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INTERACTION BETWEEN PAIRED-PULSE FACILITATION AND LONG-TERM POTENTIATION OF MINIMAL EXCITATORY POSTSYNAPTIC POTENTIALS IN RAT HIPPOCAMPAL SLICES: A PATCH-CLAMP STUDY

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Abstract—Long-term potentiation is an experimental paradigm used to study synaptic plasticity and memory mechanisms. One similarity between long-term potentiation and memory is the existence of several distinct phases. However, our preliminary quantal analysis did not reveal essential differences in expression mechanisms of the early (<1 h) and later (up to 3 h) phases of long-term potentiation. The data were compatible with presynaptic mechanisms of both phases. Another approach to distinguish between presynaptic and postsynaptic mechanisms is analysis of interaction between long-term potentiation and presynaptic paired-pulse facilitation. Such analysis had been previously done mainly with recordings of field potentials reflecting the activity of large neuronal populations. Only the early potentiation phase had been previously analysed with recordings from single neurons. The results from different groups were contradictory. In the present study, minimal excitatory postsynaptic potentials were recorded from CA1 pyramidal neurons of rat hippocampal slices. Paired-pulse facilitation ratios were calculated for various periods (up to 2–3 h) following induction of long-term potentiation. The ratio persistently decreased in the majority of neurons following long-term potentiation induction. The decrease in the paired-pulse facilitation ratio correlated with the magnitude of long-term potentiation and with the initial (pretetanic) facilitation ratio. Therefore, the general results of the present analysis was similar with the results of the quantal analysis: it is consistent with a strong involvement of presynaptic mechanisms in maintenance of both early and late phases of long-term potentiation. However, individual neurons could show variable changes in the paired-pulse facilitation, e.g., increases at late (>0.5–1 h) periods after tetanus. Calculations of partial correlations and regression analysis indicated that positive correlation between potentiation magnitude and initial (pretetanic) paired-pulse facilitation tended to increase in the late potentiation phase (1.5–2.5 h post-tetanus) indicating that different mechanisms are involved in the early (0.5 h post-tetanus) and the late phase of long-term potentiation.

The findings are compatible with involvement of presynaptic mechanisms in both the early and late phases of long-term potentiation. However, the results suggest that contribution of changes in release probability and in effective number of transmitter release sites may differ during the two phases. It is suggested that activation of silent synapses and increases in the number of transmission zones due to pre- and postsynaptic structural rearrangements represent important mechanisms of the late phase of long-term potentiation. © 1998 IBRO. Published by Elsevier Science Ltd.

Key words: long-term potentiation (LTP), phases, paired-pulse facilitation, excitatory postsynaptic potentials, CA1, rat.

Long-term potentiation (LTP) is a popular experimental model extensively used to study mechanisms of memory.[7,33,34,57] Induction of LTP in the hippocampus, specifically in the CA1 area, has been well studied, but mechanisms of LTP expression (maintenance) are still a matter of vigorous debate. There is no general agreement whether LTP expression is primarily due to pre- or postsynaptic changes, or both.

One hypothesis[7,14,43,44] suggests that presynaptic mechanisms are primarily responsible for the early (<1 h after induction) LTP1 phase, but thereafter (during LTP2 phase), changes in the sensitivity of subsynaptic receptors become important.

Quantal analysis techniques[42,54–56] failed to detect essential differences between LTP1 and LTP2 phases.[27,58] No essential increases in the quantal efficacy during LTP2 were found[27,58] contrary to what is predicted from the above hypothesis on delayed changes in postsynaptic receptor sensitivity.[7,14,43,44] Another traditional method to

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; EPSP, excitatory postsynaptic potential; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PPF, paired-pulse facilitation.

distinguish between pre- and postsynaptic locations of LTP mechanisms is based on analysing changes in paired-pulse facilitation (PPF) following LTP induction.[38]

PPF is an increase in a second "testing" postsynaptic response elicited shortly after a first "conditioning" response. The underlying mechanism is an increased probability of transmitter release to the second presynaptic volley due to a residual Ca^{2+} from the first volley.[26,70] The presynaptic mechanism of PPF is supported by numerous data known for various synaptic junctions[70] including hippocampal synapses.[6,19,21,22,66,67] Therefore, PPF ratio should decrease following LTP induction if changes in presynaptic release probability contribute to LTP maintenance.[38]

The initial study[38] with recordings of field potentials from *in vivo* dentate gyrus found no persistent (>10 min post-tetanus) changes in the PPF ratio. However, the result was challenged by more recent studies in the same region[12,29] which showed persistent PPF reduction correlated with LTP magnitude.[12] Similarly, in CA1 early studies of field potentials found no PPF changes after LTP induction.[11,40,49,69] However, decreases in PPF were often obtained when it was calculated from intracellular recordings of small ("minimal") excitatory postsynaptic potentials (EPSPs).[30,59] It had been suggested[59] that intracellular EPSPs were a more accurate measure of PPF changes because, unlike field potentials, they were less contaminated by polysynaptic (including inhibitory) events and could not be contaminated by generation of postsynaptic spikes. However, significant changes in PPF have not been obtained in some more recent intracellular studies.[1,19,37]

In a previous examination of PPF/LTP relations with field potential recordings[28] we explored a possibility that the above inconsistencies are caused by the fact that Kuhnt and Voronin[30,59] used minimal stimulation currents which might activate inputs with properties different from those activated by stronger stimuli used in other PPF studies[1,37,40,49,69] (but see Ref. 19 with minimal stimulation). Therefore, strong test stimuli were used and early parts of the initial slopes of field potentials were measured.[28] Variable changes in PPF ratio had been found in different experiments, but the averaged PPF showed a significant decrease. The decrease was positively correlated with LTP magnitude (as it had been shown previously with intracellular recordings)[30] and inversely correlated with the pretetanic PPF ratio.[48] A detailed regression analysis[28] suggested that the correlation between PPF changes and its pretetanic value was secondary and resulted from two stronger primary correlations. The primary correlations were: (i) between PPF changes and LTP magnitude[30] and (ii) between LTP magnitude and the pretetanic PPF ratio[59] (compare with a somewhat similar correlation in a different synaptic system[68]). The correlation between PPF changes and LTP magnitude prevailed during the initial 10 min following tetanization while the correlation between LTP magnitude and the pretetanic PPF ratio prevailed 40–60 min later. In general, the correlation analysis suggests that the early (10–20 min) and late (>40–50 min) LTP phases are mediated by different mechanisms but both include presynaptic changes.

The aim of the present study was to expand the previous analyses of PPF/LTP interaction using lasting (up to 3 h) recordings of minimal EPSPs from single neurons. We hoped to find additional confirmations of the postulated difference between mechanisms of LTP1 and LTP2 at the single neuron level and to test the above hypothesis on the nature of this difference.[7,14,43,44] To facilitate comparison with the majority of previous studies, we blocked $GABA_A$-mediated inhibition that had not been done in our previous studies of PPF/LTP interaction.[28,30,59]

EXPERIMENTAL PROCEDURES

Experiments were performed on hippocampal slices from five- to six-week-old male Wistar rats according to standard procedures.[2] The rats were bred at the Federal Institute of Neurobiology, Magdeburg, Germany. The slices were superfused (3 ml/min) with a solution containing (in mM): NaCl 124, KCl 1.5, $MgSO_4$ 1.3, $CaCl_2$ 2.45, KH_2PO_4 1.25, $NaHCO_3$ 25, glucose 10 and 100 μM picrotoxin at 30°C. Tetrodotoxin (2 nM) was added to prevent seizure activity. Recording patch pipettes were filled with a solution containing (in mM): K^+ gluconate 135, KCl 5, $MgCl_2$ 2, HEPES 10, glucose 20 (pH 7.2). EPSPs were recorded in the current-clamp mode from CA1 pyramidal cells. They were evoked by double-pulse minimal stimulation of stratum radiatum (0.2 ms stimulus duration, 50 ms within each pair, 6 or 8 s between pairs). LTP[7,43] was induced by three trains (1 s, 100 Hz with 0.4–0.6 ms stimulus duration, 20 s intervals) accompanied by additional 20 mV depolarization. Between 40 and 300 responses were collected before tetanization and up to 2000 responses thereafter. Covariance measures based on the principal component analysis[9] (so-called "first principal component scores") were used to evaluate the EPSP magnitudes according to a computer algorithm considered elsewhere.[2] As a distinction from the traditional peak amplitude or slope measurements which use comparatively narrow windows, the component score uses measurements over a broad window covering the whole of the initial slope and the EPSP peak. Therefore, the component scores are preferable for minimal EPSPs because, having a larger empirical base, they are more reliable and less dependent on the noise than slopes or peak amplitudes.[9] Our control evaluations showed that this measure gave very similar and strongly correlated estimates of PPF and PPF changes as compared to the traditional peak amplitude or EPSP slope measurements. For brevity, the first component scores ("covariance amplitudes") will be termed "amplitudes".

PPF was calculated as the ratio between the mean amplitudes of the second (EPSP2) and the first (EPSP1) responses. The PPF ratios were calculated from plateau regions[25,61] which were defined as continuous regions without a significant amplitude drift over 40–300 trials (typically 120–200 trials). The amplitudes of both the EPSP1 and EPSP2 were normalized to the "baseline amplitude" (i.e. the mean amplitude over the pretetanic plateau region). "PPF change" was expressed as the difference between the percentage of the ratio of the post-tetanic PPF to baseline PPF and 100%, i.e. 0 corresponded to no change in PPF. "LTP magnitude" was defined similarly: as the difference between the percentage of the ratio of the post-tetanic EPSP1

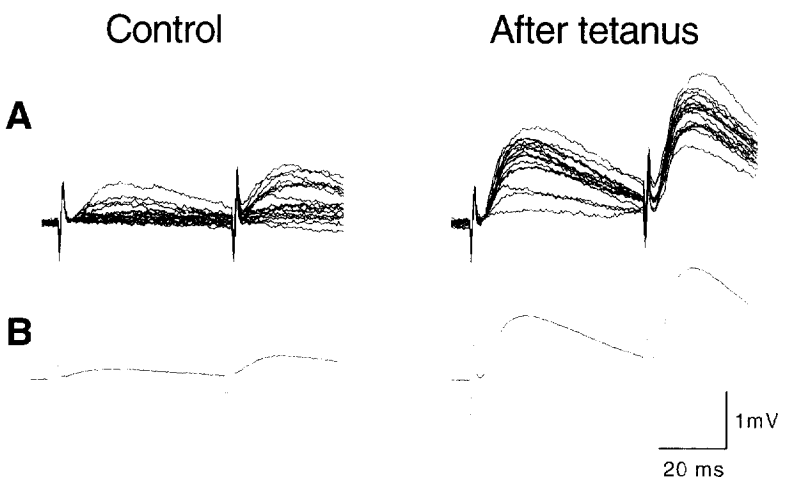

Fig. 1. Examples of single (A) and averaged EPSPs recorded before tetanization (Control) and following tetanization (After tetanus). Note large increases in the average amplitudes after tetanus due to both the disappearance of apparent response failures and an increase in the number of large responses.

amplitude to baseline amplitude and 100%. The data are given as means ± S.E.M.). A *t*-test was used and *P* values <0.05, if not otherwise specified, were taken as significant.

Correlation and regression analyses were performed according to standard methods[45] with calculation of both Pearson's product moment correlation coefficient (r) and the partial correlation coefficient. The latter describes the relation between a dependent and an independent variable and excludes the influence of a third variable.[45] Multiple regression analysis,[4] which assumes that the relation between four variables has the form: $y=b_0+b_1x_1+b_2x_2+b_3x_3$, was also done. Regression coefficients b_i were calculated together with their significance levels. For better comparison of influences of different variables, "standardized regression coefficients"[4] were used: BETAi=(Standard deviation of x_i)/(Standard deviation of y).

RESULTS

Variability of post-tetanic paired-pulse facilitation changes

Figure 1 demonstrates an example of recorded single (Fig. 1A) and averaged EPSPs (Fig. 1B) before (Control) and after tetanus which induced large LTP. Both LTP magnitudes and changes in PPF ratios varied in different neurons. Figure 2 shows measurements from four neurones with various LTP magnitudes (upper graphs, dots) and different PPF changes (lower graphs, triangles). Following LTP induction, PPF either persistently decreased (Fig. 2A, C), or showed either variable changes with a transient (<1 h) reduction (Fig. 2B) or predominant increases (Fig. 2D).

In spite of the variations in the post-tetanic PPF changes in individual neurons (Fig. 2), the averaged data (Fig. 3B) show that PPF decrease was the most typical consequence of LTP induction. In Fig. 3A, post-tetanic changes in both EPSP1 (dots) and EPSP2 (squares) are shown for comparison with PPF changes in Fig. 3B. Figure 3B shows that the average PPF decrease persisted throughout the post-tetanic recording period which was at least 1 h (up to 2 h for the majority of the cells and up to 3 h for seven neurons).

Relationships between the mean time-courses of paired-pulse facilitation changes and long-term potentiation

Correlation of paired-pulse facilitation changes with LTP magnitude have been previously established from intracellular recordings for the initial 0.5 h period following LTP induction[30,59] and also from field potential recordings for the initial 1 h post-tetanus.[28] In the present study, we explored similar correlations over longer periods included presumed LTP1 (<1 h) and LTP2 (1–3 h) phases (see Introduction). First of all, we compared changes in the EPSP amplitudes (Fig. 4, left column) and PPF (Fig. 4, right column) for groups with different LTP magnitudes and time-courses. The following five groups were isolated: cases with a small (0–50%) LTP magnitude during initial 0.5 h (Fig. 4A), with a decremental potentiation (Fig. 4B) and with a persistent LTP of three different magnitude ranges (Fig. 4C–E). Figure 4A–E (right column) shows that the average PPF changes mirrored inversely the post-tetanic amplitude changes in the different groups (Fig. 4A–E, left columns) in spite of the above illustrated variations in individual cases (Fig. 2).

Correlations between pretetanic paired-pulse facilitation ratios, changes in paired-pulse facilitation and long-term potentiation magnitudes

The dependence of PPF changes on LTP magnitudes was additionally supported by the correlation analysis. Figure 5A plots the respective individual values for three initial time-periods (0.5, 1 and 1.5 h). Table 1 presents r also for later time-periods and

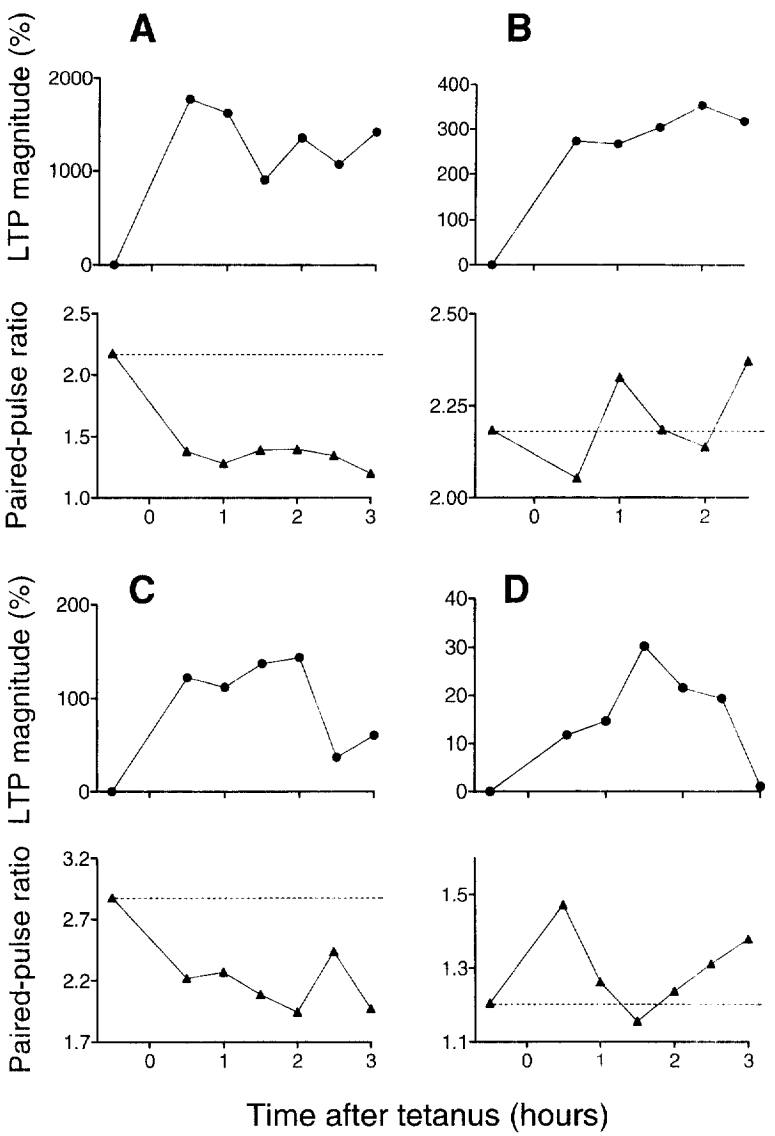

Fig. 2. Variability of changes in paired-pulse facilitation. LTP magnitudes (upper graphs, dots) and changes in PPF (lower graphs, triangles) in four neurons with different LTP magnitudes. LTP magnitudes were calculated for the first EPSPs in the paired-pulse paradigm. The sample sizes were n=40, 100, 120 and 200 (for A–D, respectively) for the pretetanic regions and n=200, 120–220, 260–300 and 240–300 for the post-tetanic regions. The dashed horizontal line, in this and other figures, indicates the pretetanic baseline. Note that PPF ratios decreased in the cases with large LTP (A) and large pretetanic PPF ratios (C) whereas it showed more variable changes in the cases with a small pretetanic PPF (B) and small LTP (D). In contrast, the increases in m (open circles in upper graphs) were persistent in cases with both large (A, B) and small (C, D) LTP.

includes data on significance levels. Highly significant inverse correlations between PPF changes and LTP magnitudes for all post-tetanic periods are evident from Fig. 5A and Table 1. Note that a sufficiently large LTP (more than three-fold) was always accompanied by PPF reduction (Fig. 5A). With a more moderate LTP, the changes in the PPF ratios were more variable and could consist in either increases or decreases. The smallest LTP magnitudes (<100%) were accompanied predominantly by increases in the PPF ratios (Fig. 5A).

It has been established from recordings of the field potentials[28,48] that changes in PPF during initial LTP periods inversely correlated with pretetanic PPF ratios. Figure 5B confirms this for the EPSP recordings (Fig. 5B, 0.5 h) and shows that the correlation tended to increase with time after tetanus (Fig. 5A, 1 and 1.5 h; see also Table 1).

The existence of the two inverse correlations—between PPF changes and LTP magnitudes (Fig. 5A) and also between the former and the pretetanic PPF ratios (Fig. 5B) predicted a positive correlation

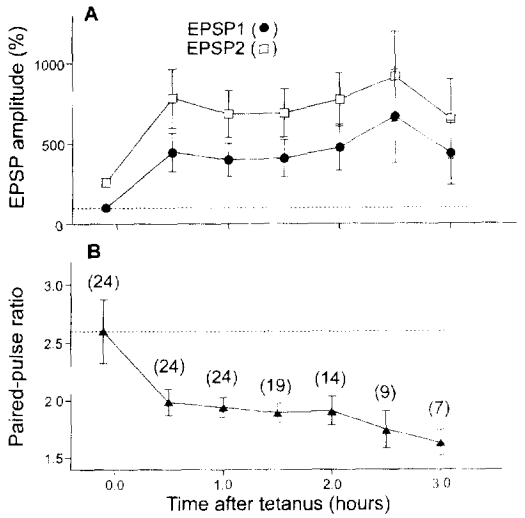

Fig. 3. Changes in EPSP amplitudes (A) and PPF ratios (B) averaged across all recorded neurons. Dots and squares in A refer to the first (EPSP1) and second (EPSP2) responses, respectively. Numbers in the parenthesis above the graph in B show the sample sizes. See Fig. 2 for additional notations. Here and in other graphs, S.E.M. values are indicated by vertical bars except when smaller than symbols.

between the pretetanic PPF ratios and LTP magnitudes[28,59] which was confirmed here for the early (0.5 and 1 h) periods and was also shown for later periods (Fig. 5C, Table 1). However, the correlation appeared to be smaller as compared to that from the field potential recordings for the initial 1 h post-tetanus.[28] Moreover, the correlation disappeared from all post-tetanic periods, except one (1.5 h), when the outlet with a very large pretetanic PPF ratio was excluded (Fig. 5C and Table 1, numbers in parenthesis). Note that for the 1.5 h period, r also significantly decreased (from 0.90 to 0.38). In contrast, the same procedure for other two correlations (Fig. 5A, B) only diminished r, but r was still statistically significant for all post-tetanic periods (Fig. 5A, B; Table 1). In general, the correlation between the pretetanic PPF ratio and LTP magnitude (Fig. 5C) appeared to be lower than the correlation between pretetanic PPF and PPF changes (Fig. 5B) in contrast to previous finding from recordings of the field potentials.[28]

To further illustrate the dependences of both LTP magnitudes and PPF changes on the initial PPF ratios we divided the data into two subsets depending on whether the pretetanic PPF ratio was small or large as it had been done for the recordings of field potentials.[28] We chose the border for this division at the pretetanic PPF ratio equal to 2 (Fig. 6). Similar to the field potential data,[28] large LTP (Fig. 6A) and a decrease in the mean PPF ratios (Fig. 6B) were observed in experiments in which the pretetanic PPF ratio was large. In experiments with smaller pretetanic PPF ratios, LTP was significantly smaller and decrementing (Fig. 6C) and it was not accompanied by significant PPF changes (Fig. 6D). The post-tetanic PPF ratio in the group with the large pretetanic PPF (Fig. 6B) appeared to be close to the mean PPF ratio in the group with the low pretetanic PPF (Fig. 6D).[28] In addition, Fig. 6 shows that in spite of the less impressive correlation between the pretetanic PPF and LTP magnitude in the present study (Fig. 5C) compared to other correlations (Fig. 5A, B) and to a previous study,[28] there was a statistically significant difference between LTP magnitudes for inputs with small (Fig. 6C, dots) and large pretetanic PPF (Fig. 6A, dots).

Partial and multiple correlation analysis differentiated early and late long-term potentiation periods

The results of the correlation analysis for different post-tetanic periods presented in Fig. 5 are summarized in Table 1. In addition, Table 1 shows r for later (2 and 2.5 h) periods with smaller sample sizes. In general, Table 1 confirms the persistence of correlations between LTP magnitudes and PPF changes, and also between the latter and the pretetanic PPF ratios. It also shows that the correlation between LTP magnitudes and pretetanic PPF ratios was less reliable and largely depended on one case with a very strong pretetanic PPF. The mutual correlations between the three variables suggest that some of these correlations might be primary while the others might represent their corollaries.

To uncover the secondary correlations, we calculated partial correlation coefficients (Table 2) which allowed the relations between two variables to be determined while eliminating the influence of a third variable.[45] However, the results (Table 2) were not as clear as the results of similar calculations for the field potential recordings.[28] The latter revealed that the correlation between the initial PPF ratio and PPF changes[48] was secondary for all post-tetanic periods (see Introduction) whereas Table 2 shows that it was comparable to other two correlations. Nevertheless, Table 2 shows that similarly to the previous data,[28] the correlation between LTP magnitudes and PPF changes for the initial LTP period (Table 2, 0.5 h) was higher than that between LTP magnitudes and pretetanic PPF ratios which was statistically insignificant. For later post-tetanic periods (1.5 and 2.5 h), the correlation between LTP magnitudes and pretetanic PPF ratios was highest, suggesting different mechanisms for the maintenance of the earlier and later phases of LTP (see Discussion).

Another approach for evaluating associations between several variables is the multiple regression analysis[4] which we used in the previous study with field potential recordings.[28] The initial (pretetanic) EPSP amplitudes were included in this analysis, in addition to the pretetanic PPF ratio, PPF change and LTP magnitude. Preliminary evaluation of the associations between the pretetanic EPSP amplitudes and other variables showed that the respective Pearson's

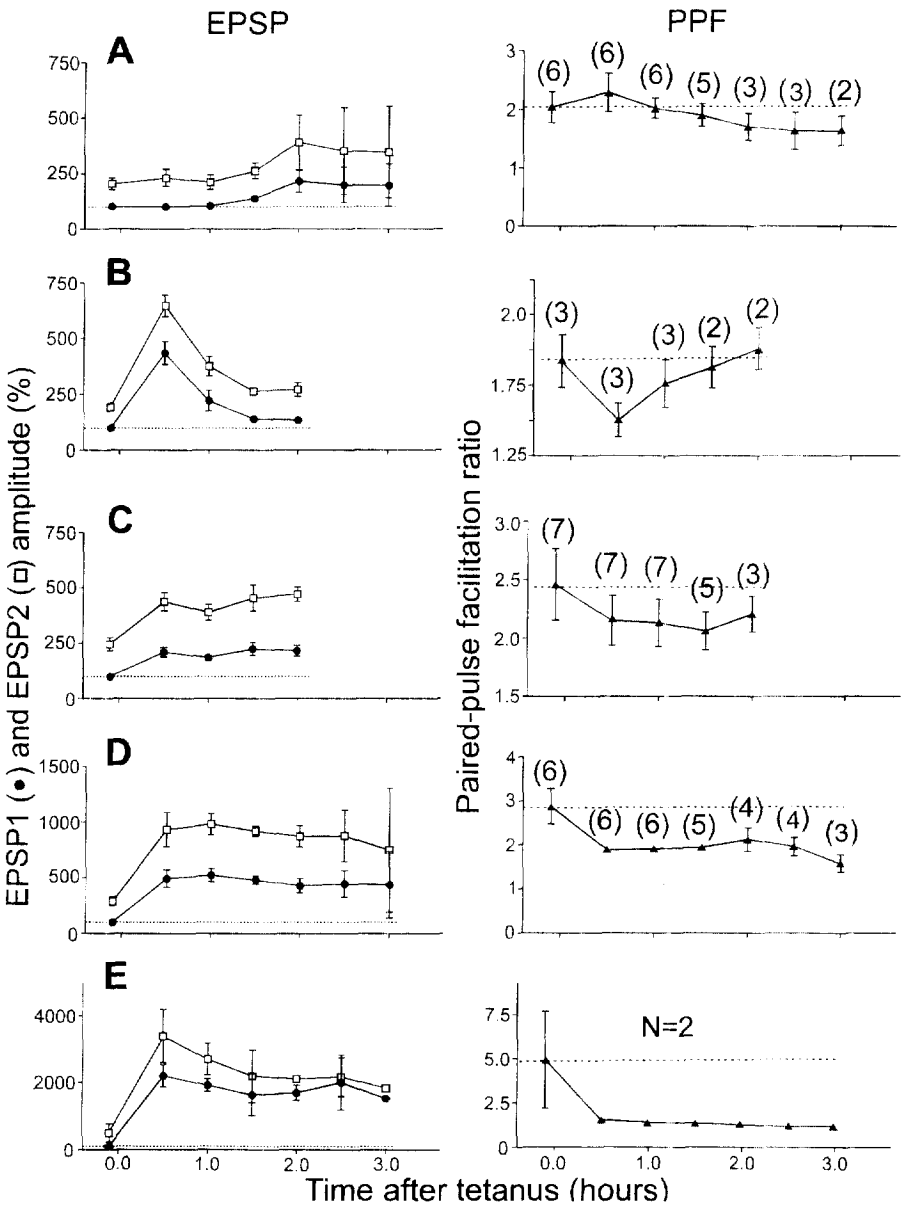

Fig. 4. Averaged changes in EPSP amplitudes (left column) and PPF ratio (right column) in groups of neurons divided according to initial LTP magnitudes and LTP time-course. The division was made according to the following criteria: LTP magnitude 0–50% (A), decremental potentiation (B), persistent LTP of 50–150% (C), of 300–800% (D) and >1400% (E). See Figs 1 and 2 for additional notations.

correlation coefficients could be as high as 0.40. This suggests that the pretetanic EPSP amplitudes might affect multiple correlations. To evaluate the relative contributions of the different factors to LTP maintenance, LTP magnitude was classed as a dependent variable and the three other values (pretetanic EPSP amplitudes, pretetanic PPF and PPF change) were treated as independent variables in the multiple regression. The analysis calculated the multiple regression coefficients, their significance levels (P) and standardized regression coefficients ($BETA_i$).[4] Figure 7 plots the standardized regression coefficients for different post-tetanic periods. Although most of the regression coefficients appeared to be statistically insignificant (Fig. 7), probably due to small samples (especially for the late LTP periods), the general pattern was similar to that in the previous study with field potentials.[28] The initial (pretetanic) EPSP amplitude (Fig. 7, open circles) did not influence LTP magnitude. The two other independent variables contributed approximately equally to the LTP magnitude during the early (0.5 h) post-tetanic period. However, the regression coefficient for the relationship between LTP magnitude and initial PPF (Fig. 7, squares) tended to increase with time. It was larger for the late post-tetanic periods (1.5 and 2.5 h)

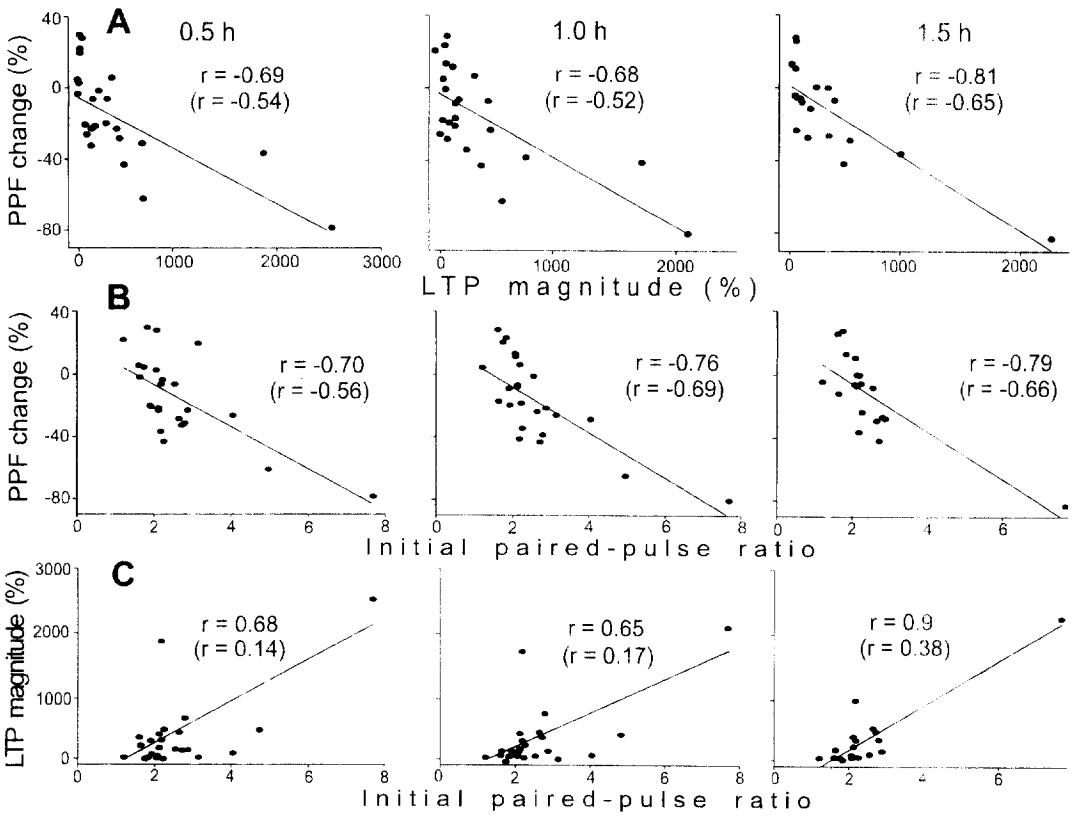

Fig. 5. Correlation analysis of relations between changes in PPF ratios, their pretetanic values and LTP magnitudes for different post-tetanic periods. (A) Correlation between LTP magnitude and PPF change. Insets here and in B and C give the coefficients of correlation (r). Numbers in parenthesis give r calculated after deletion of the outlying case with the largest LTP and initial PPF. (B) Correlation between pretetanic PPF ratio and LTP magnitude. A and B show that PPF decreased in most of the cases, but it could increase, especially when LTP magnitude (A) and pretetanic PPF ratio (B) were small. (C) Correlation between LTP magnitude and PPF change. Note that unlike the plots in A and B, the correlation practically disappeared when the case with the largest pretetanic PPF was deleted (numbers in parenthesis). The best-fit linear regression lines are given. In A: $y=0.84-0.03x$ (0.5 h), $y=0.55-0.04x$ (1 h), $y=5.0-0.04x$ (1.5 h); in B: $y=20-13x$ (0.5 h), $y=23-15x$ (1 h), $y=25-15x$ (1.5 h); in C: $y=357+317x$ (0.5 h), $y=281+268x$ (1 h), $y=416+337x$ (1.5 h). See Table 1 for significance levels of r.

as compared to the early periods (0.5 and 1 h) and was statistically significant 1.5 h following LTP induction.

DISCUSSION

Paired-pulse facilitation decreases correlated with long-term potentiation magnitudes suggest presynaptic involvement in maintenance of both long-term potentiation phases

The present study demonstrates significant PPF changes following LTP induction in CA1. Together with similar previous result for the same[28,30,48,59] and other hippocampal areas[12,49,69] it indicates a strong involvement of presynaptic mechanisms in LTP maintenance. The present correlation analysis expands the previous ones[12,28,30,48,59] and demonstrates strong correlations of PPF changes both with LTP magnitudes and pretetanic PPF. These findings suggest a reason why several groups[1,11,19,37,40,49,69] failed to reveal a significant PPF/LTP interaction. Because of the above correlations, small and bidirectional PPF changes could be missed when LTP was relatively small or could be masked when cases with small LTP magnitudes are averaged with those in which LTP was large.[28,48] Notice that here and previously[28,30,59] we used experimental conditions favourable for induction of a strong LTP. Similarly to most of the other authors (but see Ref. 19) we did not include in our analysis experiments in which LTP failed to be induced. We used picrotoxin with the aim to test whether the difference between our previous studies of the PPF/LTP interaction[28,30,59] and those of others[1,11,19,37,40,49,69] could depend on different states of inhibition. Comparison with the previous data[28,30,59] shows that the general pattern of PPF changes and respective correlations did not depend on $GABA_A$ inhibition blockage. In addition, data of others[48] show that the general results is not influenced by $GABA_B$-mediated inhibition as well. Further reasons for the lack of PPF changes in other publications have been recently discussed,[28] and the

Table 1. Correlation coefficients and their significance levels for relations between changes in paired-pulse facilitation, initial (pretetanic) paired-pulse facilitation and magnitudes of long-term potentiation

Correlated values	0.5 h	1 h	1.5 h	2 h	2.5 h
LTP/PPF_{ch}	−0.69	−0.68	−0.81	−0.77	−0.87
P	<0.001	<0.001	<0.001	<0.001	<0.001
	(−0.54)	(−0.52)	(−0.65)	(−0.50)	(−0.50)
P	<0.01	<0.01	<0.001	<0.01	<0.01
PPF_{ch}/PPF_{in}	−0.70	−0.76	−0.70	−0.80	−0.88
P	<0.001	<0.001	<0.001	<0.001	<0.001
	(−0.56)	(−0.69)	(−0.66)	(−0.58)	(−0.66)
P	<0.01	<0.001	<0.001	<0.01	<0.01
LTP/PPF_{in}	0.68	0.65	0.90	0.75	0.90
P	<0.001	<0.001	<0.001	<0.001	<0.001
	(0.14)	(0.17)	(0.38)	(0.10)	(0.12)
P	n.s.	n.s.	<0.05	n.s.	n.s.

The Pearson product-moment correlation coefficients (r) are given for six post-tetanic periods. The number of measurements n=24 for 0.5 and 1 h and 19, 14 and 9 for the three later periods, respectively. Numbers in the parentheses are the respective data for the same samples but without one item with the largest pretetanic PPF, LTP and PPF change (the outlet in Fig. 5). PPF_{ch}, changes in PPF; PPF_{in}, initial PPF; P, significance levels (two-tailed test); n.s., not significant (P>0.05).

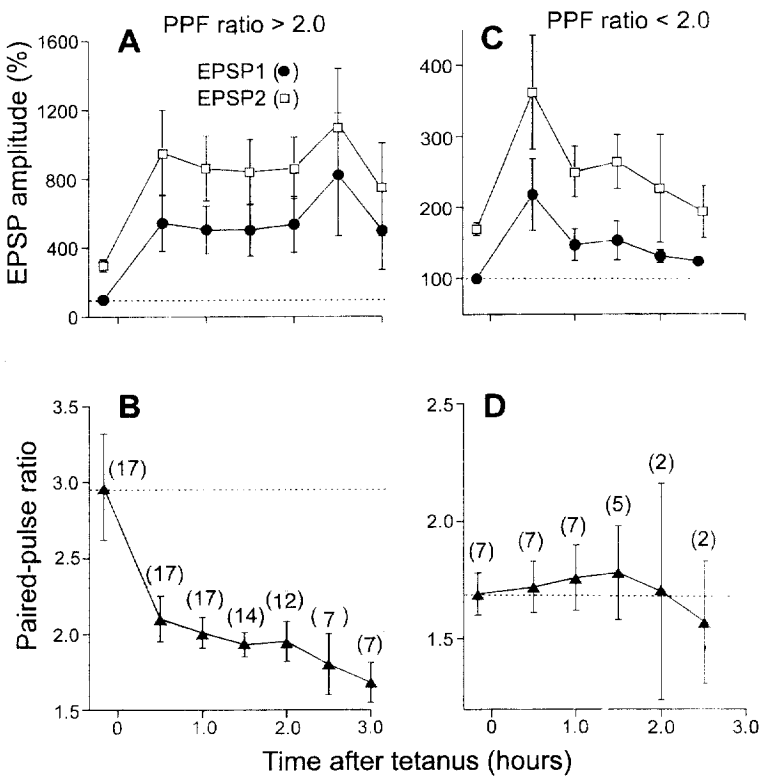

Fig. 6. Graphs showing dependences of post-tetanic changes in EPSP amplitudes (A, C) and changes in PPF ratios (B, D) on the pretetanic PPF ratio. All data were divided into two groups depending on whether the pretetanic PPF ratio was above (A, B) or below 2. (C, D) Note that the average PPF decreased in inputs with larger pretetanic PPF ratios (B) while low pretetanic PPF ratios were associated with the lack of essential post-tetanic changes in PPF (D). Note that LTP magnitudes (A, C, dots) also differed in these groups.

noted variable post-tetanic PPF changes (Fig. 2B, D) could mask PPF changes when they are averaged over long post-tetanic periods. In general, the present and previous works[12,28,30,48,59] stress that considerations of the LTP/PPF interaction should include adequate correlation analysis to be conclusive.

The major aim of the present work was to extend the analysis of PPF/LTP interaction with recordings of intracellular EPSPs over lasting periods (up to 2–3 h post-tetanus) including both the initial (LTP1) and later (LTP2) phases. The main result was that the average PPF decrease was similar for the two LTP

Table 2. Partial correlation coefficients for relations between changes in paired-pulse facilitation, initial (pretetanic) paired-pulse facilitation and magnitudes of long-term potentiation

Correlated values	0.5 h	1 h	1.5 h	2 h	2.5 h
LTP/PPF_{ch}	−0.41	−0.38	−0.37	−0.43	−0.38
P	<0.05	n.s.	n.s.	<0.05	n.s.
PPF_{ch}/PPF_{in}	−0.44	−0.57	−0.24	−0.53	−0.45
P	<0.05	<0.01	n.s.	<0.01	<0.05
LTP/PPF_{in}	0.38	0.28	0.72	0.35	0.57
P	n.s.	n.s.	<0.001	n.s.	<0.01

See Table 1 for the number of measurements and other notations.

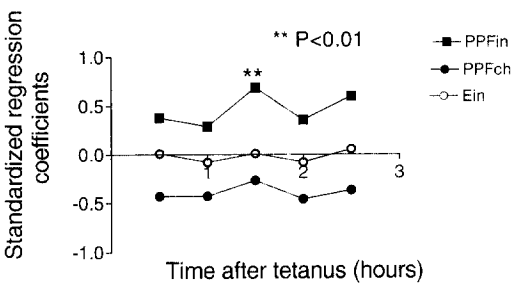

Fig. 7. Plots of standardized regression coefficients calculated for different post-tetanic periods. The multiple correlation analysis was used to investigate relations between the dependent variable, LTP magnitude, and three independent variables: pretetanic paired-pulse facilitation (PPF_{in}, squares), pretetanic EPSP1 amplitude (E_{in}, open circles) and changes in paired-pulse facilitation (PPF_{ch}, dots). Asterisks mark time-periods when the respective coefficient of multiple regression ($BETA_i$, see Experimental Procedures) was significant ($P<0.01$). See Fig. 3B for sample sizes. The negative correlation between LTP magnitude and PPF change was high during the early period after induction (0.5 h) whereas the positive correlation between LTP magnitude and pretetanic PPF ratio was largest 1.5 h post-tetanus.

phases. The easiest interpretation of this result is that presynaptic changes are strongly involved in the maintenance of both LTP1 and LTP2.

Differences in early and late phases of long-term potentiation revealed by the correlation analysis

We confirmed the existence of correlations between changes in PPF and LTP magnitudes[12,28,30] as well as between PPF changes and pretetanic PPF,[28,48] and extended these findings to the later (1–2.5 h) post-tetanic periods. The correlations strengthen the above conclusion that mechanisms responsible for the maintenance of both LTP1 and LTP2 include a presynaptic locus. In addition, the present regression analysis of intracellular EPSPs corroborated general results of the previous study[28] on the varying temporal relationship between the above correlations: LTP magnitude appeared to be more closely related to the pretetanic PPF ratio during LTP2 as compared to LTP1. This suggests that different presynaptic mechanisms are responsible for the two LTP phases. Therefore, both quantal analysis[27,58] and PPF/LTP interaction suggest contribution of presynaptic mechanisms to LTP2. However, whereas no differences in quantal parameters changes were found between LTP1 and LTP2, the regression analyses of PPF/LTP interaction revealed some differences.

Implications to synaptic mechanisms of long-term potentiation maintenance

The present study does not allow us to specify the nature of the differences between mechanisms of LTP1 and LTP2 but allows consideration of some hypotheses suitable for further experimental verifications. Several putative mechanisms of LTP maintenance have been recently discussed[28] and we shall expand this discussion here on the basis of recent observations.

The PPF/LTP interactions indicate changes in presynaptic release probability (P). However, the relation between PPF and LTP could vary. Sometimes PPF changes varied in time despite a relatively stable LTP (Fig. 2B, D), PPF changes could be absent despite a significant LTP (Fig. 5A, see also Ref. 30) and PPF could increase, especially when LTP was small (Fig. 5A). These observations suggest involvement of other mechanisms in addition to "pure" changes in P. One possibility, which is supported by previous quantal analysis of LTP1 periods[30,54,55,56] (but see Refs 8 and 50) and by recent quantal analysis of LTP2 phase[27,58] is an increase in the number of effective release sites (n). This is attractive because it explains the variations in PPF changes. For example, post-tetanic increases in PPF could be explained by larger PPF in the new release sites as compared to the pre-existing release sites.[48] The temporary variations in the PPF changes (Fig. 2B, D) or in above correlations (Fig. 7) could be accounted for by delayed increases in n (see below).

We have discussed two mechanisms[28] to explain the suggested increase in n: type 1, a consequence of increased P in the release sites with very low initial P, and type 2, the appearance of new active sites (transmission zones). The concept of "silent" synapses and fibres has been described previously in the context of LTP mechanisms.[3,10,15,24,30,35,39,52,56,60,63] Considering the type 1 synaptic modifications, we shall term respective modifiable silent sites "type 1" or "presynaptically silent" to distinguish them from

"postsynaptically silent" sites[15,24,35] of types 2. The existence of "presynaptically silent" synapses is indirectly supported by broad P variations in the hippocampus.[23,46,61] Crustacean neuromuscular junction provides one example of a glutamatergic synapse with "presynaptically silent" release sites.[3] There is evidence in favour of their existence in the hippocampus[53,54,56,60,63] as well including our recent preliminary results[2,58] of application of the principal component analysis to minimal EPSPs. Type 1 sites would not contribute significantly to the baseline EPSP, but some of them would increase pretetanic PPF ratio contributing to the second response in the paired-pulse paradigm.[2,51,53,60] Their presence could explain some inconsistencies between the present (Fig. 5C) and previous correlation analysis.[28] The correlation between the pretetanic PPF ratio and LTP magnitude was the largest of the three studied correlations according to the data obtained with the field potential recordings.[28] but it was virtually non-existent here when the experiment with the largest initial PPF was ignored (Fig. 5C, numbers in parenthesis). The discrepancy could be understood because (i) the procedure of selection of minimal EPSPs can be biased diminishing the proportion of the "presynaptically silent" synapses which is not the case for the recordings of field potential and (ii) the outlet in Fig. 5 represented a rare case close to the "presynaptically silent" synapse: it showed a very small baseline response amplitude (42 μV) and a high proportion of response failures (92%). This confirms that the correlation between the pretetanic PPF and LTP magnitude depends at least partially on activation of presynaptically silent synapses. Note that both the present (Fig. 7) and especially previous regression analysis[28] suggest that the weight of the above correlation tended to increase over the time-periods corresponding to the presumed LTP2 development. Therefore, activation of presynaptically silent synapses may represent an essential mechanism of LTP2. This possibility is supported by recent principal component analysis.[2,58]

Note that the involvement of type 1 modifiable synapses can not directly explain the delayed increase in glutamate receptor sensitivity.[7,14,43,44] Recent data[15,24,35] suggest that, at least in the developing hippocampus, "postsynaptically silent" (type 2) synapses may exist with only *N*-methyl-D-aspartate (NMDA) type of glutamate receptors active which produce no appreciable EPSPs in CA1 at resting membrane potential. The type 2 synapses became effective following 1 Hz stimulation paired with strong postsynaptic depolarization. These data[15,24,35] support the hypothesis[31] that LTP is associated with fast formation (or recovery) of glutamate receptors of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) type. In principle, the receptor formation can explain the increase in m and decreases in both the coefficient of variation and the number of failures estimated by the quantal analysis (see reviews in Refs 55 and 56). However, the fast formation of AMPA-type receptors was not proved to be universal, and a former proponent of this hypothesis[31] found recently an alternative explanation[32] of his previous results.[31] It should also be mentioned that the respective works[24,35] have several limitations. First, this mechanism was supported so far only for the developing hippocampus,[24,35] and it has been shown[15] that the number of the presumed "postsynaptically silent" sites dramatically decreased in early ontogenesis. This type of LTP-like change does not require dendritic spines[15] and altogether may have different mechanisms from LTP in the adult hippocampus. Second, the testing stimulation could depress the recorded responses before the conditioning procedure. This should happen with 1–2 Hz testing,[24,35] but could also occur at a less frequent (0.1 Hz) stimulation[15] in the immature brain. Third, the recording electrodes were filled with Cs^{2+} which could influence presynaptic release following its possible extrusion from the recorded neuron during conditioning depolarization. A conversion of silent synapses into active ones following Cs^{2+} application has been directly demonstrated.[17] Fourth, there is no strong evidence to the suggestion that both types of the glutamate receptors belong to the same synapse. For example, no evidence was given to decide whether the recorded responses were mono- or polysynaptic. Fifth, numerous data independent of quantal analysis and analysis of PPF/LTP interaction strongly suggest involvement of presynaptic mechanisms in LTP.[7,36,47] The data include demonstrations of increased NMDA receptor-mediated responses.[13] It is not clear why no such increases have been found in the above publications.[15,24,35] In addition, the PPF/LTP interaction which is naturally expected from a presynaptic mechanism is not easy to explain by the above hypothesis on AMPA receptor changes. In this case, it should signify that the "postsynaptically silent" synapses have a lower PPF (higher P) as compared to the active synapses. However, it does not seem to be compatible with the published observations[15,24,35] showing numerous response failures. Therefore, the contribution of this mechanism in the adult hippocampus could be only secondary and explain only the cases with small average LTP associated with no changes or decreases in PPF. The above hypothesis does not solve the problem of differences in LTP1 and LTP2 mechanisms because most of the respective data are related only to the first few minutes following induction of LTP-like changes[35] with only a few observations expanding to further 20–30 min.[15,24] In general, we suggest that conversion of postsynaptically silent synapses into active ones represents a mechanism additional to the major presynaptic mechanism of LTP1 based on increased presynaptic release. Both mechanisms can manifest themselves in apparent increases in n. The modification of the postsynaptically silent synapses can explain fast (within several

minutes post-tetanus) increases in AMPA responses described recently.[18]

The mechanisms of more delayed increases in the glutamate sensitivity[14,43,44] and generally mechanisms of LTP2 could include simultaneous post- and presynaptic modifications. Recently[28,57] we discussed this problem considering both LTP2 (up to 2–3 h post-tetanus) and later (LTP3) phase. LTP3 depends on protein synthesis[7,41,43] and could be explained by formation of new synapses.[5,65] In contrast, LTP2 which includes more rapid changes in n could be explained by separation of existing transmission zones into two or more parts.[16,20,57] It was noted[28,57] that the time-period required for similar structural re-organizations (spinule formation) in the retina[64] is about 30–40 min which is compatible with LTP2 development.[7,14,43,44] Our preliminary data[2,58] showed appearance of new EPSP components with delays of 20–50 min supporting the postulated mechanism of LTP2.

CONCLUSIONS

Lasting recordings (up to 2–3 h following LTP induction) of EPSPs from pyramidal neurons of the CA1 hippocampal slices confirmed that PPF often decreased after LTP induction. The decrease correlated with LTP magnitude and was especially prominent when LTP was large. The averaged PPF decrease was similar for the initial (<1 h) and later (up to 3 h) LTP phases. However, time-courses of PPF changes in individual neurons, detailed correlation and regression analyses of the relations between LTP magnitude, pretetanic PPF ratio and PPF changes suggest differences between mechanisms of LTP1 and LTP2. It is assumed that an increase in transmitter release probability is primarily responsible for maintaining LTP1. This mechanism overlaps with mechanisms responsible for LTP2 which may include simultaneous post- and presynaptic modifications which increase the number of synaptic transmission zones.

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