Changes in quantal parameters of EPSCs in rat CA1 neurones *in vitro* after the induction of long-term potentiation

C. Stricker, A. C. Field and S. J. Redman *

Division of Neuroscience, John Curtin School of Medical Research, Australian National University, GPO Box 334, Canberra ACT 0200, Australia

1. Long-term potentiation (LTP) was induced in EPSCs evoked in CA1 pyramidal neurones of young rats *in vitro* by extracellular stimulation of stratum radiatum. Low frequency stimulation was paired with postsynaptic depolarization to induce LTP, using whole-cell recording techniques.

2. Sufficient control and potentiated records were obtained under stable recording conditions to allow a quantal analysis of eleven EPSCs. The fluctuations in amplitude of all eleven EPSCs were quantized before conditioning stimulation, and they remained quantized after LTP induction, usually with an increased quantal variance.

3. Quantal current was increased by conditioning for nine out of eleven EPSCs. The increase in quantal current was correlated with the percentage increase in the EPSC. For only two EPSCs could the entire potentiation be attributed to an increase in quantal current.

4. The amplitude fluctuations of five control EPSCs could be described by binomial statistics, but after conditioning the binomial description held for only one of these EPSCs. For this EPSC, conditioning caused the release probability to increase from $0{\cdot}39 \pm 0{\cdot}05$ to $0{\cdot}47 \pm 0{\cdot}02$.

5. Quantal content was increased by conditioning stimulation for ten out of eleven EPSCs. The increase in quantal content was correlated with the percentage increase in the EPSC. However, for only four EPSCs could the entire potentiation be attributed to an increase in quantal content.

6. Most EPSCs were evoked with a high proportion of response failures. The probability of response failures decreased in eight out of eleven EPSCs following the induction of LTP. There was a negative correlation between the change in the probability of response failures and the amount of LTP.

7. The minimal number of sites at which transmission occurred increased for ten out of eleven EPSCs following LTP induction. Increases in the minimal number of active sites following conditioning were associated with decreases in the probability of response failures for seven out of eleven EPSCs.

8. The induction of LTP usually resulted in changes in the time course of the EPSCs. Cable analysis using a passive compartmental model of a CA1 pyramidal cell suggested that these time course changes were associated with shifts in the average electrotonic location of the active sites following LTP induction, rather than being caused by an increased duration of synaptic current.

9. LTP expression involves postsynaptic modifications to enhance the synaptic current at active sites. New sites are recruited, and our data cannot be used to determine if this is a result of a pre- or a postsynaptic change. Evidence for an increase in release probability was found for one EPSC.

* To whom correspondence should be addressed.

The locus of expression of long-term potentiation (LTP) in the hippocampus remains a controversial issue. There has been renewed interest in the use of quantal analysis to help resolve this question, because it has the potential to separate presynaptic and postsynaptic changes in transmission. The interpretational difficulties that arise when applying this technique to transmission at central synapses have been reviewed (Korn & Faber, 1987, 1991; Redman, 1990) and to some extent they have been ignored in many of the recent attempts to apply quantal analysis to the expression of LTP (reviewed in Bliss & Collingridge, 1993). Consequently, there has been much confusion surrounding the results of these investigations, with some reports attributing all the potentiation to postsynaptic changes (Foster & McNaughton, 1991; Kullmann, 1994); others concluding that the changes are entirely or predominantly presynaptic (Bekkers & Stevens, 1990; Malinow & Tsien, 1990; Malinow, 1991; Voronin, Kuhnt & Gusev, 1992; Stevens & Wang, 1994), while a combination of pre- and postsynaptic changes were reported by Kullmann & Nicoll (1992); Larkman, Hannay, Stratford & Jack (1992) and Liao, Jones & Malinow (1992). Much of this confusion can be traced back to differences in the methodology used in the various analyses and to the simplifying assumptions made about the statistics of the release processes.

The companion paper (Stricker, Field & Redman, 1996) provided an unambiguous decision pathway for determining the most appropriate and parsimonious statistical description of the transmission process generating EPSCs at synapses on CA1 pyramidal neurones. The quantal currents and the number of active sites were determined for nineteen EPSCs generated by a quantal transmission process (from fifty EPSCs analysed). This paper reports on the analysis of eleven of these nineteen EPSCs, for which LTP was induced and sufficient records were obtained, using the same statistical procedures as those in the companion paper. The results indicate that the induction of LTP causes an increase in quantal current, but this increase is insufficient to account for all of the potentiation. LTP induction also increases the quantal content of the EPSC, and this is generally accompanied by a decrease in the probability of response failures, an increase in the number of sites at which transmission occurs, and an indication that release probabilities increase at some of the sites that were active from the outset. The relative contributions of increases in quantal content and quantal current to the potentiation vary considerably for each EPSC. The results provide further support for the results of previous reports (Kullmann & Nicoll, 1992; Larkman *et al.* 1992; Liao *et al.* 1992) implicating both pre- and postsynaptic changes to the expression of LTP.

METHODS

The preparation of slices, electrophysiological techniques and quantal analysis procedures were described in the companion paper (Stricker *et al.* 1996).

Conditioning of synaptic transmission to induce LTP involved pairing 2 Hz stimuli to the axons in stratum radiatum (with the same stimulus strength as that used to collect EPSCs under control conditions) with depolarization at the soma to −20 or 0 mV. Fifty to one hundred stimuli were used. Conditioned responses were then recorded for as long as stable recording conditions could be maintained. Stationarity in the conditioned records was examined using the same criteria as those described in the companion paper. Analysis was performed only on those sections of recordings for which the conditions for stationarity were satisfied.

The access resistance sometimes changed during conditioning, and this changed the amplitude and time course of recorded currents. The peak amplitudes of the control and the conditioned EPSCs were formed into probability density functions (p.d.f.s) without correcting their amplitudes for the effect of access resistance. The results of analysis of these p.d.f.s (Figs 1 and 2) have been presented without correction for attenuation by the access resistance. In all subsequent analysis (Figs 3–6 and Table 1) all amplitudes, time courses and the amount of potentiation have been adjusted to the values calculated for zero access resistance. (This calculation was described in Stricker *et al.* 1996.) The scaling factors are given in Table 1, and they are very similar for both control and conditioned recordings for all but one EPSC (07/01/92 C2). Thus, even if the calculation of this scaling factor contains some inaccuracy this will have little effect on the amount of potentiation, or the relative change in quantal current. Error bars associated with a parameter indicate ± S.D.

The quantal content of an EPSC was calculated by dividing its mean amplitude by its quantal current. The use of quantal content as a measure of the presynaptic contribution of an EPSC is not predicated on the amplitude distribution being Poisson.

The data files used in this analysis may be obtained by electronic transfer on request to C. S. at Christian.Stricker@anu.edu.au.

RESULTS

The results are based on quantal analysis of eleven EPSCs for which LTP was induced. These results were obtained from attempts to condition each of the nineteen quantal EPSCs analysed in the companion paper. The other eight EPSCs described in that paper did not provide useful results after conditioning, because LTP was not maintained or the EPSC depressed (5) or because the recording was lost before sufficient conditioned records were collected (3). The eleven sets of conditioned responses were put through the same analysis procedures as those described in the companion paper for the control EPSCs. All eleven EPSCs were best described by a quantal model.

The results illustrated in Fig. 1 are typical of the results obtained for most of the eleven EPSCs. The peak amplitudes of the EPSCs recorded during the control period

and after conditioning are shown in Fig. 1*A*. The horizontal bars indicate the periods from which data were taken to create the p.d.f.s shown in Fig. 1*B* and *C*. The optimal fit to the control p.d.f. is a uniform binomial distribution with more failures than predicted by the binomial release model. The quantal current is 1·7 ± 0·2 pA, the release probability is 0·29 ± 0·05, there is negligible quantal variance and the number of sites at which transmission occurs (n) is five. The probability of failures additional to those predicted by the binomial model is 0·22 ± 0·04 and there is a zero offset of 0·2 ± 0·1 pA. The peak currents and their probabilities as predicted by this model are shown in Fig. 1*D*, together with confidence limits.

Conditioning the EPSC resulted in a maintained enhancement of about 500% for 20 min during which stable recording was maintained. It should be obvious from the clear peaks in the p.d.f. of the conditioned EPSCs (Fig. 1*C*) that the separation between the peaks is greater than for

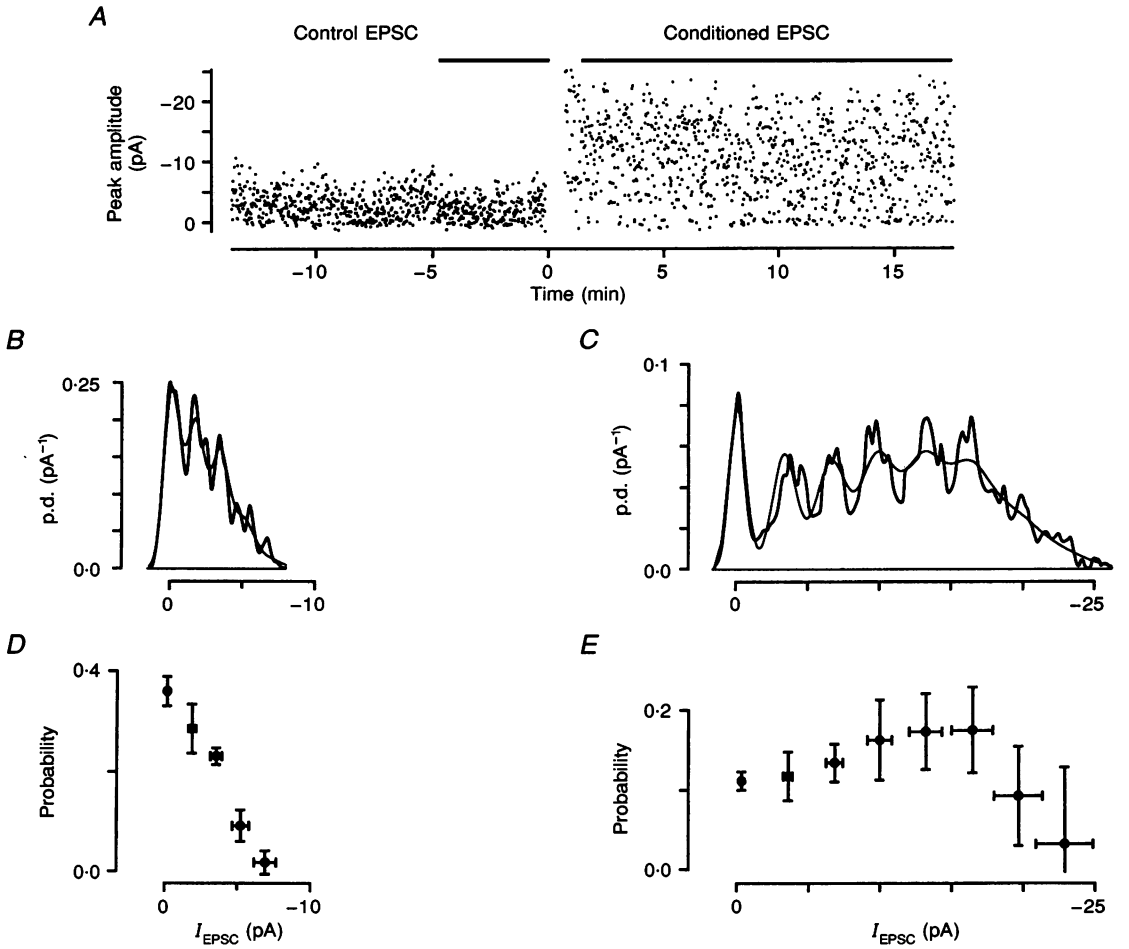

Figure 1

A, peak amplitudes of control and conditioned EPSCs, recorded over 13 and 18 min, respectively. Conditioning stimulation commenced at time zero. The horizontal bars indicate the periods when the EPSC amplitudes remained stationary. Data were taken from these periods to create the control p.d.f. (*B*) and the conditioned p.d.f. (*C*). The p.d. (probability density) at a particular current is the p.d.f. evaluated at that current. 343 records were obtained from the control EPSCs and 1049 records from the conditioned EPSCs. σ_n was 0·6 pA for both sets of records. The Gaussian kernels used for the control and conditioned p.d.f.s had σ values of 0·15 and 0·13 pA, respectively. The thin line in *B* is the optimal fit to the p.d.f., which corresponds to a quantal model with negligible quantal variance and with binomial release statistics except for the high incidence of response failures. The thin line in *C* is the optimal fit to the p.d.f. for the conditioned responses. This corresponds to a quantal process, with quantal variance, and with no constraints on the statistics of release. *D* and *E*, discrete amplitudes and their associated probabilities, together with confidence limits, for both the control (*D*) and conditioned (*E*) EPSCs.

the control p.d.f., that the number of clearly distinguishable peaks had increased, and that the probability of response failures had decreased. The optimum model for the p.d.f. of the conditioned EPSC is a quantal process with eight discrete amplitudes. Quantal variance is required, but no constraints can be attached to the release probabilities. The quantal current is 3·2 ± 0·3 pA, the coefficient of variation (c.v.) is 0·19 ± 0·12 and the offset from zero is 0·3 ± 0·1 pA. The probabilities attached to the peak currents are (in order of increasing amplitude): 0·11 ± 0·01, 0·12 ± 0·03, 0·13 ± 0·02, 0·16 ± 0·05, 0·17 ± 0·05, 0·18 ± 0·05, 0·09 ± 0·06 and 0·02 ± 0·10. The peak currents and the probabilities attached to them are indicated in Fig. 1*E*. The optimal model to fit this p.d.f. is largely determined by the locations and probabilities of the first six peaks. The error in the amplitude associated with each peak is set by the error for the first quantal increment, and is magnified by the progressive addition of quanta. The error in probability is determined by the sample size associated with each peak. Table 1 (entry 30/03/92 C1) provides full details of the analysis of the EPSC in Fig. 1. Note that all currents in Table 1 have been corrected for the effects of access resistance and are larger than those given above. For this EPSC, conditioning resulted in an increase in the quantal

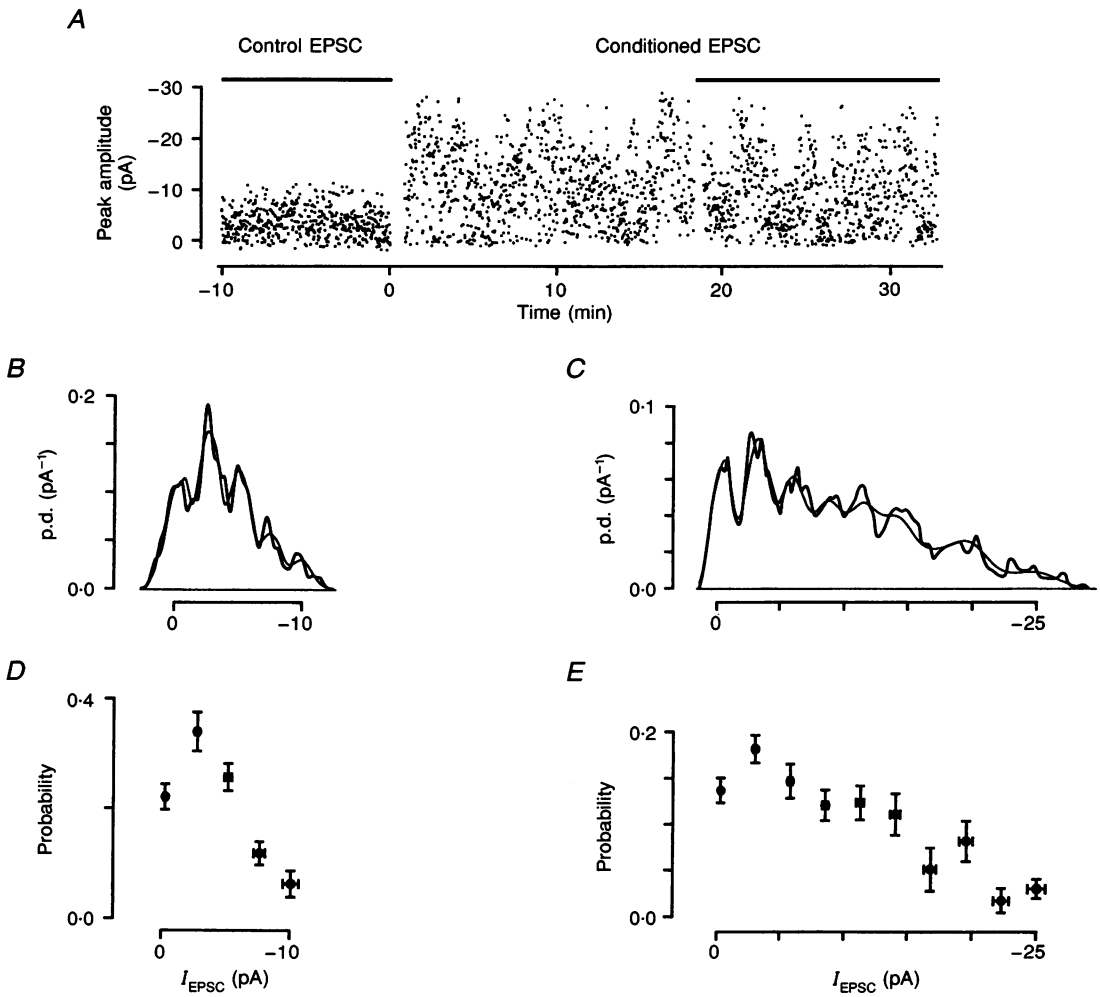

Figure 2

A, peak amplitudes of the control EPSCs, recorded over 10 min, and peak amplitudes of the conditioned EPSCs, recorded over 2 periods of 18 and 14 min, separated by a 1 min interval. Details of the analysis of the EPSCs from the control and the second recording period (indicated by the horizontal bars) are given in *B–E*. 526 control EPSCs and 777 conditioned EPSCs were obtained to generate the p.d.f.s shown in *B* (control) and *C* (conditioned). σ of the Gaussian kernel used to form the p.d.f. in *B* was 0·20 pA, and 0·18 pA for the p.d.f. in *C*. σ_n for control EPSCs was 0·9 and 0·7 pA for the conditioned EPSCs. The thin line in *B* is the optimal fit to the control p.d.f. This was obtained for a quantal process, with negligible quantal variance, and with no constraints on release statistics. The thin line in *C* is the optimal fit for this p.d.f., which was obtained for quantal transmission, quantal variability, and with no constraints on release statistics. *D* and *E* indicate the discrete amplitudes and their associated probabilities, together with confidence limits, for both the control (*D*) and conditioned (*E*) EPSC.

Table 1. Details of the analyses of each EPSC, before and after conditioning

Experiment	N	t (min)	σ_n (pA)	Mean (pA)	LTP (%)	R_A (MΩ)	R_I (MΩ)	T_R (ms)	T_{HW} (ms)	Sc	Dist	K	Q (pA)	c.v.	m	P_0
12/12/93 C1 C	416	−9	1·2	−7·6 ± 7·1	—	25·8	65·5	2·4	16·2	2·5	0·6	3	−9·5 ± 0·3	—	0·8 ± 0·0	0·52 ± 0·08
P	731	26	1·5	−20·7 ± 17·5	273 ± 15	25·5	61·9	1·8	8·5	2·7	0·6	6	−11·7 ± 0·1	—	1·8 ± 0·1	0·37 ± 0·02
18/12/93 C2 C	437	−10	1·1	−7·1 ± 5·7	—	8·6	107·0	2·6	10·2	1·4	0·5	6	−4·5 ± 0·2	—	1·6 ± 0·1	0·25 ± 0·03
P	1100	25	1·1	−17·5 ± 12·5	246 ± 11	10·2	98·7	2·4	9·0	1·6	0·5	12	−5·1 ± 0·4	—	3·4 ± 0·3	0·11 ± 0·01
P	687	43	1·2	−31·4 ± 19·0	440 ± 20	11·6	102·4	2·5	10·0	1·6	0·5	12	−7·4 ± 0·4	0·15 ± 0·04	4·3 ± 0·2	0·07 ± 0·01
07/01/92 C1 C	526	−10	0·9	−5·1 ± 4·1	—	6·5	79·6	1·8	9·7	1·4	0·4	5	−3·4 ± 0·2	—	1·5 ± 0·1	0·22 ± 0·02
P	936	18	0·8	−15·6 ± 10·4	306 ± 13	7·0	74·4	2·3	8·9	1·4	0·7	9	−4·3 ± 0·2	0·24 ± 0·05	3·6 ± 0·2	0·13 ± 0·01
P	777	33	0·7	−10·9 ± 8·2	214 ± 9	6·2	67·8	2·5	9·8	1·2	1·2	10	−3·3 ± 0·1	0·16 ± 0·04	3·3 ± 0·1	0·14 ± 0·01
07/01/92 C2 C	333	−10	0·7	−9·9 ± 7·3	—	9·6	51·7	3·1	12·8	1·4	1·1	8	−3·2 ± 0·3	—	3·1 ± 0·3	0·13 ± 0·04
P	494	18	1·0	−20·3 ± 11·9	250 ± 11	20·3	46·3	2·6	12·7	2·2	0·9	7	−7·1 ± 0·8	0·37 ± 0·12	2·9 ± 0·3	0·04 ± 0·03
15/01/92 C1 C	581	−10	0·9	−7·9 ± 6·4	—	20·0	97·4	2·3	16·2	2·0	0·4	4	−5·3 ± 0·7	0·39 ± 0·16	1·5 ± 0·2	0·23 ± 0·06
P	1435	26	1·2	−10·5 ± 9·3	134 ± 5	16·1	101·8	2·6	16·5	1·8	0·5	9	−5·1 ± 0·5	—	2·1 ± 0·2	0·27 ± 0·02
30/03/92 C1 C	343	−14	0·5	−3·4 ± 3·1	—	14·8	87·8	3·8	16·1	1·6	0·8	5	−2·7 ± 0·3	—	1·3 ± 0·2	0·36 ± 0·03
P	1049	30	0·5	−18·0 ± 10·9	529 ± 28	14·0	116·1	3·2	14·3	1·7	0·6	8	−5·5 ± 0·5	0·19 ± 0·12	3·3 ± 0·3	0·11 ± 0·01
01/04/92 C3 C	263	−12	0·4	−8·0 ± 6·2	—	14·2	53·2	2·6	15·8	1·7	0·8	11	−2·6 ± 0·2	0·12 ± 0·12	3·1 ± 0·3	0·19 ± 0·03
P	494	18	0·7	−32·8 ± 18·8	411 ± 22	16·8	48·0	2·1	10·1	2·1	0·7	17	−4·8 ± 0·2	0·08 ± 0·07	6·8 ± 0·4	0·06 ± 0·01
07/04/92 C1 C	349	−9	0·7	−7·8 ± 3·5	—	21·0	75·6	4·5	21·3	1·8	1·0	6	−3·4 ± 0·1	—	2·3 ± 0·1	0
P	936	18	1·0	−13·1 ± 8·0	168 ± 5	23·9	88·5	5·7	25·0	1·9	1·1	9	−4·2 ± 0·5	—	3·2 ± 0·4	0·03 ± 0·02
11/12/91 A2 C	458	−9	0·6	−9·6 ± 6·5	—	13·5	129·3	1·8	16·2	1·8	0·3	8	−3·5 ± 0·2	0·13 ± 0·06	2·7 ± 0·2	0
P	934	27	0·7	−22·5 ± 17·0	234 ± 9	13·5	94·3	2·0	8·5	1·7	0·4	19	−3·5 ± 0·1	0·06 ± 0·03	6·5 ± 0·2	0·10 ± 0·01
18/12/91 A1 C	827	−15	0·6	−3·1 ± 4·2	—	16·1	117·6	1·7	11·2	1·9	0·3	7	−2·7 ± 0·2	—	1·1 ± 0·1	0·65 ± 0·02
P	1743	31	0·5	−13·0 ± 13·1	414 ± 22	23·1	108·3	2·8	12·3	2·1	0·4	9	−3·0 ± 0·1	0·23 ± 0·03	2·1 ± 0·1	0·30 ± 0·01
P	1341	93	0·6	−10·4 ± 8·9	332 ± 17	23·4	96·1	2·9	11·4	2·1	0·5	6	−3·0 ± 0·2	0·33 ± 0·04	1·7 ± 0·1	0·23 ± 0·01
13/01/92 A1 C	849	−15	0·6	−3·2 ± 2·6	—	12·1	67·4	1·8	14·2	1·7	0·4	5	−2·6 ± 0·2	—	1·2 ± 0·1	0·41 ± 0·04
P	974	31	0·6	−10·0 ± 8·2	317 ± 12	12·3	59·3	2·8	11·7	1·6	0·8	10	−3·1 ± 0·1	0·21 ± 0·07	3·3 ± 0·2	0·21 ± 0·01

C and P, control and potentiated responses, respectively; N, number of responses and t, duration of each recording (time zero, time of conditioning; negative times, time prior to conditioning when the control recordings began and positive times, time when the recording of conditioned responses ended). For some EPSCs, there was more than 1 period when conditioned responses were recorded. σ_n, S.D. of the noise; Mean, mean ± S.D. of evoked responses; LTP, amount of potentiation after conditioning; R_A, access resistance; R_I, input resistance; T_R and T_{HW}, 10–90% rise time and half-width, respectively; Sc, scaling factor applied to quantal current to compensate for effect of access resistance; Dist, electrotonic distance from soma to calculate synaptic site on a reduced apical dendrite; K, number of discrete amplitudes in evoked response; Q, quantal current, after scaling for effect of access resistance; c.v., quantal variance; m, quantal content; P_0, probability of response failures. Values are given as means ± S.D.

current, an increase in the c.v., an increase in the quantal content, an increase in the minimum number of sites at which transmission occurred, and a decrease in the probability of response failures.

In a second example, illustrated in Fig. 2, the control and conditioned EPSCs were recorded as shown in Fig. 2*A*. In the first recording period of 18 min postconditioning, the average increase in the EPSC was 306%. In a second period of 19–33 min postconditioning, the average increase in the EPSC over the control EPSC was 214%. The optimal model of transmission, as determined by the p.d.f. of the control EPSC (Fig. 2*B*) is a quantal process with no quantal variability, and with no constraints on release probabilities. The quantal current is 2·5 ± 0·2 pA, $K = 5$, and the probabilities attached to the five amplitudes (starting with the failure peak) are 0·22 ± 0·02, 0·34 ± 0·04, 0·26 ± 0·02, 0·12 ± 0·02 and 0·06 ± 0·02. There is no zero offset.

The conditioned EPSCs could also be described by a quantal process (Fig. 2*C*) with a small increase in quantal current to 2·8 ± 0·1 pA, but with a pronounced increase in quantal variance (c.v. = 0·16 ± 0·04). After correction for the effects of access resistance, the change in quantal current is not significant (Table 1, 07/01/92 C1). The minimum number of sites at which transmission occurred increased to nine, and the probability of failure decreased to 0·14 ± 0·01. The optimal fit to the p.d.f. in Fig. 2*D* did not allow any constraints to be placed on the release statistics. The probabilities attached to the nine amplitudes, beginning with the smallest were: 0·18 ± 0·01, 0·15 ± 0·02, 0·12 ± 0·02, 0·12 ± 0·02, 0·11 ± 0·02, 0·05 ± 0·02, 0·08 ± 0·02, 0·02 ± 0·01 and 0·03 ± 0·01. The offset from zero was 0·3 ± 0·1 pA. Table 1 (entry 07/01/92 C1) gives full details of the analysis of the EPSC in Fig. 2, together with the results of the analysis of the EPSC in the first postconditioning period. Note that all currents in Table 1 have been corrected for the effects of access resistance, and differ somewhat from the values given above. In summary, the increase in the EPSC illustrated in Fig. 2 following conditioning was largely through an increase in the minimum number of sites at which transmission occurred, and a decrease in the probability of failures.

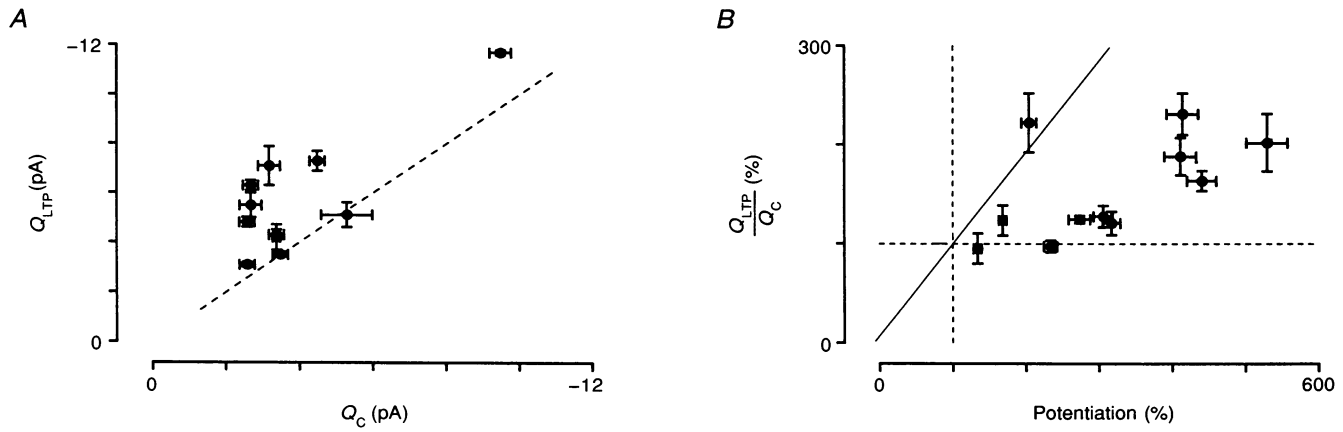

Figure 3

A, quantal current for the conditioned EPSC (Q_{LTP}) *vs.* the quantal current for the control EPSC (Q_C). Both currents have been corrected for the effects of access resistance by calculating the quantal current for an access resistance of zero. The dashed line indicates no change. *B*, relative change in quantal current *vs.* the percentage increase in the EPSC following LTP induction. The continuous line indicates equal changes, and the dashed lines indicate no change.

These two examples broadly illustrate the changes that occurred in the fluctuations of the eleven EPSCs following conditioning. Table 1 provides details of quantal parameters obtained before and after conditioning for each EPSC. The quantal description was preserved following conditioning for all EPSCs. Quantal current increased for all but two EPSCs. Quantal variance usually increased. The number of peaks in the conditioned response, indicative of the minimum number of sites at which transmission occurred, increased for nine out of eleven EPSCs. The probability of response failures decreased for eight out of eleven EPSCs. Release probabilities could be determined for only one EPSC, as the p.d.f.s for the remaining ten EPSCs were more dispersed than would occur for any known release model. However, quantal content, which is usually regarded as an index of presynaptic strength, increased for all but one EPSC.

Maintenance of LTP during prolonged recordings

The mechanisms supporting the maintenance of LTP are believed to change with time (reviewed in Bliss & Collingridge, 1993). One recording was maintained for 90 min postconditioning. The quantal currents and quantal content were extracted from these data for the control period (15 min), the first 30 min following conditioning, and the period 70–93 min postconditioning. These quantal parameters are shown in Table 1 (18/12/91 A1). The quantal current did not change after conditioning and remained constant over the subsequent 90 min of recording. The quantal variability was negligible in the

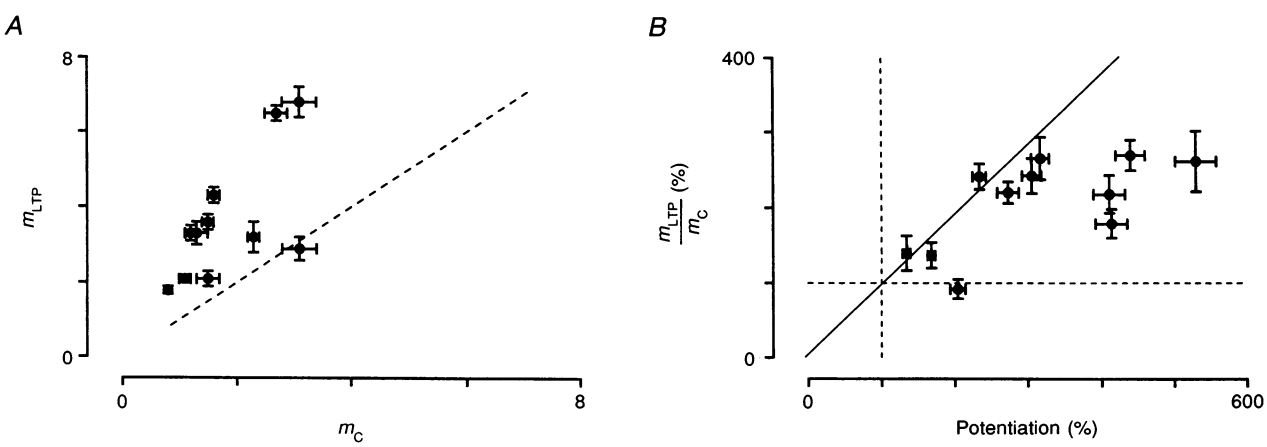

Figure 4

A, quantal content of the EPSC following conditioning (m_{LTP}) *vs.* the quantal content of the control EPSC (m_C). The dashed line indicates no change. *B*, relative change in quantal content *vs.* the percentage increase in the mean EPSC after the induction of LTP. The continuous line indicates equal changes, and the dashed lines indicate no change.

control EPSC, and increased to 0·23 ± 0·03 for the first analysis period, and to 0·33 ± 0·04 for the second period. The quantal content also increased after conditioning, and decreased slightly in the second recording period by a similar amount (15%) to the decrease in the average EPSC (20%). LTP was followed in another two EPSCs (43 min, 18/12/93 C2, and 33 min, 07/01/92 C1; Table 1). Recordings were analysed from two different periods for each EPSC, and the amount of LTP in each period varied for each EPSC (from 310 to 250% and from 220 to 390%). In both recordings, quantal content was greater than the control value throughout the entire postconditioning period. However, for each of the two recordings, quantal current differed from its control value in only one of the recording periods. The quantal current was different in each recording period for both these EPSCs, while the quantal content changed for one EPSC. These results suggest that changes in quantal current and quantal content can occur (in both directions) at times other than the period when conditioning takes place. The possibility that such lability could be present in unconditioned EPSCs when periods of non-stationary behaviour were evident (e.g. Fig. 1*B* of Stricker *et al.* 1996) could not be examined, because insufficient records were available to allow for reliable statistical analysis. Ideally, control and conditioned records should be evoked at < 1 Hz, to avoid the possibility of inducing long-term changes, such as those reported by Dudek & Bear (1992).

Changes in quantal current

Quantal currents of the enhanced EPSCs were corrected for access resistance, in the same manner as for the control EPSCs (described in Stricker *et al.* 1996). There were significant increases in nine out of eleven quantal currents, as shown in Fig. 3*A*. The change in quantal current is correlated with the amount of LTP (Fig. 3*B*; $r = 0{\cdot}58$, significance level of the linear correlation (α) = 0·06). The increase in quantal current was insufficient to account for all of the enhancement of the EPSCs, except for two EPSCs which registered the smallest and third smallest increase following conditioning. The quantal current after conditioning is correlated with the quantal current of the control EPSC ($r = 0{\cdot}8$, $\alpha < 0{\cdot}01$). However, there is no correlation between the increase in quantal current and its control value (results not shown). Nor was there any correlation between the increase in quantal current and the initial quantal content.

Changes in release probabilities

For all but one EPSC (12/12/93 C1) there was no statistical model of the release process to which we could fit the

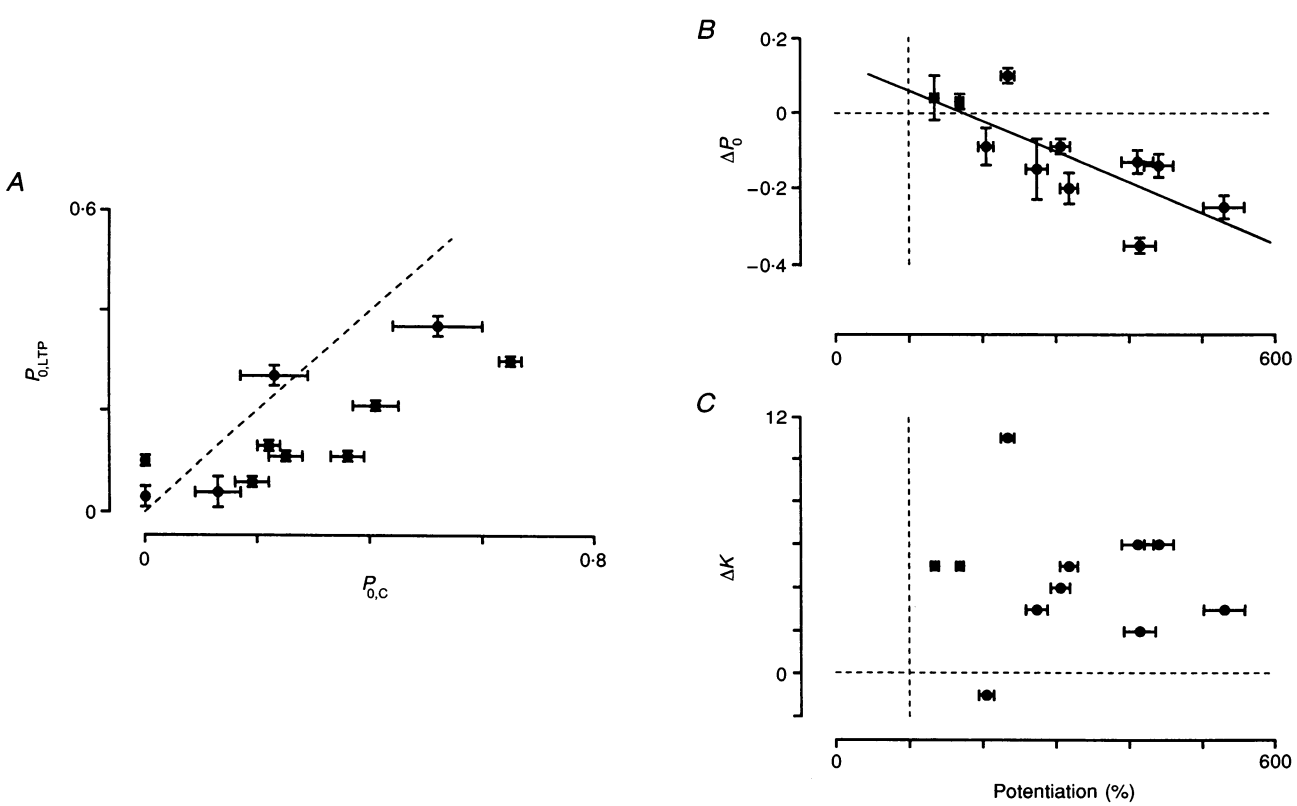

Figure 5

A, probability of response failure after conditioning ($P_{0,LTP}$) *vs.* P_0 for the control EPSC ($P_{0,C}$). *B*, change in the probability of failure responses in the EPSC following conditioning *vs.* the percentage potentiation. The continuous line is the regression line. *C*, change in K plotted against the percentage potentiation. For *A–C*, the dashed lines indicate no change.

observed p.d.f.s, and we were unable to calculate release probabilities. Binomial statistics could be used to describe the release process for the above EPSC before and after conditioning, but only after allowing for excess failures. The release probability increased from $0{\cdot}39 \pm 0{\cdot}05$ to $0{\cdot}47 \pm 0{\cdot}02$, and n increased from two to five.

Changes in quantal content

As release probabilities could only be calculated for one EPSC, we have used quantal content as an indicator of presynaptic effectiveness. Quantal content will increase if there is an increase in the number of sites at which transmission occurs or if release probabilities increase. Figure 4*A* shows the quantal content of the conditioned EPSC plotted against the quantal content of the control EPSC. After conditioning, the quantal content increased for all but one EPSC, and the enhanced and the control values of quantal content are correlated ($r = 0{\cdot}67$, $\alpha = 0{\cdot}02$). The increase in quantal content is correlated with the increase in the EPSC following conditioning ($r = 0{\cdot}66$, $\alpha = 0{\cdot}02$), as shown in Fig. 4*B*. The increase in quantal content was sufficient to account for all the potentiation of the EPSC in four out of eleven experiments.

Changes in the probability of response failures

Response failures in CA1 EPSCs occur more frequently than predicted by conventional models of release (Stricker *et al.* 1996). The reasons are not clear, and they are discussed in the companion paper and in Allen & Stevens (1994). In conventional release models, the probability of response failures (P_0) will decrease if release probabilities increase at some or all of the release sites. In the results presented below, we have assumed that changes in P_0 are an indicator of changes in release probabilities. P_0 decreased for all but three EPSCs following LTP induction (Fig. 5*A*). The amount of LTP was negatively correlated with the change in P_0 (Fig. 5*B*; $r = -0{\cdot}76$, $\alpha < 0{\cdot}01$). Note, however, that for two EPSCs LTP was accompanied by an increase in P_0. When release probabilities increase at sites where the probability of release was negligible before conditioning, the minimum number of sites at which transmission can be detected (K) will increase. For all but one EPSC, LTP was

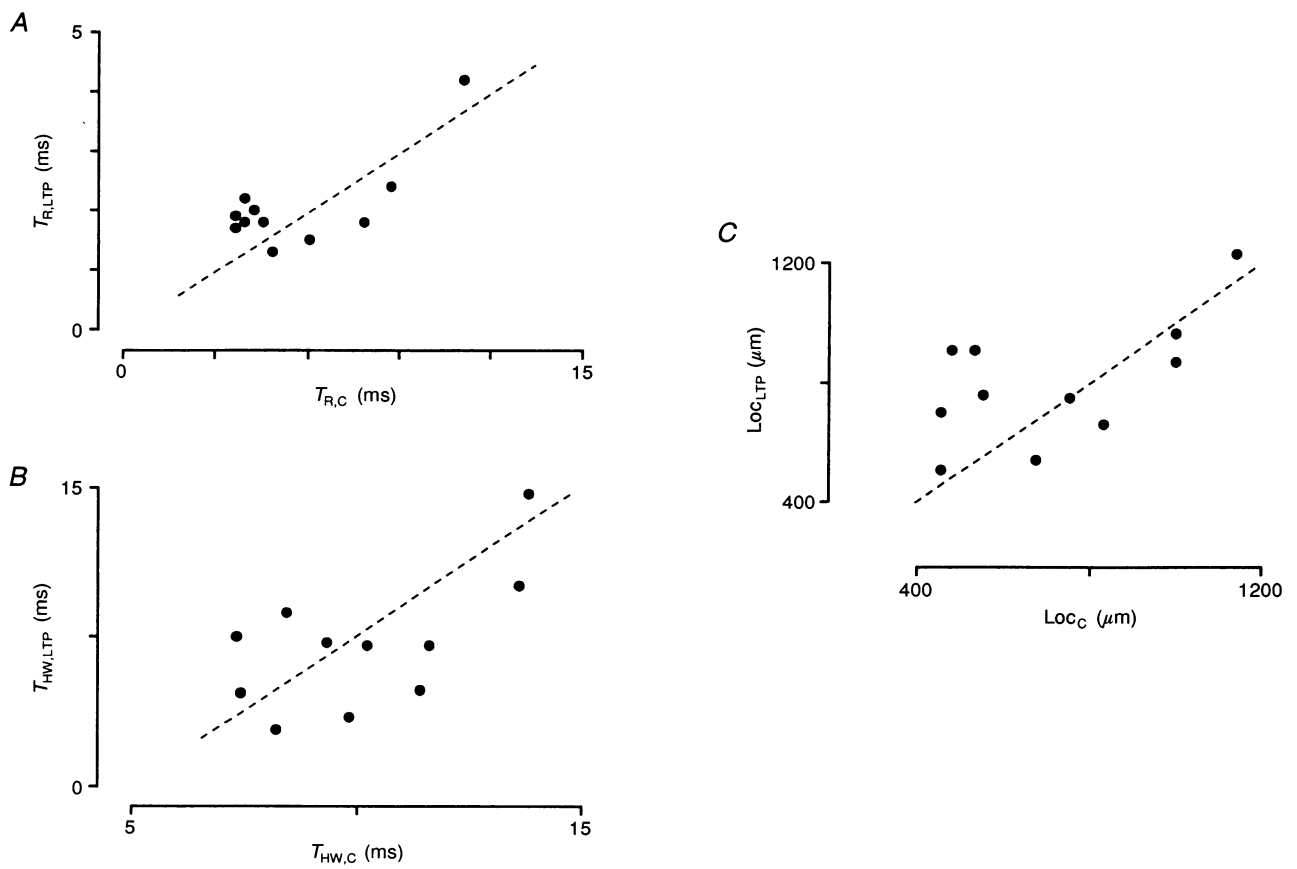

Figure 6

The 10–90% rise time (T_R; *A*) and the half-width (T_{HW}; *B*) of the averaged time course for the control and the potentiated EPSCs. *C* shows the calculated electrotonic locations (Loc) of the synaptic site (in terms of distance from the soma) on a reduced apical dendrite of a reconstructed CA1 pyramidal cell (Major, Evans & Jack, 1993) for the time courses of the control and the conditioned EPSCs. The dashed lines indicate no change.

accompanied by an increase in K (Fig. 5C). However, there was no correlation between the increase in K and the amount of potentiation.

Changes in time course following LTP induction

The 10–90% rise time and the half-width were measured for the averaged EPSC, recorded under control conditions and after inducing LTP. The results are shown in Fig. 6A and B. Changes in input resistance following LTP induction were usually negligible, and this implies that no change occurred in the membrane time constant. Thus, changes in time course can be attributed to changes in synaptic current duration and/or changes in the average electrotonic location at which transmission occurs. The control EPSCs with the smallest rise times and half-widths mostly had their rise times and half-widths prolonged by LTP induction. Several control EPSCs with larger rise times and half-widths had these shape indices reduced during LTP. Assuming that these changes in shape indices represent shifts in the average synaptic location on an equivalent apical dendrite (weighted by the probabilities of transmission at each active site) the new synaptic locations were calculated (as described in Methods in Stricker *et al.* 1996). The results are shown in Fig. 6C. The spatial realignments are small for many of the EPSCs, and are probably within the errors involved in this calculation. However, there are four proximal EPSCs for which the average synaptic location became more distal by 200 μm or greater. For this to occur, the synaptic sites activated during LTP must have been located more than 200 μm distal to the focus of synaptic activity under control conditions.

DISCUSSION

The results provide clear evidence that quantal current is increased following the induction of LTP. The contribution this makes to the enhancement of the synaptic current varies for different experiments, but it is usually insufficient to account for all of the potentiation. The results also provide clear evidence that more active sites are involved in transmission following conditioning, and that response failures become less frequent.

Changes in quantal current

The finding that increases in quantal current partially contribute to the increased synaptic current during LTP is in agreement with the results from previous quantal analyses of LTP, using different techniques (Kullmann & Nicoll, 1992; Larkman *et al.* 1992; Liao *et al.* 1992). However, there have been many other investigations of this issue where the conclusion has been that LTP can be totally explained by increases in quantal current, or totally explained by presynaptic changes (reviewed in Bliss & Collingridge, 1993). There was no significant increase in quantal current for two of the eleven potentiated EPSCs. An increase in quantal current alone could account for the entire potentiation for only two EPSCs.

It has been proposed that the extent by which the quantal current changes depends on the size of the unconditioned quantal current (Liao *et al.* 1992) or on the unconditioned release probability (Larkman *et al.* 1992, using binomial release statistics). We found no correlation between the percentage increase in quantal current and its initial value. As the amplitude fluctuations of most EPSCs could not be described by a binomial process, we were unable to examine the reported correlation between change in quantal current and the release probability before conditioning. When the initial quantal content was used instead of initial release probability, no correlation was found. The result obtained by Larkman *et al.* (1992) appeared to be highly dependent on the use of 4 mM extracellular Ca^{2+} to raise the initial release probabilities.

One of the remarkable results is that all eleven quantal EPSCs retained their quantal description after conditioning, albeit with an increased c.v. of the quantal current for six out of eleven EPSCs. For one EPSC (15/01/92 C1), the c.v. decreased after conditioning. This means that similar increases in quantal current occurred at those active sites where transmission took place before conditioning. It also implies that the quantal current (at the soma) generated at any sites that became active following conditioning must be similar to the enhanced quantal current from the sites that were involved in transmission from the outset. Similar observations were made by Liao *et al.* (1992). Some of the enhanced quantal currents have c.v.s in the range 0·2–0·4, allowing for variability in quantal current between sites over roughly a twofold range. Even so, these results imply that the number of functional channels at all of the active sites is constrained within a fairly narrow range, in both the unpotentiated and potentiated states. This discussion assumes that there is sufficient glutamate in a single vesicle to ensure full occupancy of all AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate) receptors on a postsynaptic density, as originally proposed by Edwards, Redman & Walmsley (1976) and Jack, Redman & Wong (1981) and supported by recent experimental evidence (Tong & Jahr, 1994). It also assumes that quantal variability arising from the stochastic opening of channels (Faber, Young, Legendre & Korn, 1992; Hestrin, 1992) is small in comparison with intersite variability, because the number of channels generating the quantal current is sufficiently large (Stricker *et al.* 1996). On this basis, the observed increase in quantal variability following LTP induction in some EPSCs is unlikely to arise from stochastic opening of channels, as the enhanced quantal current is generated by the gating of more channels than in the unpotentiated state. Changes in intersite variability following conditioning is the most likely cause of the changes in quantal variability.

Changes in EPSC time course

Enhancement of synaptic current during LTP in the 3–6 h time period postconditioning (referred to as LTP1 in Bliss & Collingridge, 1993) is believed to involve post-translational modification of channel proteins, possibly by phosphorylation (reviewed in Bliss & Collingridge, 1993). Currents could be enhanced by channels opening to a higher conductance state, by remaining open for a longer period, by increasing their probability of opening, by a decrease in receptor desensitization, or by some combination of these modifications. An increase in channel open time prolongs the time course of an EPSC, as well as increasing its peak amplitude. The time course changes illustrated in Fig. 6 show prolongation of synaptic currents for proximally generated EPSCs, while the time course of distally generated EPSCs was shortened for all but one EPSC. If the increase in quantal current was due to an increased duration of synaptic current, the EPSCs should all show increases in rise time and half-width, assuming that new sites for transmission (following conditioning) were not always located more proximally than old sites.

Simulations using a compartmental model of a CA1 neurone (described in Methods in Stricker *et al.* 1996) indicate that a 50% increase in peak EPSC at the soma requires an increase in the time-to-peak conductance at the synapse from 0·5 to 0·9 ms, for synapses located 0·3–0·8 λ from the soma. A change in the time-to-peak conductance alters the rise time and half-width of the EPSC. The rise time at the soma would then increase by 60% for a synapse at 0·3 λ and by 20% for a synapse at 0·8 λ, with smaller changes in the half-widths. While changes of these magnitudes were observed in the time course of some of the conditioned EPSCs, they did not show consistent increases in rise time and half-width. The most likely explanation for these shifts in time course is that the time course of synaptic current is not prolonged during LTP induction. Instead, the average electrotonic location of the active sites changes, as new sites become involved in transmission and as release probabilities are altered at old sites.

Changes in quantal content: presynaptic modifications

There was a strong correlation between the amount of LTP and the increase in quantal content. The quantal content was increased by LTP induction in all but one EPSC. The conventional interpretation of this result is that the conditioning stimulation caused the probability of transmitter release to increase at some or all of the activated release sites. As a result, the probability of response failures decreased, and sites that rarely released transmitter before conditioning became more active. We found no evidence to support previously published data showing that the increase in quantal content was inversely correlated with the initial quantal content (Larkman *et al.* 1992; Liao *et al.* 1992).

A decrease in the probability of response failures and an increase in quantal content following LTP induction has been reported by many groups (Malinow & Tsien, 1990; Bekkers & Stevens, 1990; Malinow, 1991; Liao *et al.* 1992; Larkman *et al.* 1992; Kullmann & Nicoll, 1992; Voronin *et al.* 1992). Some of these reports attribute almost the entire enhancement of the EPSC to these presynaptic changes (Malinow & Tsien, 1990; Bekkers & Stevens, 1990; Voronin *et al.* 1992). Our results do not support claims that all the potentiation is due entirely to an increased quantal content. This occurred for only two of eleven potentiated EPSCs. For one potentiated EPSC, there was no increase in quantal content.

Response failures

In many of the reports referred to above, as well as for the data in this paper, the probability of response failure is greater than would be expected from a conventional release model. Allen & Stevens (1994) provided indirect evidence that response failures are likely to be caused by failure to release transmitter following activation of the axon terminals, rather than by failure to reliably excite the axon or by conduction block. This position is supported by the results obtained from two experiments (Stricker *et al.* 1996) where intracellular stimulation of CA3 pyramidal cells was used to evoke EPSCs in CA1 pyramidal neurones. There was a very high incidence of response failures in both of these experiments as well as in similar experiments reported by Malinow (1991).

Stevens & Wang (1994) have argued that LTP can be accounted for entirely by a decrease in response failures. The results in this paper do not support their position. Firstly, we have shown that increases in quantal current are needed, in addition to increases in quantal content. Secondly, the results in Fig. 5*A* contain three EPSCs for which LTP is accompanied by either no change or an increase in response failures.

Do changes in the frequency of response failures indicate changes in release probabilities?

An entirely different interpretation of the unusually high incidence of response failures has been suggested by Liao *et al.* (1992) and by Kullmann (1994). These authors propose that in the unpotentiated state, there are many active sites at which the only openable channels are coupled with NMDA receptors. The same sites contain clusters of AMPA receptors but the channels coupled with these receptors cannot be opened until they are converted to a functional state during LTP induction. When this happens, new AMPA-mediated currents originate from these sites. When the synaptic responses are AMPA currents recorded at −70 mV, this process is indistinguishable from one where the receptors have not been modified but the release site adjacent to them begins to release transmitter. This idea, which places all the synaptic modification at the

postsynaptic membrane, fits with the results of Manabe & Nicoll (1994) using the open-channel blocker for NMDA receptors (MK-801), who showed that when transmitter release is assayed using only NMDA currents, presynaptic release probabilities do not change following conditioning stimuli.

This scheme can only account for an increase in quantal content arising from the recruitment of new sites for transmission. Increases in quantal content caused by increases in release probabilities at sites where release took place before conditioning are the result of genuine presynaptic modulation. Except for one EPSC, we were unable to show that changes in release probabilities occurred at previously active sites, because our data were incompatible with a release model having conventional statistics. The exception (12/12/91 C1, Table 1) could be modelled with binomial release statistics. For this EPSC, the release probability increased following conditioning from 0·39 ± 0·05 to 0·47 ± 0·02 and the number of release sites increased from two to five. The binomial model has been used by others (Malinow & Tsien, 1990; Bekkers & Stevens, 1990; Larkman *et al.* 1992; Voronin *et al.* 1992) to claim that release probabilities at previously active sites are increased by LTP induction. It should be pointed out that when the number of sites at which AMPA currents are generated is increased following conditioning, this does not necessarily imply that NMDA currents and hence transmitter release, were present at these sites prior to conditioning. A retrograde messenger is necessary to alter release probabilities at terminals from axons stimulated during conditioning, but this diffusible messenger for each site needs not to have arisen from the spine immediately postsynaptic to the activated axon terminal. Other sites at which AMPA and NMDA currents were present before conditioning could provide the retrograde message to sites that were genuinely silent before conditioning (Bonhoeffer, Staiger & Aertsen, 1989). Thus, an increase in the number of sites at which AMPA currents are generated following conditioning cannot be used as evidence that at the apparent silent sites before conditioning transmitter release occurred and NMDA currents were generated.

Additional evidence that release probabilities are modulated comes from four EPSCs for which the change in the probability of response failure was the opposite of what was expected from the change in the minimum number of release sites (Fig. 5*B* and *C*). For three of these EPSCs, the minimum number of active sites increased, while response failures either became more frequent (2 EPSCs) or did not change in frequency. The reverse occurred for another EPSC. These results can only be explained if some release probabilities are altered. For the first three EPSCs, some must decrease. For the last EPSC, some must increase. These results imply that the increase in quantal content following LTP induction cannot be due entirely to initiating transmission at previously quiescent sites, as the scheme proposed by Liao *et al.* (1992) and Kullmann (1994) requires.

The results in Fig. 5*B* and *C* suggest further that changes at all of the synaptic sites subjected to the conditioning stimulation are not homogeneous. In two EPSCs failures became more frequent after conditioning, and for one EPSC, LTP was accompanied by a decrease in the number of active sites. One interpretation of these results is that while some release probabilities are increased, some are decreased, while the overall effect is an increase in quantal content.

Summary

A rigorous and systematic procedure has been developed to determine the quantal parameters of EPSCs before and after the induction of LTP. This analysis has shown that LTP expression involves postsynaptic modifications to enhance the synaptic current at active sites. It has also shown that new sites become involved in transmission after the induction of LTP. This result is supported by cable analysis using the time course of EPSCs before and after conditioning to show that for some EPSCs, the locus of synaptic transmission on the dendritic tree is altered. We were unable to specify the mechanism by which new sites were activated. The conventional explanation would be that inactive release sites were converted to sites that release transmitter with reasonable frequency. However, the proposal by Liao *et al.* (1992) that the new sites appear after a batch of AMPA receptors is brought into operation at sites where previously there were no functional AMPA receptors present but where transmitter release did occur is equally compatible with most of the data. We were able to provide evidence from one EPSC for modulation of release probabilities at sites where release occurred prior to conditioning.

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