A common rule governs the synaptic locus of both short-term and long-term potentiation

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Background: At synapses between neurons in the brain, transmitter molecules are released from presynaptic terminals in multi-molecular packets called quanta. Excitatory synapses in the CA1 region of the hippocampus show a long-lasting increase in strength known as long-term potentiation (LTP), which may be important for some kinds of learning and memory. LTP can involve an increase in the number of quanta released, or in the size of the response each quantum produces in the postsynaptic cell, or both, depending on the initial condition of the synapse. These synapses also show two forms of brief potentiation: post-tetanic potentiation (PTP), which lasts for a minute or less and involves only modifications at the presynaptic terminal, and short-term potentiation (STP), which lasts rather longer. The significance of STP, the mechanisms whereby it is produced and its relationship to other forms of potentiation are poorly understood. We have studied STP electrophysiologically using slices of the rat hippocampus maintained *in vitro*.

Results: We found that STP, like LTP, can involve increases in either the number of quanta released, or their postsynaptic effect, or both. The rule governing the relative contribution from these two mechanisms appears to be the same as operates during LTP. Both the presynaptic and postsynaptic changes can develop equally rapidly and so must involve fast-acting messenger systems.

Conclusions: STP seems to be a separate phenomenon from PTP, but appears closely related to LTP. The rapidity of its onset may require a reappraisal of current understanding of the messenger systems involved in bringing about changes in synaptic strength.

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Background

At most synapses in the brain, which are chemical synapses, the presynaptic and postsynaptic structures are separated by a narrow gap, the synaptic cleft (Fig. 1) [1]. An electrical impulse, or action potential, in the presynaptic cell causes transmitter molecules to be released from the presynaptic terminal and bind to specific receptors on the postsynaptic membrane. The receptors, in many cases, have ion channels that open transiently in response to transmitter binding, and cause a potential change in the postsynaptic cell. If the direction of this potential change is depolarizing, it will make the cell more likely to fire action potentials, and the synapse is termed excitatory.

For many years, theoreticians have suggested that changes in the functional strength of individual synapses could underlie at least some forms of memory and learning. One of the most influential ideas was formulated by Hebb [2], who proposed a scheme for memory storage by synaptic modification based on the rule that synapses linking two cells that are both active at the same time will be strengthened. This theoretical proposal took on new significance with the discovery, some 20 years later, of a biological phenomenon within the mammalian brain that appeared to represent such a rule in action. This is the phenomenon known as long-term potentiation (LTP), a long lasting, use-dependent increase in synaptic strength that was first demonstrated for excitatory synapses in the hippocampus [3], a structure known to be important for memory. LTP exhibits the features of associativity and specificity required for the implementation of the Hebb rule, and has since been reported for a number of other synaptic connections within the brain. A large research effort has been directed towards understanding both the requirements for triggering LTP and the mechanisms that bring about the increase in synaptic strength [4].

Perhaps most progress has been made with the first of these issues. It has been shown that the primary trigger for LTP, at least for excitatory synapses in the CA1 region of the hippocampus, is a local elevation of the cytosolic Ca^{2+} concentration in the postsynaptic cell. Usually, the extra Ca^{2+} enters through a particular type of transmitter receptor, the NMDA (N-methyl-D-aspartate) receptor. NMDA receptors only admit Ca^{2+} if they bind transmitter and at the same time experience depolarized potential levels. Thus they implement the requirement for simultaneous presynaptic and postsynaptic activity proposed by Hebb. Many excitatory synapses are made onto spines, the narrow necks of which may restrict the spatial spread of Ca^{2+} [5,6], ensuring that neighbouring synapses are not affected and so conferring specificity (see Fig. 1). Thus we have at least an elementary understanding of the key features involved in LTP induction.

To elucidate the mechanisms by which the strengthening of the synapse is achieved, we must look in a little more detail at normal synaptic function. It is thought

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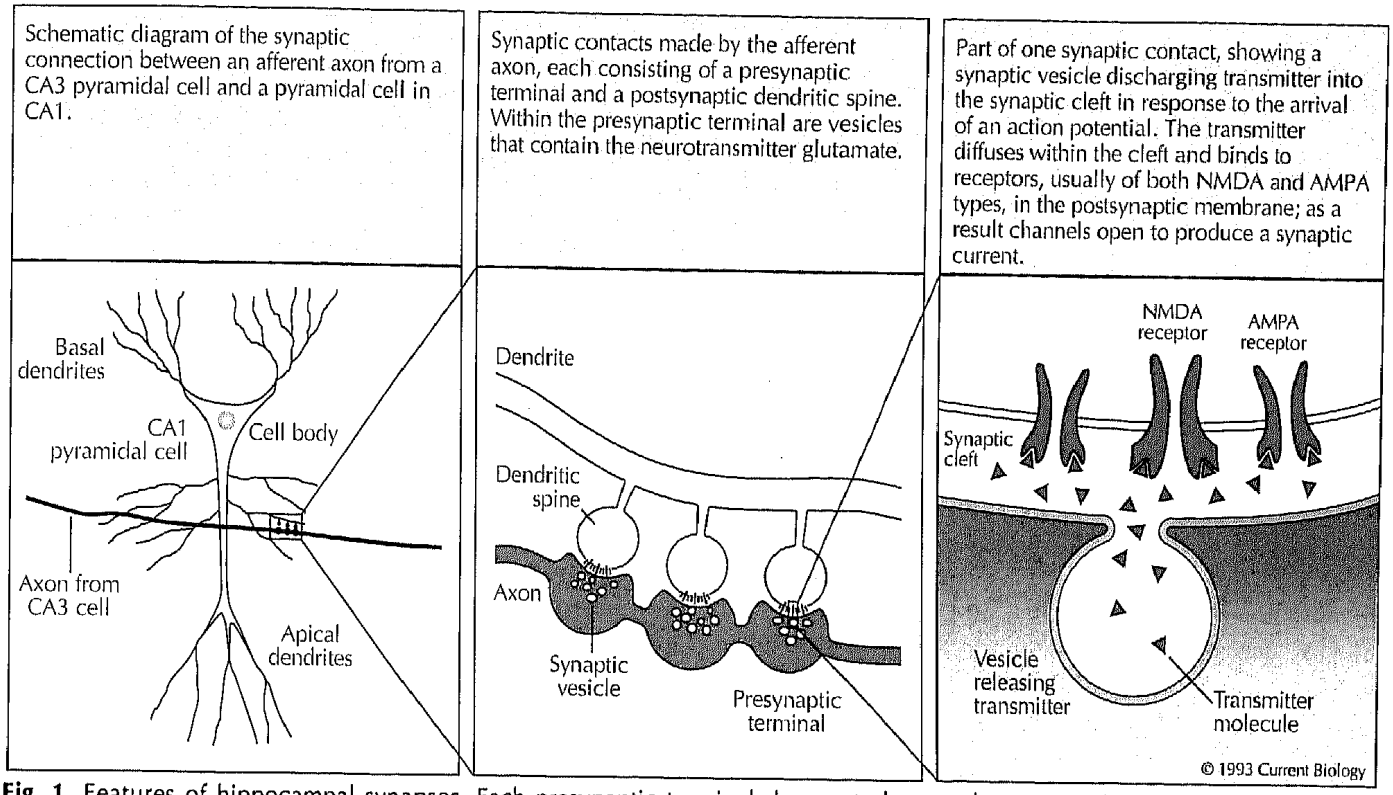

Fig. 1. Features of hippocampal synapses. Each presynaptic terminal does not always release transmitter every time an action potential arrives. On different occasions one, two, three or possibly none of the terminals may release. This gives rise to stepwise trial-to-trial fluctuations in the size of the synaptic current or potential change recorded in the postsynaptic cell.

that transmitter is released from the presynaptic terminal in discrete packets of approximately equal size, known as quanta [7]. The packets are released from a limited number of specialized release sites in a probabilistic manner, so that the number of quanta released, and hence the size of the synaptic potential produced, fluctuates from trial to trial. Quantal analysis is the statistical analysis of these trial-to-trial fluctuations, and can ideally provide estimates of the number of release sites (n), their probability of release (P) and the size of the postsynaptic potential or current produced by a single quantum, known as the quantal size (v_1). We can consider several ways by which an individual synapse could be made stronger, which include: first, increasing the number of functional release sites (n); second, increasing the probability (P) that each site will release a quantum when an action potential arrives; third, increasing the amount of transmitter released in each packet or quantum; fourth, increasing the size of the synaptic current or potential produced by the postsynaptic receptors in response to a given amount of transmitter.

The first three factors would normally be regarded as involving the presynaptic elements of the synapse, while the fourth involves changes to only the postsynaptic cell. Much research effort has been spent in trying to distinguish between these factors and something of a classic scientific 'tug-of-war' has developed between proponents of presynaptic as opposed to postsynaptic mechanisms. Initial results favoured the presynaptic camp, with reports that increased amounts of glutamate (the likely transmitter at these synapses) were released after LTP induction [8]. The pendulum swung back following elegant work showing that different types of receptors were affected differentially during LTP, suggesting a postsynaptic locus [9,10]. A heavy, and some thought final, blow was struck by the presynaptic side in showing that the statistical pattern of fluctuations in synaptic response was consistent with a mainly presynaptic locus [11,12]. The presynaptic camp has probably remained in the ascendant, in spite of further compelling evidence on both sides ([13–14] postsynaptic; [15] presynaptic; for review see [16]).

Apart from making an intriguing controversy, these issues are important for understanding the molecular mechanisms and messenger systems responsible for generating LTP. Postsynaptic changes could be brought about by changing the properties of the receptors, which may be modulated by phosphorylation [17–19] involving various kinase and phosphatase enzymes, blockade of several of which is known to affect LTP. Presynaptic mechanisms are especially interesting, as we know that LTP is triggered postsynaptically. This means that some form of message must be passed across the synaptic cleft in the retrograde direction, that is, contrary to the conventional direction of information flow across the synapse. Several interesting candidate messengers have been proposed, including arachidonic acid [20] and, perhaps surprisingly, the gases nitric oxide [21,22] and carbon monoxide [23,24].

Detailed quantal analysis has always been problematic at central synapses [25,26], and has been relatively slow to contribute to this debate. In 1991, we proposed [27] that the explanation for the low variability in the size of quanta that we observed, at least over short time scales, might be that each packet of transmitter contains

perhaps several thousand transmitter molecules, but leads to the opening of only about 100 postsynaptic channels. Thus there was usually an excess of transmitter available, so that variations in the number of molecules from packet to packet would have only a minor effect on the size of the postsynaptic response. This conclusion made the third factor in the list above an unlikely mechanism for the large changes seen in LTP, but left the other three options open.

An important breakthrough came with the demonstration that, within the same region of the hippocampus, some synapses could show presynaptic LTP, some postsynaptic LTP and others a mixture of both [28]. With hindsight, this might seem a less than surprising result, but it was of real importance in breaking the stalemate of the 'pre- versus post-' debate. This finding was soon confirmed by reports from our laboratory [29] and by Liao *et al.* [30], and now the great tug-of-war can be said to have ended in a draw. The latter two studies also provided clues as to what might determine the type of potentiation a particular synapse would show.

LTP is only one of a series of use-dependent changes that hippocampal synapses can undergo, all of which may be important in learning (or forgetting). These can include perhaps several types of long-term depression (LTD), and two briefer forms of potentiation known as post-tetanic potentiation (PTP) and short-term potentiation (STP). PTP is a very short-lasting (30 seconds to 5 minutes) enhancement observed immediately after an episode of high-frequency presynaptic firing, known as a tetanus. From work at other synapses, it is thought that PTP is both triggered and expressed presynaptically, and involves an increase in release probability (the second factor in the list above). STP can last slightly longer, but its mechanism and its relationship to PTP and LTP have always been unclear. Of particular interest is the fact that blockade of a variety of messenger and enzyme systems thought to be involved in LTP leaves STP apparently intact although abolishing LTP [4].

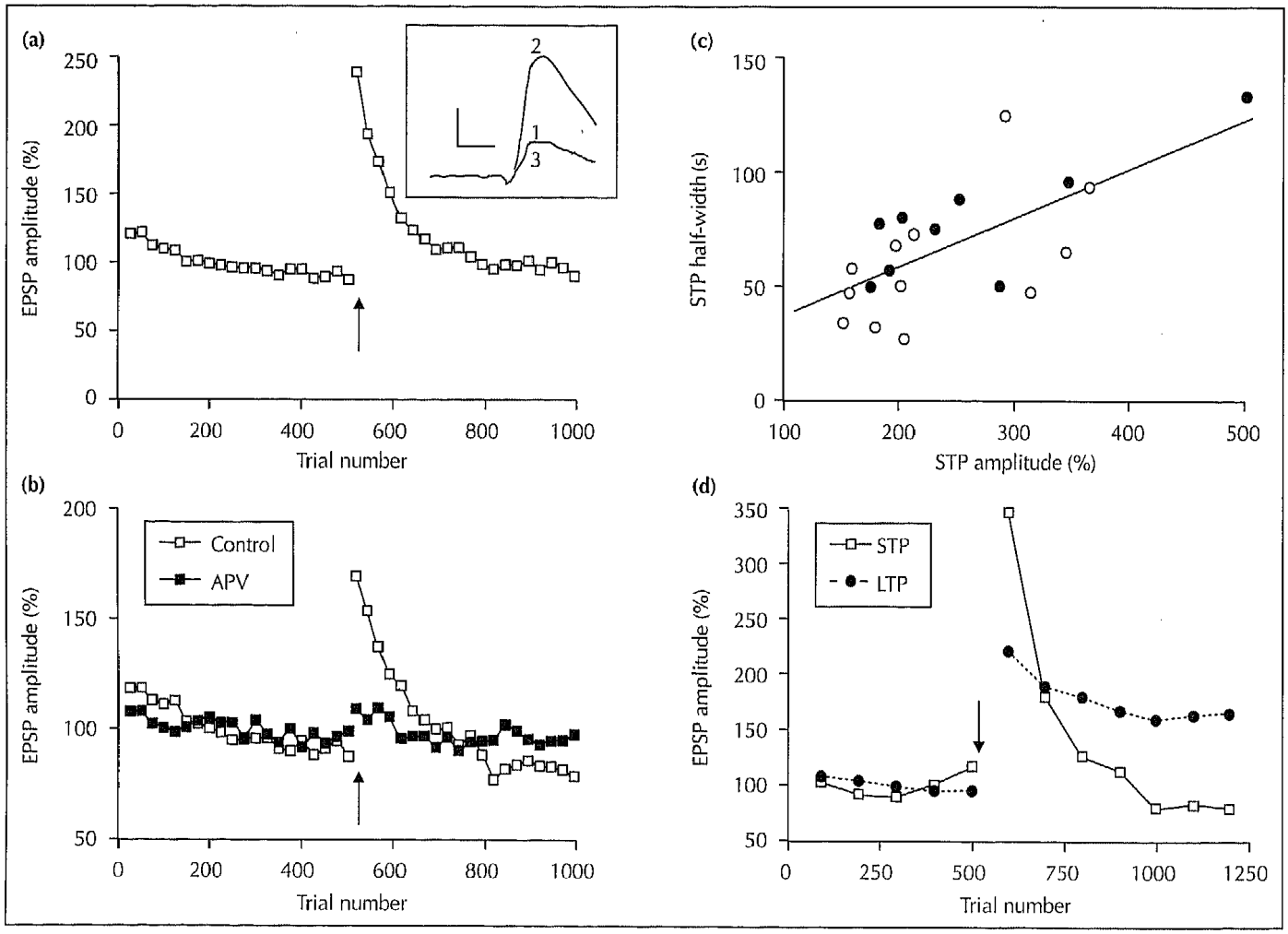

Fig. 2. Properties of STP. **(a)** Mean time course of STP for all 22 examples induced (at arrow) by either method. Inset: averages of 50 waveforms for an example EPSP taken from: 1, baseline period before induction; 2, immediately after induction; and 3, when EPSP had returned to baseline level (scale 0.5 mV, 10 ms). **(b)** Sensitivity of STP to APV: the control graph shows the mean time course of STP for all 22 experiments using the current injection method in normal medium, 10 of which showed substantial STP; the APV graph shows the mean for 12 similar experiments performed in the presence of 100 μM D–L APV, none of which showed substantial STP. **(c)** Correlation between STP duration (measured at half-maximal amplitude) and maximum amplitude (normalized to baseline). Open symbols, tetanus; filled symbols, current injection. Correlation coefficient for all points, 0.65; $p < 0.001$. **(d)** Comparison between the largest example of STP induced by tetanus (solid line) with the mean time course of 13 examples of tetanus-induced LTP from our previous study (dotted line) [29]. The STP example shows much greater initial enhancement yet decays more rapidly than LTP.

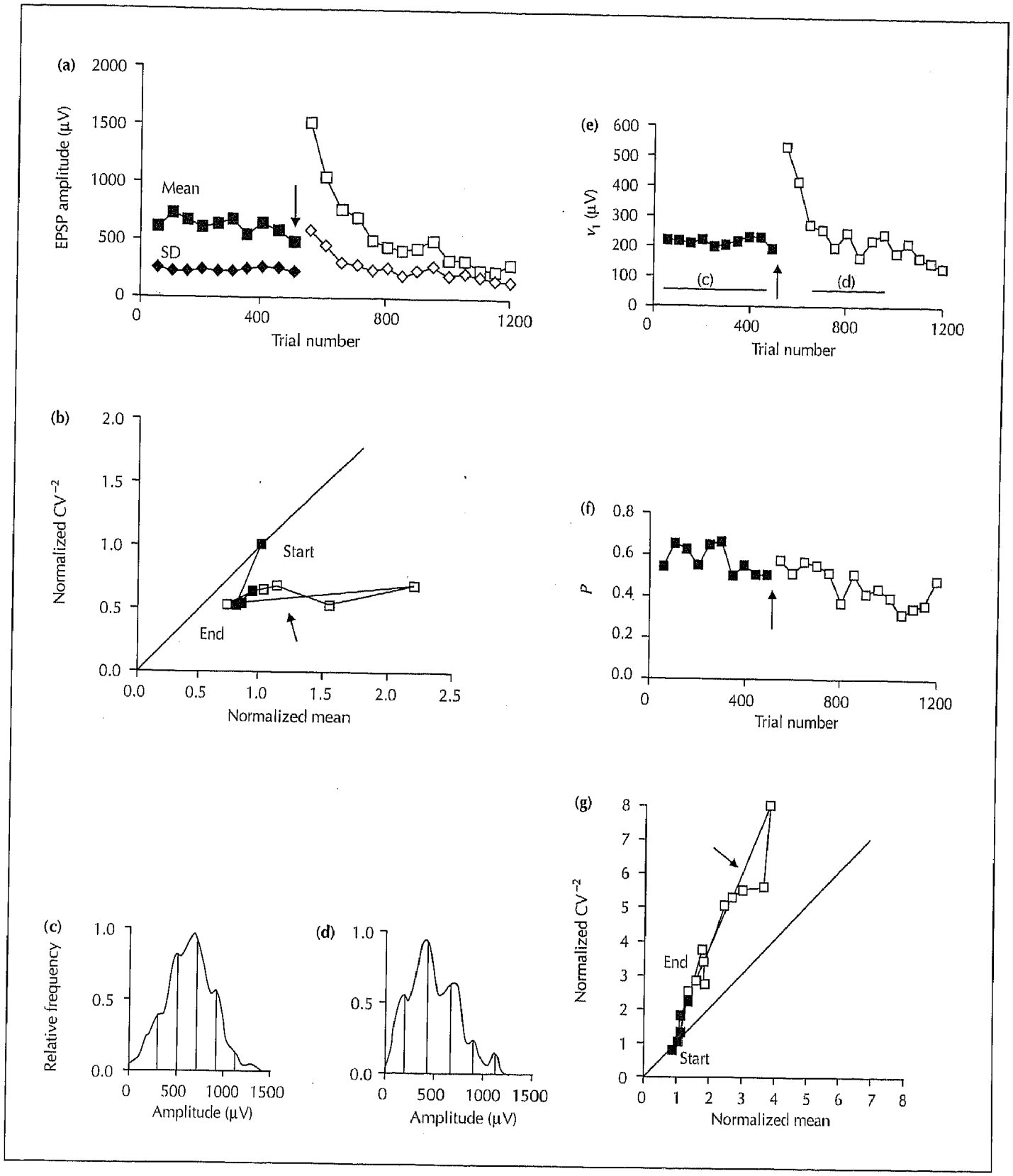

Fig. 3. Quantal analysis of STP. The analysis of one example EPSP is shown in panels a–f. **(a)** Time course of changes in EPSP mean (squares) and standard deviation (SD, diamonds), calculated every 50 trials. STP was induced by the tetanus method at the time indicated by the arrow. **(b)** Graph of $1/CV^2$ against mean for 500 trials straddling the induction of STP. The trajectory after induction is virtually horizontal, for a greater than two-fold change in the EPSP mean. This is strong evidence for the enhancement being predominantly postsynaptic. **(c)** Amplitude histogram for trials 1–460 (before tetanus). The peak spacing (vertical bars) indicates a quantal size of approximately 220 μV. **(d)** Histogram for 300 trials (650–950) during the late stages of STP, indicating a quantal size of approximately 250 μV. Autocorrelation scoring [29] indicates that the likelihood of these histograms having been generated by sampling artifact from smooth distributions was low ($p < 0.043$ for (c) and $p < 0.031$ for (d)). **(e)** Time course of the changes in v_1 (the quantal size) predicted by the binomial approximation method (see below), using $n = 5$. Note that v_1 increases by a factor of more than two following the tetanus (arrowed). The epochs used for the histograms in (c) and (d) are indicated by horizontal bars. **(f)** Time course of changes in P (release probability) predicted by the binomial method. Note that P tends to decrease throughout the whole period, with little change after the tetanus. **(g)** Graph of $1/CV^2$ against mean for an example of STP induced by the current injection method. The $1/CV^2$ trajectory is steeper than the diagonal, indicating a predominantly presynaptic change, and typical of changes in P rather than n.

In the present study, we have applied the range of quantal analysis techniques that we used previously for the study of LTP to STP. We induced STP by two different methods, only one of which involved a presynaptic tetanus, and by comparing the two we hoped to clarify the relationship between STP and PTP. But our main goal was to see if STP could involve both presynaptic and postsynaptic changes, like LTP. The clear conclusion from this study is that STP has far more in common with LTP than with PTP, and that in the light of this, we must consider carefully the results of some of the messenger and enzyme blockade studies.

Results

Description of STP

Small excitatory postsynaptic potentials (EPSPs; mean peak amplitude before STP, 0.69 ± 0.26 mV) were evoked by extracellular stimulation at 1 Hz, and, after a baseline period, potentiation was induced by either the tetanus or current injection methods (see Materials and methods). As the current injection method did not involve any change in the rate of presynaptic stimulation, it would not be expected to induce any PTP. The tetanus method might, of course, produce PTP, but after induction we paused for 1 minute before recording was recommenced, to allow PTP to decline. Under these conditions, the time course of the STP induced by both methods was similar, and the mean time course for all 22 examples is shown in Figure 2a. The mean initial increase in amplitude was by a factor of 2.4 ± 0.9, but the duration was quite brief (mean width at ½ amplitude, 67 ± 27 seconds). The STP induced by the current injection method was essentially abolished by the selective NMDA-receptor antagonist APV (Fig. 2b), indicating that it was NMDA-receptor dependent, as has been reported previously [31–33]. The duration of the STP was correlated with its initial magnitude (Fig. 2c), as reported by Malenka [32]. The largest tetanus-induced STP example showed a larger initial potentiation than was typical for LTP, but decayed much more rapidly (Fig. 2d).

Quantal analysis

The application of the quantal analysis procedures to an example of STP is illustrated in Figure 3. The details of the procedures and the assumptions on which they depend are given in Materials and methods. After a tetanus, this EPSP showed a greater than two-fold enhancement that decayed back to baseline within about six minutes (Fig. 3a). The trajectory of the graph of $1/CV^2$ against mean for trials immediately after induction was almost exactly horizontal (Fig. 3b), indicating an essentially postsynaptic change. An amplitude histogram from before the tetanus indicated a quantal size of 220 μV (Fig. 3c). This value, together with the measured EPSP mean and variance, gave the number of release sites (n) as five from the binomial approximation method.

The predicted time course for the quantal size (v_1) and release probability (P), assuming that n remained constant throughout, are shown in Figures 3e and 3f. The quantal size briefly increased to over 500 μV, while P hardly changed following the tetanus. After the STP had largely decayed, an amplitude histogram indicated a quantal size of about 250 μV (Fig. 3d), which was consistent with the prediction of the binomial approximation method. Thus, the various analysis procedures were in agreement and indicated that potentiation in this case was almost entirely postsynaptic. We repeated this analysis for the other 21 EPSPs and found that most showed mainly postsynaptic changes but some were largely presynaptic. Figure 3g shows the $1/CV^2$ graph for an example of presynaptic STP induced by the current injection method; the trajectory is steeper than

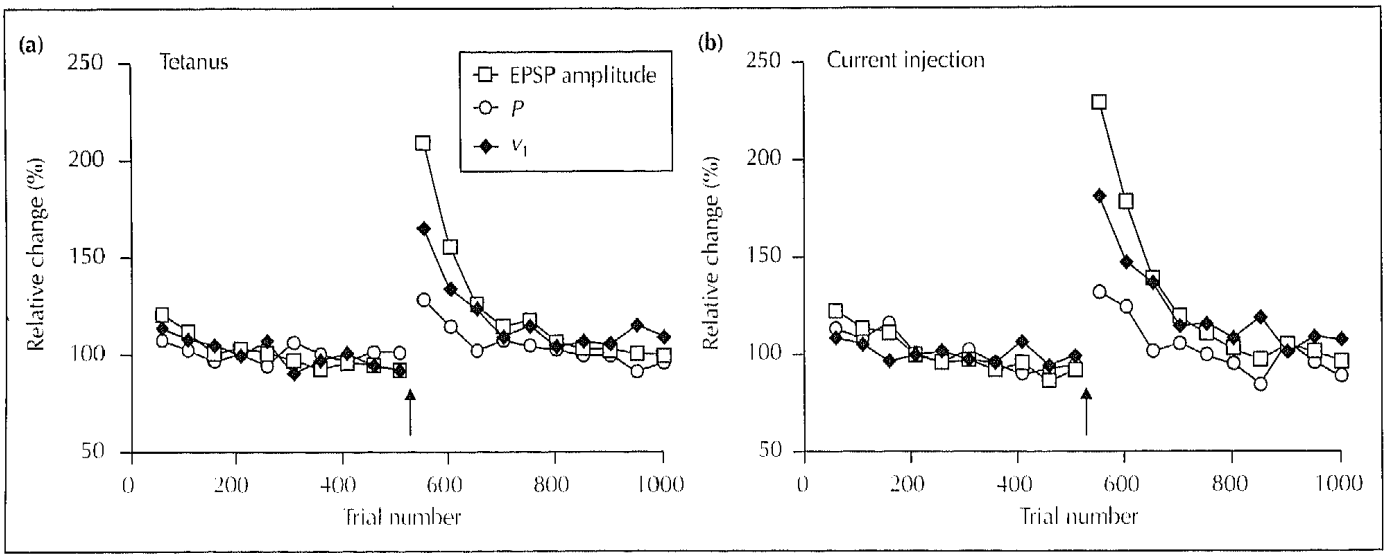

Fig. 4. Comparison of the two induction methods. Group mean time courses of changes in EPSP mean (open squares), v_1 (filled diamonds) and P (open circles), obtained using the binomial approximation method, for STP induced by tetanus **(a)** or current injection **(b)**. There are no obvious differences in the overall patterns of changes between the two induction methods, but it must be remembered that there is wide variation between EPSPs within each group. Both presynaptic and postsynaptic changes develop rapidly in both cases.

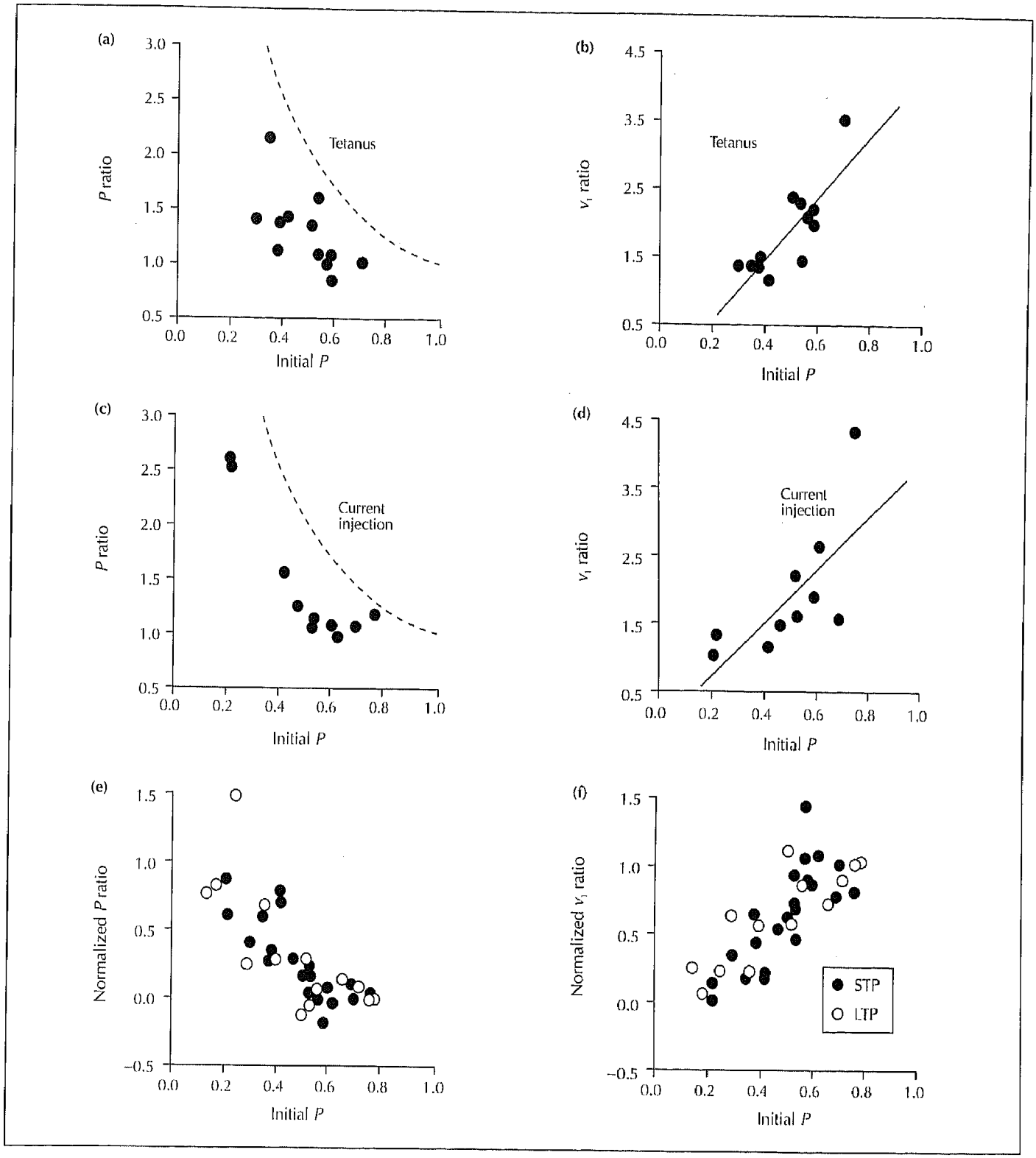

Fig. 5. Evidence that the initial release probability is a major factor determining the relative importance of presynaptic and postsynaptic changes. Panels **(a)–(d)** show that the relative contribution of changes in P and changes in v_1 to the overall enhancement is correlated with the initial P for each EPSP. P ratios were calculated as P for the 50 trials immediately after STP induction divided by P for the 50 trials immediately before, which was also taken as initial P; v_1 ratios were calculated in the same way. For both the tetanus (a, b) and current injection (c, d) groups, P ratios were negatively and v_1 ratios positively correlated with initial P. Dotted curves in (a) and (c) show the theoretical upper limit for P ratios, given that P cannot exceed 1. The least-squares linear correlation coefficients were – 0.61, 0.82, – 0.89 and 0.71 for (a)–(d), respectively. **(e, f)** The P ratios were normalized for the differing magnitude of enhancement shown by different EPSPs according to: normalized P ratio = (P ratio – 1)/(EPSP mean ratio – 1), where the EPSP mean ratio is the ratio of the EPSP mean peak amplitude for the 50 trials after STP induction to the 50 trials immediately before induction. The v_1 ratios were treated in the same way. Data for both methods of STP induction were pooled and the normalized P ratios ((e), filled circles) and v_1 ratios ((f), filled circles) plotted as functions of initial P. The correlation coefficients were 0.81 (e) and 0.78 (f). The corresponding values for 13 examples of the early stages of tetanus-induced LTP from our previous study [29] are also shown for comparison (open circles). Note that the correlations for STP and the early stages of LTP are very similar, suggesting that similar mechanisms may operate in both. Values for later stages of LTP, after STP would have declined, still showed similar correlations (not shown). Correlations with initial m, rather than initial P, gave similar results (not shown).

current injection method; the trajectory is steeper than the diagonal in this case (contrast with Fig. 3b).

The mean time course of the changes in EPSP mean peak amplitude, release probability (P) and quantal size (v_1) are shown separately for the tetanus (Fig. 4a) and current injection (Fig. 4b) groups. Both the presynaptic and postsynaptic changes developed rapidly and there were no obvious differences between the two groups.

For LTP, it has been shown that the initial condition of the synapse is an important factor influencing the relative contributions of presynaptic and postsynaptic changes to the overall potentiation [29,30]. To see if this was also true for STP, we calculated the ratios of the binomial parameters P and v_1 immediately after and before STP induction for each EPSP, and plotted these as functions of initial P (Fig. 5a–d). Both STP groups showed clear correlations. EPSPs with low initial release probabilities showed increases in release probability, whereas those with high initial release probabilities showed predominantly increases in quantal size. Variation in the magnitude of the STP between EPSPs may have contributed to the scatter in these graphs, so we normalized the P and v_1 ratios by the change in EPSP mean (Fig. 5e, f). This resulted in improved correlations, and the relationships obtained for STP were very similar to those for LTP, normalized in the same way, from our previous study [29]. The P and v_1 ratios were also correlated with initial quantal content, m (data not shown; $m = nP$), as has been shown for LTP [30]. The size or duration of the STP was not correlated with the initial release probability or quantal content.

Discussion

Several forms of short-term increase in synaptic strength have been known for many years, and these have been investigated particularly thoroughly at the vertebrate neuromuscular junction. These include, in order of increasing duration: facilitation, augmentation and PTP [34]. All of these are thought to be expressed presynaptically. The relatively slow clearance of the Ca^{2+} that enters the nerve terminal during repetitive stimulation may be at least one of the mechanisms underlying these phenomena. The elevated Ca^{2+} levels that thus persist in the terminal lead to increased numbers of quanta being released in response to subsequent action potentials — this is known as the residual Ca^{2+} hypothesis [35].

It is perhaps not surprising that, in earlier work on the hippocampus, STP was equated with PTP and thought to be entirely presynaptic [36]. This did lead to some anomalies, such as the suggestion that hippocampal PTP did not require tetanic stimulation and showed associativity [37]. Recent work has tended to link STP with LTP rather than PTP. It has been shown that STP can be blocked by APV, and so presumably involves the activation of NMDA-type receptors [31,33]. It has also been shown that PTP in the hippocampus lasts for only 30–40 seconds at 30 °C [33]. In this study, we directly compared examples of STP that had been induced by two different methods, one using a tetanus, the other not involving a tetanus or indeed any change in presynaptic stimulation rate at all. We could find no differences in the STP produced by the two methods. This suggests that STP is quite distinct from PTP, and also suggests that, like LTP, STP is triggered postsynaptically.

If STP is triggered postsynaptically, how is it expressed? In our earlier study of LTP, we found that the trial-to-trial fluctuations at these synapses conformed approximately to binomial statistics, and the changes during LTP could be accounted for without requiring a change in n. Thus, the major changes were in P (the second factor in the list given in the Background section) and in quantal size (the fourth factor in the list). We went on to look for any correlations that might indicate the operation of some rule determining the type of LTP that particular synapses might show. We found that synapses which had quite a high P in the baseline period showed mainly a change in quantal size during LTP, while those initially with a low P showed mainly an increase in P. Liao *et al.* [30] showed a similar correlation.

In the present study, we have used the same combination of quantal analysis procedures that we previously applied to LTP to show that STP can also involve both presynaptic and postsynaptic changes. Furthermore, the same rule for determining the relative contributions of these two types of change seems to apply to both STP and LTP. Why should presynaptic release probability influence the locus of the potentiation induced? For a given rate of stimulation or induction protocol, the value of P is likely to set the amount of Ca^{2+} entering the postsynaptic spine. This may influence the combination of messenger systems that are triggered. Additionally, presynaptic terminals that are already releasing with high probability may be less likely to respond to messengers calling for increased release. On the other hand, the rule might be broken if prolonged or vigorous induction procedures, perhaps giving rise to very large potentiations, were used. It might then be possible to take a synapse with an initially low P, first induce an increase in P and then go on to induce an increase in quantal size as well.

Our findings would seem to confirm the close relationship between STP and LTP. Recent work by Malenka and colleagues [32,38] has suggested that both STP and LTP are triggered by a rise in Ca^{2+} in the postsynaptic cell. Relatively small or brief rises in Ca^{2+} produce STP, whereas larger or longer rises cause LTP. As the threshold for STP induction is thus apparently lower than that for LTP, it might be expected that LTP would always be accompanied by STP, or even that STP and the early stages of LTP are one and the same. As Stevens [26] put it "STP could just be a form of LTP that

did not quite 'catch'." Our present results would certainly be consistent with this notion.

If STP and LTP are so similar, it might be expected that they would share many common mechanisms, but here there seems to be a problem. A great deal of recent work into the mechanisms of LTP expression has involved the use of blocking agents to inhibit the various messenger systems and regulatory enzymes that might be involved in up-regulation of the presynaptic and postsynaptic elements of the synaptic machinery. There is now a list of kinases, such as protein kinase C, CAM-kinase II and tyrosine kinase, blockade of any one of which will abolish LTP [39–41]. Similarly, blocking the synthesis of any of the currently favoured retrograde messenger candidates, such as arachidonic acid, nitric oxide or carbon monoxide, or scavenging the messenger molecules themselves from the synaptic cleft, also blocks LTP [21–24,42]. However, a common feature of nearly all of these experiments is that the blockers leave STP intact. Most of the blockers only have a significant effect on potentiation five minutes or more after its start.

In the present study, we have found evidence that both the presynaptic and postsynaptic components of STP develop rapidly and are usually maximal as soon as we recommence recording, about one minute after induction. One possible explanation is that, in all of these studies, the blockade has been incomplete in some way, and enough of the relevant messenger activity has survived to permit STP, but not LTP, to occur. This explanation cannot be ruled out, but we believe it to be unlikely, partly because the initial magnitude of the STP that we observe is no smaller than the initial size of LTP induced under similar circumstances. A much more exciting possibility is that there exist further intracellular and intercellular messengers, not yet identified or explored, that can act more quickly than the current candidates. These would bring about the rapid presynaptic and postsynaptic changes seen during STP, and would probably operate during the early stages of LTP as well.

Conclusions

We induced STP by two different methods: one used tetanic stimulation whereas the other did not involve any alteration to the presynaptic firing rate. There were no obvious differences in the form of the STP produced by the two methods. This suggests that STP is quite distinct from PTP and is probably triggered postsynaptically, like LTP. Quantal analysis indicated that STP induced by either method could involve an increase in the probability of transmitter release (a presynaptic modification), or an increase in the quantal size (probably postsynaptic) or a combination of the two. As for LTP, the initial condition of the synapse appeared to be a major factor in determining the relative importance of the two mechanisms. Synapses with an initially low release probablility showed mainly a presynaptic change and *vice versa*. The presynaptic and postsynaptic changes were usually maximal by one minute after the end of the induction procedure. Recent work on LTP has shown that blockade of any of a range of intercellular and intracellular messenger systems can prevent the expression of LTP. However, in most studies the blockade takes effect relatively slowly and leaves STP apparently intact. The present work suggests that novel, faster-acting messenger systems may need to be considered.

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Materials and methods

Experimental procedures

Transverse hippocampal slices were prepared from young adult rats (Sprague–Dawley, 120–180 g) and maintained in an interface-type recording chamber at 34 °C in medium containing: 124 mM NaCl; 2.3 mM KCl; 26 mM $NaHCO_3$; 10 mM glucose; 4 mM $MgSO_4$; 4 mM $CaCl_2$; 50 μM picrotoxin; 0.5 mM glutamine. Voltage recordings were made from the stratum pyramidale of the CA1 region using sharp electrodes containing 2 M $KMeSO_4$. Small EPSPs were evoked at 1 Hz via a bipolar electrode placed in the stratum radiatum, and 500 trials were recorded as baseline. Signals were filtered at 1 kHz, digitized at 5 kHz and recorded on computer disk. Potentiation was induced either by: (1) the 'tetanus method', tetanizing the small EPSP simultaneously with a larger EPSP for five episodes of 0.2 s at 100 Hz at 15 second intervals, followed by a 1 minute pause before resuming 1 Hz test stimulation; or (2) the 'current injection method', continuing a 1 Hz stimulation while depolarizing the postsynaptic neuron to about – 40 mV by steady current injection (2–2.5 nA) for 40 seconds. Using the tetanus method, clear ($\geq$ 50 %) STP was induced on 12 out of 158 attempts; LTP was induced on a further 13 occasions and these have been described previously [29]. STP could be clearly distinguished from LTP, and appreciable post-tetanic potentiation was not observed under these conditions. The current injection method never induced LTP, but STP occurred on 10 out of 22 attempts. The peak amplitude of the EPSP on each trial and the baseline noise distribution were measured as described previously [27].

Quantal analysis procedures.

We used the same combination of analysis procedures as in our earlier study to quantify the relative contribution of presynaptic and postsynaptic change during LTP [29].

Amplitude histograms: Zones of data yielding histograms with approximately equally spaced peaks from before and also, in most cases, after STP induction were selected. The EPSP amplitudes were binned finely, and the histograms smoothed using a moving Gaussian filter [27]. The mean histogram peak separation during these zones was taken to be the quantal size. In 21 out of the 22 examples of STP it was possible to measure peak spacing in histograms both before and after STP.

$1/CV^2$ graphs: The EPSP peak amplitudes were divided into epochs of usually 50 trials and the EPSP mean, standard deviation (SD, corrected for noise) and coefficient of variation (SD/mean) were calculated for each epoch. Graphs of $1/CV^2$ against mean were then produced. The trajectory of such graphs gives an indication of whether the change in mean was due to presynaptic or postsynaptic factors. Postsynaptic changes give trajectories close to the

horizontal, whereas presynaptic ones give trajectories along or steeper than the diagonal. This type of graph has been used in the study of LTP [11,12,29], and the mathematical basis for its use and interpretation is given in the first two of these papers. There are circumstances in which it can give misleading results [43], particularly if the change in mean is small, if multiple fibres are stimulated unreliably or if quantal sizes and release probabilities are very different at different sites. For all our EPSPs, we obtained at least one histogram with clear peaks, indicating that quantal variance was low and the quantal size was similar at each site. We found no evidence of intermittent stimulation (such as bimodal histograms or large failures peaks). In our previous study [29] there was good alignment between quantal size estimates from histograms and those obtained using the binomial approximation method (see below), suggesting that release probabilities are similar between sites for these synapses. Thus we believe that the difficulties that can arise with the use of these graphs are unlikely to be serious here.

Binomial approximation

In our previous study [29], we showed that transmitter release at these synapses and the changes in quantal size during LTP could be approximated using binomial statistics with n held constant. We have assumed that this will also be true during STP. For such a description:

$$\text{EPSP mean} = nPv_1 \quad (1)$$

$$\text{EPSP SD} = [nP(1-P)]^{1/2}v_1 \quad (2)$$

For any epoch for which an amplitude histogram was available, the quantal size estimated from the histogram was substituted for v_1 in equations (1) and (2) above, together with the EPSP mean and SD. The equations were then solved to obtain n and P for that epoch. n was then assumed to remain constant for all epochs, and the measured EPSP mean and variance for each epoch were used to obtain the time course of P and v_1 for the whole recording period. These assumptions appear to be valid during LTP, as the quantal sizes derived from histograms before and after potentiation aligned closely with v_1 values obtained from the binomial approximation method. We have no means of verifying this for STP, as the transient nature of the potentiation meant that histograms with reasonable sample sizes and stationarity could not be obtained during the potentiation.

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