance increase was maximal (*B*), and following recovery (*C*). The time course of the variance increase is illustrated in *D*. *E*, this epoch of potentiated EPSPs was associated with a 50·1% decrease in the mean interval between spontaneous miniature EPSPs greater than 0·75 mV in amplitude (from 186 ± 29 to 85·3 ± 6·9 ms).

DISCUSSION

Our results demonstrate that depolarizing current pulses can trigger epochs of potentiated EPSPs which have a stereotyped time course and which are quantitatively similar to spontaneously occurring epochs. Our results also demonstrate that a similar depolarization-related potentiation can be observed in the presence of TTX, implying that a component of the potentiation is due to plasticity at the granule cell–mossy cell synapse. This discussion will focus on the nature of the variance measurements used to quantify the potentiation of spontaneous EPSPs and the possible mechanisms involved in DRP.

Basis of membrane potential variance

Membrane potential variance reflects changes in the amplitude and frequency of spontaneous voltage fluctuations. In mossy cells, these fluctuations are almost exclusively depolarizing and appear to be EPSPs based on their pharmacology and their reversal potentials. The amplitude of spontaneous potentials increased when the membrane was hyperpolarizing and individual spontaneous depolarizations could trigger action potentials when the neurone was depolarized. These observations are consistent with those of Scharfman (1992) who found that these spontaneous depolarizing events were abolished by the non-NMDA receptor antagonist CNQX; Monaghan & Cotman (1985) have demonstrated extremely low levels of NMDA receptor binding in the rat hilus.

It is possible that these large spontaneous depolarizations result from intrinsic (within the mossy cell) regenerative Ca^{2+} spikes which could occur spontaneously or could be triggered by smaller EPSPs. However, there are several lines of evidence that argue against this possibility. First, we often observed very large-amplitude EPSPs that were subthreshold for triggering Na^{+} action potentials (e.g. when the membrane was strongly hyperpolarized with injected current). Second, the frequency of large-amplitude spontaneous depolarizations did not decrease when the membrane was held at hyperpolarized potentials; if these depolarizations resulted from spontaneous or EPSP-triggered intrinsic regenerative responses, the probability of eliciting the events would be expected to decrease when the membrane is hyperpolarized. We also never triggered EPSP-like potentials in response to depolarizing pulses.

Origin of spontaneous EPSPs in mossy cells

There is strong evidence that most of the spontaneous EPSPs recorded in mossy cells arise from activity at granule cell–mossy cell synapses. Spontaneously occurring EPSPs closely resemble monosynaptically evoked EPSPs in granule cell–mossy cell paired recordings (Scharfman *et al.* 1990), where the amplitudes of both spontaneous and granule cell-mediated EPSPs were extremely large (often >15 mV). The large amplitude of EPSPs recorded in mossy cells is not consistent with distal, electrotonically remote synapses; rather such large-amplitude PSPs often occur in somatic or proximal dendritic sites, as has been shown for the termination of granule cell axons ('mossy fibres') onto hilar mossy cells (Amaral, 1978; Ribak *et al.* 1985). A majority of the spontaneous EPSPs appear to be due to spiking in the presynaptic mossy fibre axon, since TTX eliminates most PSPs. It is not clear, however, whether spontaneous spiking is generated in the soma/dendritic region of granule cells or is restricted to the synaptic terminal region. Previous studies (Fricke & Prince, 1984; Scharfman & Schwartzkroin, 1990; Scharfman *et al.* 1990; Stanley, Otis & Mody, 1992) have consistently failed to demonstrate spontaneous spiking in granule cell somata, which are generally found to be very hyperpolarized at rest. These findings suggest that the locus of the TTX-sensitive spiking is either in a small subpopulation of spontaneously active granule cells or is restricted to the axons or terminals of granule cells.

It is also possible that spontaneous EPSPs are due to spontaneous spiking in other excitatory neurones that contact mossy cells. Anatomical evidence has demonstrated two other potential sources of this input which would be expected to be preserved in a 400 μm slice preparation: inputs from CA3 pyramidal cells and other hilar mossy cells. In a recent study, Scharfman (1994) recorded monosynaptically coupled pairs of CA3 pyramidal cells and mossy cells, and showed that the amplitude of the unitary EPSP evoked by this pathway (i.e. from CA3 to mossy cell) was significantly smaller than that evoked at the granule cell–mossy cell synapse. The large amplitude of most spontaneous EPSPs in mossy cells would thus appear to be too large to have originated from CA3 neurones. One study of paired recording from two different mossy cells (Scharfman *et al.* 1990) failed to reveal a physiological connection between mossy cells.

DRP epochs are stereotyped responses

Variance measurements demonstrated that epochs of potentiated EPSPs occurred with stereotyped patterns. In most neurones, both the percentage increase in variance and the time course of the variance change were similar in successful trials. Even more striking was the similarity in amplitude and kinetics of the variance changes that occurred spontaneously and those evoked by injected current pulses. The marked similarity in these responses strongly suggests that DRP epochs result from an endogeneous mechanism and are not due to an artifact of the applied current pulses. Further evidence supporting this hypothesis came from the observation that the DRP epoch can be elicited by stimuli generated within the slice itself.

A surprising feature of DRP was the high frequency of treatments with depolarizing current pulses which failed to potentiate EPSPs. The explanation for the high rate of failures is unknown. The stimulus parameters (duration and amplitude of the injected current pulses) were similar

in both successful and unsuccessful trials. There was a moderate negative correlation between the basal variance and the amplitude of DRP-related variance increase in successful trials, suggesting that unsuccessful trials might be expected when resting variances are tonically elevated. The basal variances tended to be higher in failures than in successful trials, but this difference was not statistically significant. We also observed that most spontaneous DRP epochs occurred during periods of relatively low variance. It is possible that the failures were due to a refractoriness in the mechanisms that underlie DRP. It is also possible that the high rate of failures was due to suboptimal stimulation protocols.

Synaptic plasticity in DRP

Depolarization-related potentiation appears to be a form of plasticity in which the trigger mechanism is purely postsynaptic, since DRP could be evoked by injecting depolarizing current pulses into a single neurone. Other methods of inducing DRP, including single depolarizing steps and recurring strong synaptic input from CA3, also produced strong depolarization in mossy cells. However, the mechanism by which depolarization might induce EPSP potentiation is unclear. Although continuous spiking during the depolarizing pulses was not necessary (as demonstrated in our previous study: Strowbridge *et al.* 1992), the abolition of Na^+-based action potentials (with TTX) resulted in epochs of potentiated miniature EPSPs that were considerably briefer than normal DRP epochs. This result suggests that action potentials evoked during DRP epochs may contribute to the maintenance of potentiation (see below).

The amplitude of the current pulses used to elicit DRP was sufficient to enable Ca^{2+} entry through voltage-sensitive Ca^{2+} channels (B. W. Strowbridge & D. W. Tank, unpublished observations). It is possible that increases in intracellular $[Ca^{2+}]$ signal other second messenger systems that modulate synaptic transmission from the postsynaptic cell. This mechanism has been proposed by Kullman *et al.* (1992) to explain a similar short-term potentiation (STP) of evoked EPSPs in CA1 pyramidal cells by depolarizing current pulses. Kullman *et al.* (1992) found that potentiation was abolished by experimental manipulations that diminished or blocked intracellular Ca^{2+} accumulations in CA1 pyramidal cells. Wyllie and colleagues (Wyllie, Manabe & Nicoll, 1994) extended these findings by demonstrating that a similar current pulse protocol (lasting 2 min) potentiated the amplitude of miniature EPSCs in the presence of TTX. Interestingly, both studies of STP in CA1 pyramidal cells and the present study found that the potentiation of EPSPs (or EPSCs) was not maximal immediately following the treatment with depolarizing current pulses. Presumably, this delay represents time needed to fully engage the biochemical or ionic mechanisms that produce the synaptic enhancement. Short-term potentiation in CA1 also can be elicited by focal application of NMDA (Collingridge *et al.* 1983; Kauer *et al.* 1988) and by short tetanizations in the presence of NMDA receptor antagonists (Malenka, 1991). In CA1 pyramidal cells, the duration of STP epochs was longer (typically 10–20 min) than the duration of DRP epochs in mossy cells. We did, however, observe longer-duration epochs of potentiated EPSPs following prolonged treatment with depolarizing current pulses in our previous study (Strowbridge *et al.* 1992). Unfortunately, since those data were not digitized continuously, we cannot exclude the possibility that the long duration was not due a series of short-duration epochs.

Increased frequency of spontaneous EPSPs in DRP

The present data support the hypothesis that the increase in variance associated with DRP was due to a combination of an increase in the amplitude as well as the frequency of spontaneous EPSPs. In each of the eight successful DRP trials examined in detail, periods of elevated variance were associated with statistically significant decreases in the mean interval between spontaneous EPSPs from 15·4 to 24·6 s^{-1}. It was unlikely that these increases in EPSP frequency were due to potentiation of subthreshold EPSPs since large changes in the holding current failed to alter the basal EPSP rate. In general, changes in the frequency of spontaneous synaptic events imply a modification of a presynaptic process, although such a change can be due to activation of latent receptor clusters in the postsynaptic neurone (Liao, Hessler & Malinow, 1995). It is not clear from these experiments whether the presynaptic structures affected were restricted to the presynaptic terminal or included polysynaptic circuits activated by mossy cell action potentials. While depolarizing current pulses still evoked increases in EPSP frequency when polysynaptic activity was suppressed (with TTX), the time course of this form of DRP was considerably shorter than DRP epochs evoked in normal ACSF (see below). This difference in time course may be due to an effect of TTX on the mossy cells which diminished the effectiveness of the depolarizing stimulus (by blocking the propagation of action potentials through the dendritic tree) or is due to the elimination of a polysynaptic component of DRP. If the increase in EPSP frequency is due to a direct effect on the presynaptic terminal, the likely mechanisms would include biochemical signals (e.g. 'retrograde' messengers) or transient changes in the ionic composition of the extracellular space surrounding the terminal.

Increased EPSP amplitude

The increase in spontaneous EPSP frequency during DRP was not sufficient to account for the magnitude of the membrane potential variance increase. We concluded, therefore, that the amplitudes of the spontaneous EPSPs were also increased during DRP. Estimated mean EPSP amplitudes doubled when the variance increase was maximal. These results were consistent with our observations of the actual voltage record which nearly always showed dramatic potentiation of EPSP amplitudes.

In several instances, the time course of the amplitude potentiation was clearly different from the time course of frequency enhancement, suggesting that DRP involves multiple mechanisms regulating different aspects of synaptic function. It is not clear from these data whether this increase in mean amplitude reflects an increased postsynaptic sensitivity or is mediated by a change in presynaptic function (e.g. an increased probability of release).

A component of DRP may be due to recruitment of spiking activity in other, synaptically coupled dentate neurones. Intracellular staining in hippocampal slices has demonstrated that mossy cells produce extensive axonal ramifications in the hilus and, less densely, in the molecular layer of the dentate gyrus (Scharfman *et al.* 1990). The postsynaptic target of the molecular layer innervation appears to be dentate granule cells (Ribak *et al.* 1985; Buckmaster *et al.* 1992). Since these pathways are present in 400 μm thick transverse slices (Buckmaster *et al.* 1992), it is possible that spiking in one mossy cell could produce suprathreshold EPSPs in other mossy cells or granule cells. These connections raise the possibility that DRP results from reverberating activity between multiple mossy cells or mossy cell–granule cell pairs. In contrast to DRP, the duration of STP epochs in CA1 pyramidal cells were similar in the presence and absence of TTX (Kullman *et al.* 1992; Wyllie *et al.* 1994).

In conclusion, these results demonstrate that depolarization of a single mossy cell can initiate a stereotyped epoch of potentiated EPSPs. A component of the potentiation appears to be due to plasticity at the granule cell–mossy cell synapse since DRP persists, albeit in a briefer form, when Na^+ spiking is blocked with TTX. In normal ACSF, variance increases reflect an increase in both the amplitude and frequency of spontaneous EPSPs, and thus may reflect recruitment of spiking activity in presynaptic granule cells or increased excitability of presynaptic terminals.

Amaral, D. G. (1978). A Golgi study of cell types in the hilar region of the hippocampus. *Journal of Comparative Neurology* **182**, 851–914.

Buckmaster, P. S. & Schwartzkroin, P. A. (1995). Interneurons and inhibition in the dentate gyrus in the rat *in vivo*. *Journal of Neuroscience* **15**, 774–789.

Buckmaster, P. S., Strowbridge, B. W., Kunkel, D. D., Schmiege, D. L. & Schwartzkroin, P. A. (1992). Mossy cell axonal projections to the dentate gyrus molecular layer in the rat hippocampal slice. *Hippocampus* **2**, 349–362.

Buckmaster, P. S., Strowbridge, B. W. & Schwartzkroin, P. A. (1993). A comparison of rat hippocampal mossy cells and CA3c pyramidal cells. *Journal of Neurophysiology* **70**, 1281–1299.

Claiborne, B. J., Xiang, Z. & Brown, T. H. (1993). Hippocampal circuitry complicates analysis of long-term potentiation in mossy fiber synapses. *Hippocampus* **3**, 115–122.

Collingridge, G. L., Kehl, S. J. & McLennan, H. (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral–comissural pathway of the rat hippocampus. *Journal of Physiology* **334**, 33–46.

Fricke, R. A. & Prince, D. A. (1984). Electrophysiology of dentate gyrus granule cells. *Journal of Neurophysiology* **51**, 195–209.

Frotscher, M., Misgeld, U. & Nitsch, C. (1981). Ultrastructure of mossy fiber endings in *in vitro* hippocampal slices. *Experimental Brain Research* **41**, 247–255.

Frotscher, M., Seress, L., Schwerdtfeger, W. K. & Buhl, E. (1991). The mossy cells of the fascia dentate: a comparative study of their fine structure and synaptic connections in rodents and primates. *Journal of Comparative Neurology* **312**, 145–163.

Ishuzuka, N., Weber, J. & Amaral, D. G. (1990). Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. *Journal of Comparative Neurology* **295**, 580–623.

Kauer, J. A., Malenka, R. C. & Nicoll, R. A. (1988). NMDA application potentiates synaptic transmission in the hippocampus. *Nature* **334**, 250–252.

Kullman, D. M., Perkel, D. J., Manabe, T. & Nicoll, R. A. (1992). Calcium entry via postsynaptic voltage-sensitive calcium channels can transiently potentiate excitatory synaptic transmission in the hippocampus. *Neuron* **9**, 1175–1183.

Liao, D., Hessler, N. A. & Malinow, R. (1995). Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* **375**, 400–404.

Malenka, R. C. (1991). Postsynaptic factors control the duration of synaptic enhancement in the area CA1 of the hippocampus. *Neuron* **6**, 53–60.

Monaghan, D. T. & Cotman, C. W. (1985). Distribution of *N*-methyl-D-aspartate-sensitive L-[^{3}H]glutamate-binding sites in rat brain. *Journal of Neuroscience* **5**, 2909–2919.

Ribak, C. E., Seress, L. & Amaral, D. G. (1985). The development, ultrastructure and synaptic connections of the mossy cells of the dentate gyrus. *Journal of Neurocytology* **14**, 835–857.

Scharfman, H. E. (1992). Blockade of excitation reveals inhibition of spiny hilar neurons recorded in rat hippocampal slices. *Journal of Neurophysiology* **68**, 978–984.

Scharfman, H. E. (1994). Evidence from simultaneous intracellular recordings in rat hippocampal slices that area CA3 pyramidal cells innvervate dentate hilar mossy cells. *Journal of Neurophysiology* **72**, 2167–2179.

Scharfman, H. E., Kunkel, D. D. & Schwartzkroin, P. A. (1990). Synaptic connections of dentate granule cells and hilar interneurons: results of paired intracellular recordings and intracellular horseradish peroxidase injections. *Neuroscience* **37**, 693–707.

Scharfman, H. E. & Schwartzkroin, P. A. (1988). Electrophysiology of morphologically identified mossy cells of the dentate hilus recorded in guinea pig hippocampal slices. *Journal of Neuroscience* **8**, 3812–3821.

Scharfman, H. E. & Schwartzkroin, P. A. (1990). Responses of cells of the rat fascia dentata to prolonged stimulation of the perforant path: sensitivity of hilar cells and changes in granule cell excitability. *Neuroscience* **35**, 491–504.

Soltesz, I. & Deschênes, M. (1992). Spiny hilar neurons of the rat in vivo. *Society for Neuroscience Abstracts* **18**, 323, 142.2.

Stanley, K. J., Otis, T. S. & Mody, I. (1992). Membrane properties of dentate gryus granule cells: comparison of sharp microelectrodes and whole-cell recordings. *Journal of Neurophysiology* **67**, 1346–1358.

STROWBRIDGE, B. W., BUCKMASTER, P. S. & SCHWARTZKROIN, P. A. (1992). Potentiation of spontaneous synaptic activity in rat mossy cells. *Neuroscience Letters* **142**, 205–210.

STROWBRIDGE, B. W. & SCHWARTZKROIN, P. A. (1992). Modulation of spontaneous EPSPs in rat mossy cells. *Society for Neuroscience Abstracts* **18**, 1339, 564.10.

WYLLIE, D. J. A., MANABE, T. & NICOLL, R. A. (1994). A rise in postsynaptic Ca^{2+} potentiates miniature excitatory postsynaptic currents and AMPA responses in hippocampal neurons. *Neuron* **12**, 127–138.

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