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Brain Research 957 (2002) 61–75

BRAIN RESEARCH

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Research report

Quantal analysis suggests strong involvement of presynaptic mechanisms during the initial 3 h maintenance of long-term potentiation in rat hippocampal CA1 area in vitro

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Accepted 15 August 2002

Abstract

Long-term potentiation (LTP) is the most prominent model to study neuronal plasticity. Previous studies using quantal analysis of an early stage of LTP in the CA1 hippocampal region (<1 h after induction) suggested increases in both the mean number of transmitter quanta released by each presynaptic pulse (*m*, quantal content) and postsynaptic effect of a single quantum (*v*, quantal size). When LTP was large, it was *m* that increased predominantly suggesting prevailing presynaptic contribution. However, LTP consists of several temporary phases with presumably different mechanisms. Here we recorded excitatory postsynaptic potentials from CA1 hippocampal slices before and up to 3.5 h after LTP induction. A new version of the noise deconvolution revealed significant increases in *m* with smaller and often not statistically significant changes in *v*. The changes in *m* were similar for both early (<1 h) and later (1–3 h) post-tetanic periods and correlated with LTP magnitude. The coefficient of variation of the response amplitude and the number of failures decreased during both early and late post-tetanic periods. The results suggest that both early (<0.5 h) and later LTP components (0.5–3 h) are maintained by presynaptic changes, which include increases in release probabilities and the number of effective release sites. In addition initially silent synapses can be converted into effective ones due to either pre- or postsynaptic rearrangements. If this occurs, our data indicate that the number and the efficacy of the receptors in the new transmission sites are approximately similar to those in the previously effective sites.

Theme: Excitable membranes and synaptic transmission

Topic: Long-term potentiation: physiology

Keywords: Quantal analysis; Rat

1. Introduction

Long-term potentiation (LTP) is a lasting (up to days and weeks) increase in postsynaptic responses induced by brief high-frequency activation (tetanization) of respective afferent fibers [8,43,45,54,60,85]. The phenomenon has been studied most extensively in the hippocampal CA1 area. Mechanisms of LTP induction are known to include activation of glutamate receptors of the *N*-methyl-D-aspartate (NMDA) type and an increase in intracellular Ca^{2+} concentration. The major mechanisms of LTP expression or maintenance depend on a lasting increase in the efficacy of excitatory synaptic transmission mediated by glutamate receptors of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) type. However, whether the increase is primarily due to pre- or postsynaptic changes (or both) remains a matter of vigorous debate (for reviews see [8,51,54,83–85]).

Several methods have been used to distinguish between pre- and postsynaptic locations of LTP mechanisms. One popular approach is fluctuation analysis of intracellular

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0006-8993/02/$ – see front matter
PII: S0006-8993(02)03600-4

excitatory postsynaptic potentials (EPSPs) based on the quantal hypothesis of synaptic transmission [36,65,83,84,96]. This approach had been firstly applied to LTP in area CA3 in vivo [81] and later has been extended to analysis of LTP in area CA1 in vitro by this [82,91] and other groups (for reviews of initial data see [51,83,84] and also [10,31,69,74,76–78]).

The traditional quantal analysis determines two major parameters: the mean quantal content (*m*) which is the average number of transmitter portions (quanta) released by each presynaptic pulse and quantal size (*v*) or quantal efficacy which is the postsynaptic potential change (or postsynaptic current) induced by action of one transmitter quantum. Although both changes in *m* and *v* have been reported, increases in *m* prevailed in a majority of the above publications when LTP was large. The changes in *v* appeared to be less consistent and could explain primarily cases with relatively small LTP (<60% increase above the pretetanic baseline [22,90]) although sometimes larger (e.g. twofold) increases in *v* had been encountered [10,50,77,78,84]. The increase in *m* suggested presynaptic changes: either in the release probability (P_r) or number of effective release sites or both. Several recent publications denied changes either in *v* or in the number of release sites [10,74,76], attributing early LTP maintenance exclusively to increased P_r.

In general, the formal quantal analysis supports a strong contribution of presynaptic mechanisms into LTP maintenance. However, the analysis has been restricted mainly to very early LTP stages, i.e. <30–40 min after its induction. Sometimes this phase is termed 'short-term potentiation', but we shall keep here an early nomenclature [61] and will term this phase 'LTP1' in distinction from a later LTP2 (up to 2–3 h) and even later LTP3 phases. One hypothesis [8,16] suggests that presynaptic mechanisms are primarily responsible for maintaining LTP1, but that thereafter, changes in sensitivity of subsynaptic receptors become important. However, other authors also consider synaptic receptor changes to be important for LTP1 [18,54,60,73].

The aim of the present study was to extend quantal analysis to LTP of up to 2–3 h corresponding to the LTP2 phase. We hoped to specify differences between mechanisms of LTP1 and LTP2 analyzing changes in quantal parameters. Specifically we tested whether estimated quantal size would increase during LTP2 as predicted from the above hypothesis on delayed changes in sensitivity of glutamate receptors [8,16]. Several modifications had been introduced as compared to the previous quantal analysis of LTP mechanisms [82,89–91]. The modifications include whole cell recordings in the current clamp mode, blockade of $GABA_A$-mediated inhibition, lasting control recordings without afferent tetanus and a new 'unconstrained' algorithm for the deconvolution analysis, which imply a priori neither a uniform quantal size for different release sites nor binomial transmitter release. A part of this work was published in abstract form [71].

2. Materials and methods

Experiments were performed on transverse hippocampal slices from 5–7-week-old male Wistar rats as previously described [72]. The slices were superfused (3 ml/min) with solution containing (in mM): NaCl 124, KCl 1.5, $MgCl_2$ 1.3, $CaCl_2$ 2.4, KH_2PO_4 1.25, $NaHCO_3$ 25, glucose 10 and 100 μM picrotoxin at 30 °C.

EPSPs were recorded from CA1 pyramidal cells using patch pipettes (2–3 MΩ) filled with a solution (in mM): K^+ gluconate 135, KCl 5, $MgCl_2$ 2, HEPES 10, glucose 20 (pH 7.2). To avoid the epileptic activity a cut was made between CA3 and CA1 regions and tetrodotoxin (TTX, 2 nM) was added in the perfusion solution [29]. We used the PATCH CLAMP L/M-EPC-7 amplifier (List-Medical, Darmstadt, Germany). Membrane potentials, not currents, were recorded. Due to lower dependence on the variations of the electrode (and generally access) resistance and the superior signal-to-noise ratio, this method provides advantages over the voltage clamp with minimal EPSP recordings [33]. Paired 0.2-ms pulses (50-ms interpulse interval, 6- or 8-s interstimulus interval) were delivered via glass or platinum–iridium stimulation electrodes positioned in stratum radiatum. In several experiments two stratum radiatum inputs were tested to the same neuron. The stimulus (20–50 μA) was adjusted to evoke just suprathreshold (minimal) EPSPs with occasional failures in response to the first pulse in the pair (EPSP1). Absence of the failures in the second response (EPSP2) confirmed reliable stimulation of at least one presynaptic axon. LTP was induced by three trains (1 s, 100 Hz with 0.4–0.6 ms stimulus duration, 20-s intervals) accompanied by 20-mV depolarization through the intracellular current injections. Hyperpolarizing current pulses (10 pA, 100 ms) were delivered via recording electrode in the time intervals between the testing stimulus to control the access resistance and cell membrane resistance. The input resistance (*R*) was evaluated as shown in Fig. 1D. Note that we used an amplifier without electronic bridge and did not balance the access resistance. Therefore, the potential drop shown in Fig. 1D was proportional to the sum of the access and membrane resistances. The membrane resistance of the recorded neuron was evaluated from the lower part of the potential drop (Fig. 1D, below the dashed line). Only data from neurons with stable (within ±20%) mean membrane resistances over the respective plateau region (see below for the definition of the plateaus regions) and with stable (within±5 mV) membrane potentials below −60 mV were used.

The experimental protocol was as follows. After stabilization of recording conditions, testing stimuli were delivered for 40–300 trials (typically 120–300 trials). Afterwards conditioning tetani were applied followed by a resumption of the testing stimuli (up to 2000 trials). The neuron was recorded as long as it remained under stable conditions as defined above. All responses were recorded

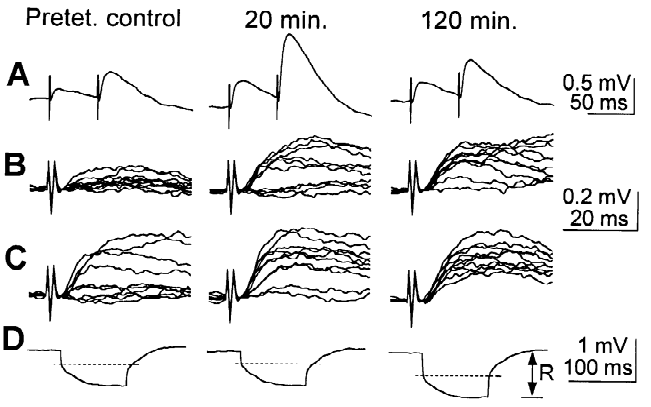

Fig. 1. Examples of whole cell recordings of EPSPs before (left column) and after LTP (two right columns). (A) Averaged ($n=50$) responses to the paired pulse stimuli. Note characteristic paired-pulse facilitation and large LTP. (B,C) Superimposed consecutive single responses ($n=10$) to the first (B) and second (C) stimuli of the pulse pair. Note decreases in the number of response failures and increases in the number of large responses in the post-tetanic sweeps as compared to the pretetanic ones. (D) averaged ($n=50$) responses to -10 pA current steps and illustration of the measurement of the general input resistance (R). The horizontal dashed line separates the voltage drops on the access resistance (upper part) and cell membrane resistance (lower part). A case is illustrated when R increased for about 30% (D, right column) but the membrane resistance did not change significantly. Recordings in the current clamp mode are not sensitive to such changes in R.

on disk at 5 kHz sampling frequency and analyzed offline. Measurements from plateaus regions [35,89–92], which were defined as continuous regions without significant amplitude drift over 40–300 trials (typically 120–200 trials) were taken for analysis. In distinction from commonly used amplitudes averaged over the EPSP peak [44–48] or over an initial part of the EPSP slope [89–92] we used a covariance measure provided by a modification [2] of the principal component analysis (PCA) that is a standard method of multivariate statistics [27,34]. The application of PCA to minimal EPSPs has been described in details previously [2,7]. Briefly, standard PCA scores were determined from a window covering the whole initial EPSP slope (sometimes including the EPSP peak). The first component score represents a correlation (covariance) between single response and average. Because this measure is strongly correlated with peak amplitude, the mean results did not depend on whether they are derived from amplitude or first PCA component measurements [7,35]. However, the latter gave a better signal-to-noise ratio using most of information containing in the response waveform [34] that is especially important for quantal analysis. The covariance amplitudes were expressed in mV and termed amplitudes for simplicity.The noise was measured in the same manner as the responses using a window of the same duration but before stimulus artefact.

A recently elaborated variant [1] of the noise deconvolution procedure [65] was applied to determine quantal parameters m and v. The algorithm used an L1-metric in the space of distribution functions for the deconvolution (minimization) procedure. The aim of the procedure was to find a discrete distribution (see bars in Fig. 4A) with the best fit to the experimental amplitudes (dotted columns in Fig. 4A). In distinction from the previously used binomial [90,91] and 'quantal' [88,89] deconvolution algorithms (for definitions see [65,83,84]) the present algorithm was 'unconstrained' in the sense that neither binomial nor generally quantal models have been assumed. As usual for noise deconvolution procedures [65] the independence of the signal and noise was assumed. We set the intrinsic coefficient of variation of v equal to $0.2v$ on the basis of previous data [21,33,40,76,78,86,95] (but see [23,28,52,59,69] for larger intrinsic quantal variances, e.g. in immature animals, cell cultures or under increased external Ca^{2+} concentration). As for any statistical analysis, an important problem was response stationarity. Because of large variations in minimal EPSP amplitudes, especially prominent after LTP [56] we selected the plateau regions (as defined above) from the pretetanic period and also from the post-tetanic periods of 15–45, 46–75 min, etc. The mean amplitudes and estimated quantal parameters from these regions were plotted in the graphs at 0.5, 1 h, etc, respectively. The control region was taken immediately before tetanus. The mean number of trials was 145 ± 32 and 224 ± 48 for pre- and post-tetanic regions, respectively. Computer simulations [2] show that such small samples can give reliable estimates of v provided $v/S_n>1.33$ with the best estimates of the mean v at v/S_n between 1.33 and 3. The mean v/S_n for 24 pretetanic regions analyzed was 2.6 ± 0.2, for 106 post-tetanic regions it was 2.9 ± 0.2. Although our deconvolution algorithm did not suggest uniform quantal efficacy for different release sites [2] we estimated a 'weighted mean quantal size' (v), which will be termed 'quantal size' for simplicity. It was determined as the weighted interval between the components (bars) of the deconvolution solution: the sum of products x_iP_i was divided by the sum of products iP_i. Here x_i is the location (distance from 0) of a component with number i and P_i is its weight. Similarly to other authors which used 'unconstrained deconvolution' or related procedures (e.g. [42]) we assumed that significant changes in the quantal efficacy even in a part of release sites should change the mean distance between the components of the solution. The mean quantal content of individual EPSPs (m) was calculated by dividing their mean amplitude (E) by v. In addition to the quantal parameters, the inverse square of the coefficient of variations ($1/(CV)^2$) was calculated. It was corrected for the background noise variance.

To obtain further insight into the mechanisms of the changes in the quantal parameters, correlation and regression analyses were performed. The Pearson's product moment correlation coefficient (r) was calculated according to standard methods [68]. In addition more sophisticated multiple regression analysis was done [5] to reveal

more precisely different contributions of changes in estimated parameters to different LTP periods. The analysis assumes that the relation between four variables has the form: $y = b_0 + b_1x_1 + b_2x_2 + b_3x_3$ and allows the evaluation of effects of one variable with exclusion of influences from other ones [5]. Regression coefficients b_i were calculated together with their significance levels. For better comparison of influences of different variables, 'standardizes regression coefficients' [5] were used: $BETA_i =$ (standard deviation of x_i)/(standard deviation of y).

LTP magnitude was defined as the difference between 100% and the ratio of the post-tetanic EPSP1 amplitude to the baseline amplitude, i.e. 0 corresponded to absence of LTP. Throughout the text means are given together with ±S.E.M. Significant differences correspond to $P<0.05$ if not specified otherwise (paired or unpaired t-test, as appropriate).

3. Results

3.1. LTP induction and persistence

LTP induction was attempted in 94 neurons but about half of them had been lost within 1 h of tetanus. Forty-six neurons were followed for at least 1 h post-tetanus, but twenty-five neurons either did not show any signs of potentiation or did not pass the selection criteria (see Materials and methods).

Complete analysis was performed for 21 neurons (24 inputs) with at least 1 h stable post-tetanic recordings and with signs of response potentiation. The majority of the neurons ($n=14$) were recorded for more than 2 h; nine, seven and two neurons were recorded for more than 2.5, 3 and 3.5 h, respectively. In addition, 20 control neurons that satisfied the selection criteria for at least 1 h, were recorded without tetanization. Responses of eleven, seven and five control neurons were followed for more than 1.5, 2 and 2.5 h, respectively.

Fig. 1A (left column) shows examples of averaged EPSPs recorded in response to paired pulses before tetanus. Superimposed single responses evoked by the first and by the second pulse (EPSP1 and EPSP2) are shown in Fig. 1B and C, respectively. Comparison of pre- and post-tetanic responses in Fig. 1B and C shows that EPSP potentiation was due to both increases in the number of large responses and decreases in the number of response failures, i.e. poststimulus traces not distinguishable from the baseline noise. Fig. 1A illustrates large paired-pulse facilitation (PPF) characteristic for hippocampal synapses under the same conditions [72]. Fig. 1D illustrates the evaluation of the general input resistance (R) from the voltage changes induced by 10 pA hyperpolarizing current pulses. Note the stability of the voltage below the dashed lines in Fig. 1D indicating the stability of the membrane resistance (see Materials and methods) in spite of about 30% increase in R later after tetanus in this particular experiment (right column). Such increase would influence a recording in the voltage mode [33] but should not significantly influence the current clamp recordings. This is supported by the relative stability of the estimated v in this neuron over more than 3 h post-tetanus (shown below together with the membrane resistance, see Fig. 4).

Fig. 2 represents summary plot for all neurons of the experimental and control groups. A large LTP persistent over all recorded period is evident in Fig. 2A. The dots and squares depict relative amplitudes of EPSP1 and EPSP2, respectively, both showing a persistent potentiation. In contrast, the control group (Fig. 2B) showed no statistically significant changes at any period with only an insignificant tendency to an amplitude increase after 2 h.

Fig. 3 illustrates varieties of LTP magnitudes and time courses. The first group (Fig. 3A) represents cases with small or absent LTP during initial (0.5 h) post-tetanic period (LTP magnitude of 0–50%). A clear delayed potentiation was evident 1.5 h following the conditioning procedure. The magnitude of the potentiation showed a strong tendency to further increase although the sample sizes (numbers in parenthesis) diminished after 1.5 h. In the small second group (Fig. 3B) EPSP amplitudes strong-

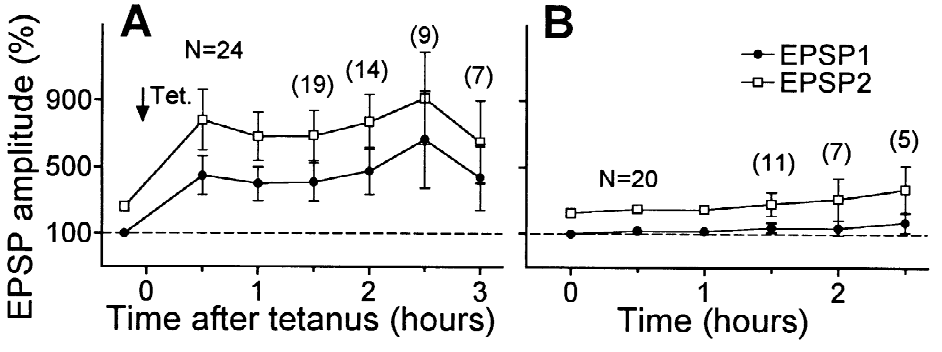

Fig. 2. Mean post-tetanic changes in the amplitudes of the first (dots) and second (squares) EPSPs in the testing pairs. (A) experimental group with afferent tetanus at time 0. (B) control group without tetanus. The numbers in the parenthesis show the number of analyzed EPSPs for the later periods. The dashed horizontal line, in this and other figures, indicates the pretetanic baseline for the first EPSP in the pair. S.E.M. values are indicated by vertical bars, except when smaller than the symbols.

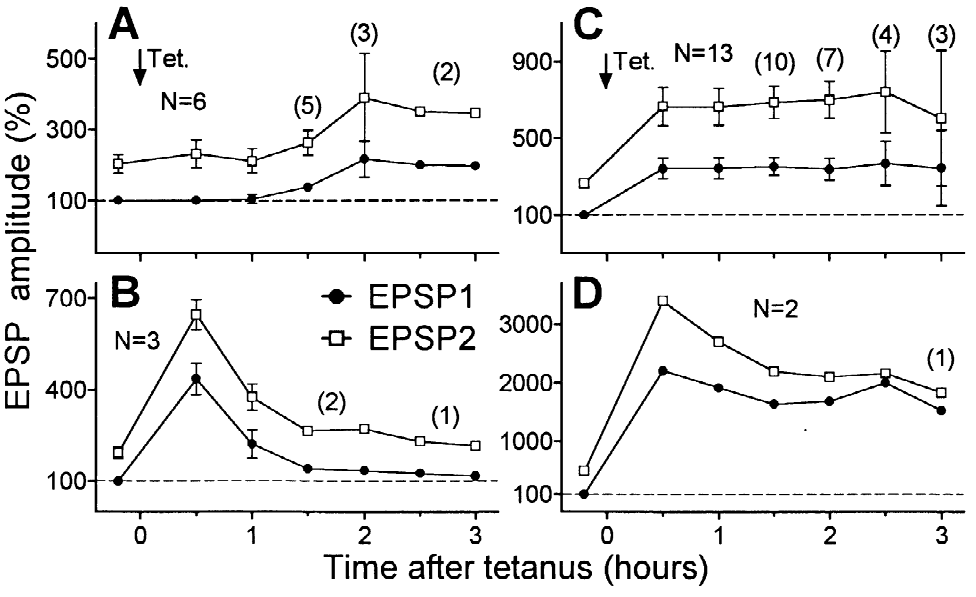

Fig. 3. Mean post-tetanic changes in the amplitudes of the first (dots) and second (squares) paired EPSPs in separate groups. All recordings were divided into four groups (A–D) according to LTP magnitude and time course during initial hour post-tetanus. (A) Cases with small (0–50%) LTP magnitudes. (B) Decremental potentiation. (C,D) Persistent LTP with magnitudes in the range of 50–800% (D) and >1400% (E). See Fig. 2 for other notations.

ly increased during the initial post-tetanic period (0.5 h) but the potentiation declined already 1 h post-tetanus. The basic group (Fig. 3C) represents the majority of the analyzed cases ($n=13$) with a persistent LTP of 50–800% magnitudes. Mean data for additional two neurons (Fig. 3D) are shown separately. They showed very large (>14 fold) LTP persistent almost without decrement over the whole recording period (2.5 h). Both neurons showed very small pretetanic responses (40 and 220 μV) with a large proportion of failures (92 and 75%, respectively) representing synapses with apparently very low initial release probability. We showed this two cases group separately because they represent very low-efficacy synapses, a kind of a border case with so-called 'presynaptically silent' synapses (see Discussion) that demand special approaches to be revealed [13,25]. Note that the basic group (Fig. 3C) showed LTP of EPSP1 (dots) of about 300%, which was not significantly different from that of the average LTP in the summary graph (Fig. 2A, dots).

3.2. Noise deconvolution analysis

An example of the deconvolution procedure used to evaluate the quantal parameters is shown in Fig. 4A. The dashed columns show experimental distributions obtained at different periods before and after LTP induction. The potentiation was accompanied by decreases in the number of response failures (see peaks around zero amplitude) and by a shift of the amplitudes to larger values as compared to the pretetanic distribution. This was a typical LTP pattern: only in two cases from our sample of fifteen experiments with large LTP (Fig. 3C and D) did the number of failures decrease without significant changes in the mean amplitude of the 'nonfailures', i.e. of positive responses clearly distinct from the noise level [10,74].

The bars in Fig. 4 show deconvolution solutions and the continuous lines show predicted (reconvolved) distributions. Note that most of the bars correspond to the peaks in the experimental distributions. Accordingly, the predicted distributions (continuous lines) fit well to the experimental data (dashed columns). Consideration of the quantal parameters (insets) shows that the post-tetanic changes in the EPSP amplitudes (represented as E in Fig. 4) were mainly due to changes in the mean quantal content (m) whereas the increase in the estimated quantal size (v) was relatively small (100–130 min post-tetanus) or even statistically not significant (10–40 min post-tetanus). Although the comparison of the early and late post-tetanic periods does show the expected increase in v later post-tetanus, the increase was not persistent as is evident from the more detailed plot of changes in v in the same experiment (Fig. 4B, v). In contrast, changes in m paralleled post-tetanic changes in the EPSP amplitude (open and closed circles, respectively).

In principle, post-tetanic increases in v could be missed if the cell membrane resistance declined after tetanus. However, in accordance with the selection criteria the membrane resistance was stable within ±20%. Fig. 4B (triangles) shows that there were no changes in the general input resistance, which could mask any significant post-tetanic increases in v.

3.3. Changes of quantal parameters in the whole sample and in separate LTP groups

Fig. 5 (left column, A and B) summarizes the post-tetanic changes in the quantal parameters for all neurons

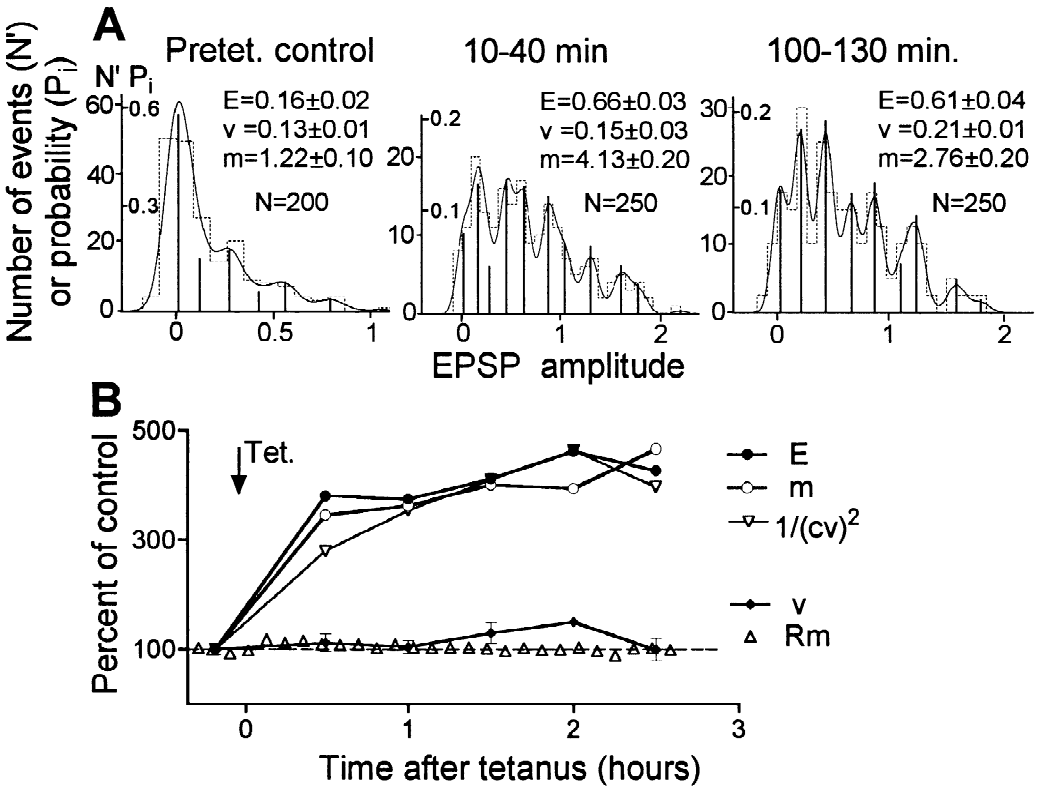

Fig. 4. Quantal analysis at various LTP periods. (A) noise deconvolution analysis before LTP induction and at two indicated periods after LTP induction in a representative neuron. Bars show deconvolved components; experimental and predictive distributions are shown as dashed columns and continuous curves, respectively. The left numbers on the ordinate correspond to the number of events (N'); the right numbers correspond to the bar heights expressed in probabilities (P_i). The insets give the mean EPSP amplitude (E), estimated quantal size (v); mean quantal content (m) and sample size (N). Values for E and v are in mV. (B) changes in EPSP amplitude (E), quantal parameters (m and v), general input resistance (B) and the inverse square of the coefficient of variation ($1/(CV)^2$) following LTP induction in the same experiment shown in (A). Note that m (open circles) increased approximately in parallel with EPSP amplitudes (crosses), whereas v was relatively stable. Note also that the general input resistance (triangles) measured as shown in Fig. 1D could vary within ±20% but its variations did not correlate with variations in the estimated v.

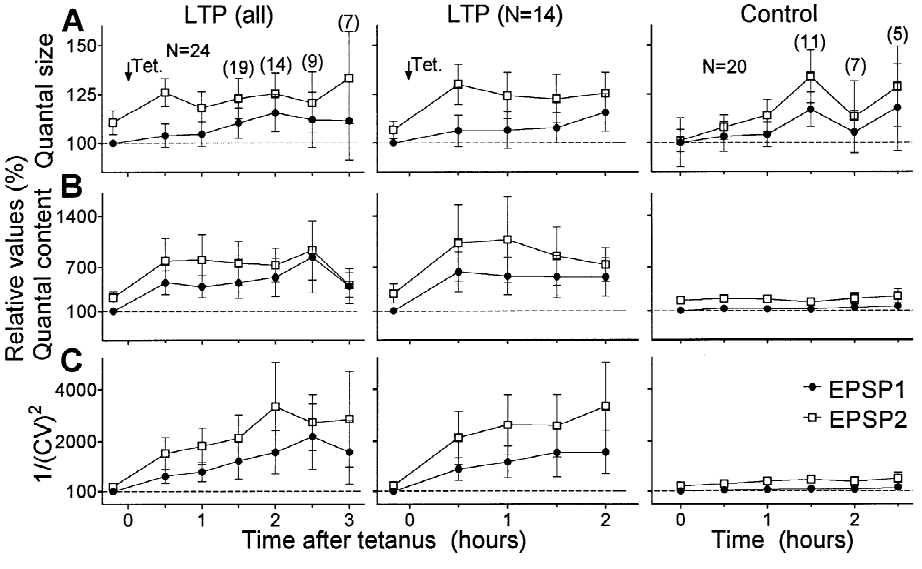

Fig. 5. Mean changes in quantal parameters (A,B) and in the inverse square of the coefficient of variation (C) of EPSP amplitudes for all inputs (left column), for neurons recorded for at least 2 h post-tetanus (middle column) and for the control group without tetanus (right column). Dots and squares represent the values for EPSP1 and EPSP2, respectively. Note persistent significant changes in the mean quantal content (B) and the coefficient of variation (C) compatible with presynaptic mechanisms of both early and late LTP phases and the lack of significant changes in the control recordings.

analyzed. Note a large (about fourfold for EPSP1) and statistically significant increase in the mean quantal content (Fig. 5B, dots) with only a weak trend to an increase in v for about 5–15% (Fig. 5A; note the differences in the ordinate scales in A and B). The trend was slightly more evident for the later post-tetanic periods (1.5–3 h, Fig. 5A, dots). To check whether this trend could depend on smaller sample sizes for the later periods, we calculated v and m only for neurons recorded for at least 2 h (Fig. 5 middle column, A and (B) respectively). The delayed increase in v (Fig. 5 A left, dots) appeared to be even smaller than for the whole group (Fig. 5A middle, dots). To further test reliability of our procedures, we estimated quantal parameters for the control group without tetanus. There was only a small and statistically insignificant trend to an increase in v at later recording periods and no changes in m (Fig. 5 right column, A and B, respectively).

Considerations of changes in the quantal parameters (Fig. 6) for the three major LTP groups described above (Fig. 3) confirm the conclusion on relative stability of v and essential changes in m following LTP induction. Note that the average m changes in different groups (Fig. 6A–C) matched the post-tetanic changes in EPSP amplitudes (Fig. 3A–C). The estimated v did not change significantly in the major groups (Fig. 6A and C) and tended to diminish in the neurons of the small group (Fig. 6B) simultaneously with the strong decrement in the potentiation in this particular group. The membrane resistances were stable within ±10% in all three neurons of this group. There was a weak trend to a small (about 20–30%) increase in v for the later LTP periods as compared to the early (0.5–1 h post-tetanus) periods in the major group but it was essentially smaller than changes in m (Fig. 6C, middle and left columns, respectively, note different y-scales).

As one more control for the validity of the procedures used, we calculated quantal parameters for the second EPSPs in the testing pairs (Fig. 6, open squares). Initial quantal size appeared to be similar to that of EPSP1 within 5–15% and the relative changes in v were practically the same. Changes of m for EPSP2 roughly repeated the changes of the same parameter for EPSP2 but were slightly smaller reflecting a decrease in presynaptic PPF after LTP as previously described ([72], see also [39]).

3.4. Changes in the coefficient of variation following LTP induction

One popular approach to distinguish between pre- and postsynaptic location of LTP mechanisms is the variance method. Using this method and the method of failures it is possible to calculate quantal parameters assuming the Poisson or binomial model of transmitter release [83,90,92]. We did not calculate quantal parameters by any of these methods because we would not like to constrain our model of transmitter release. Instead, following many other authors (for reviews see [19,83,84]) we constrained ourselves to calculations of changes in the inverse squared coefficient of variations (Figs. 4B, 5C and 6, right column). The results showed significant increases in this value after LTP comparable with the increases in m. Similar changes are traditionally interpreted as evidence in favour of presynaptic contribution (but see [19]). No significant changes in the coefficient of variation were found in the control group without tetanus (Fig. 5C, right column).

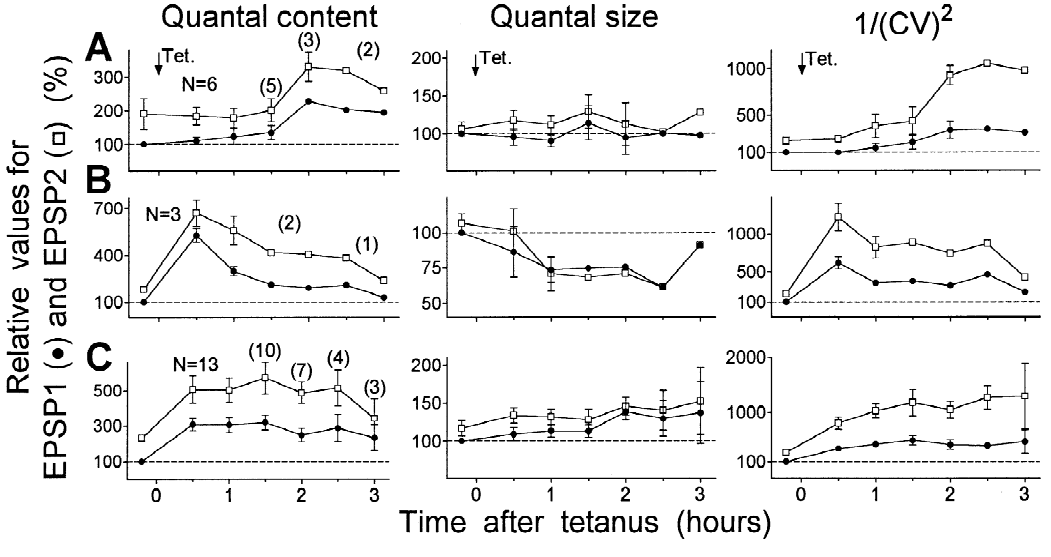

Fig. 6. Changes in quantal parameters (two left columns) and in the inverse square of the coefficient of variation (right column) for three groups separated according to LTP magnitude or time course as in Fig. 3A–C, respectively. Note persistent changes in the quantal content, which paralleled EPSP amplitude changes in different groups (see Fig. 3 for comparison). In contrast, quantal size showed variable changes with only a small increase in one group (D), suggesting a small (if any) contribution of postsynaptic mechanisms to LTP. Note different y-scales for the quantal content and quantal size.

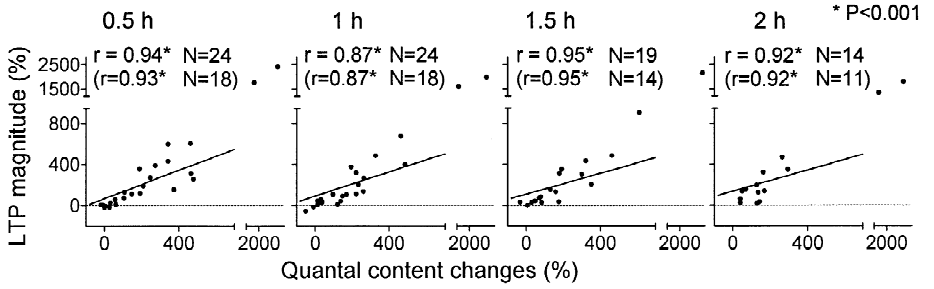

Fig. 7. Correlation analysis of relations between LTP magnitudes and changes in the quantal content at various periods after LTP induction. The coefficients of correlation (r) are given for all inputs and for inputs with statistically significant LTP during initial 0.5 h (numbers in parenthesis). Note that LTP magnitudes strongly correlated with changes in the quantal content.

3.5. Correlation analysis of changes in quantal parameters

Fig. 7 shows a large and highly statistically significant correlation between LTP magnitude and changes in m during initial 2 h after tetanus. Table 1 expands the data to the recording period of up to 3 h post-tetanus. Note that the coefficient of the correlation (r) was very similar for different periods except 1 h when it was slightly smaller. The correlation was not changed when cases without significant LTP during the early post-tetanic period were excluded (Fig. 7, number in parenthesis). In contrast to the changes in m (Fig. 7 and Table 1) the small post-tetanic changes in v did not show any significant correlation to the LTP magnitude (Table 1). It should be noted that increases in v for $>20\%$ were rare. The small differences of r from 0 at the earlier periods (Table 1) were created by decreases in the estimated v in some experiments with small LTP magnitudes. The changes in m and v did not correlate to each other at any post-tetanic period (Table 1). The lack of this correlation is expected from the other two relationships in Table 1, but it is contrary to the expectation from the hypothesis on the substitution of early presynaptic mechanisms of LTP by later postsynaptic ones (see Introduction).

Pretetanic control values of m correlated inversely with LTP magnitude. The correlation was moderate for 0.5 h and 1 h ($r=-0.47$ and -0.43, respectively, see Table 1) and slightly larger for 1.5 and 2 h post-tetanic periods ($r=0.52$ and 0.56). However, again, the difference was small and did not suggest any difference in underlying mechanisms. Several groups reported similar correlations for the early (about 30 min after induction) LTP periods [48,50,77,78,84]. However, unlike some other groups [48,50] we found no statistically significant correlation between LTP magnitude and initial quantal size as well as between changes in v and its initial value (data not shown). The latter result is similar to that of Sticker et al. [77,78].

Fig. 8A summarizes the changes in the EPSP amplitude and quantal parameters. It stresses large changes in m (black bars) that are absent in the control recordings without tetanus (Fig. 8B). Fig. 8 shows also that the changes in m were similar for different post-tetanic periods. In an attempt to reveal different contributions of changes in m and v to different LTP periods, we used multiple regression analysis that gives a possibility to evaluate effects of one variable with exclusion of influences from other ones [5]. LTP magnitude (Fig. 8A, open bars) was classed as a dependent variable whereas the changes in m and v were treated as independent variables in the multiple regression. The analysis calculated the multiple regression coefficients, their significance levels and standardized correlation coefficients ($BETA_i$, see Materials and methods). Fig. 8C plots the standardized correlation coefficients for different post-tetanic periods. The plot supports the above observations: it shows the

Table 1
Correlation coefficients for relations between changes in quantal size, mean quantal content and magnitudes of long-term potentiation

	Correlated values					
	0.5 ($n=24$)	1 h ($n=24$)	1.5 h ($n=19$)	2 h ($n=14$)	2.5 h ($n=9$)	3 h ($n=7$)
m_{ch} vs.LTP	0.94**	0.87**	0.95**	0.92**	0.98*	0.95*
v_{ch}vs. LTP	0.33	0.35	0.09	0.03	0.18	0.13
v_{ch}vs. m_{ch}	0.16	0.06	−0.06	−0.15	0.05	−0.18
m_{in}vs. LTP	−0.47*	−0.43*	−0.52*	−0.56	−0.52	−0.41
v_{in}vs. LTP	0.06	−0.01	0.03	0.13	0.1	−0.27

The Pearson product–moment correlation coefficients (r) are given for six post-tetanic periods. The number of measurements are given in parenthesis for each period. v_{ch}, changes in quantal size; m_{ch}, changes in the quantal content; LTP, magnitude of long-term potentiation. Significance levels (two-tailed test): *, $P<0.01$; **, $P<0.001$.

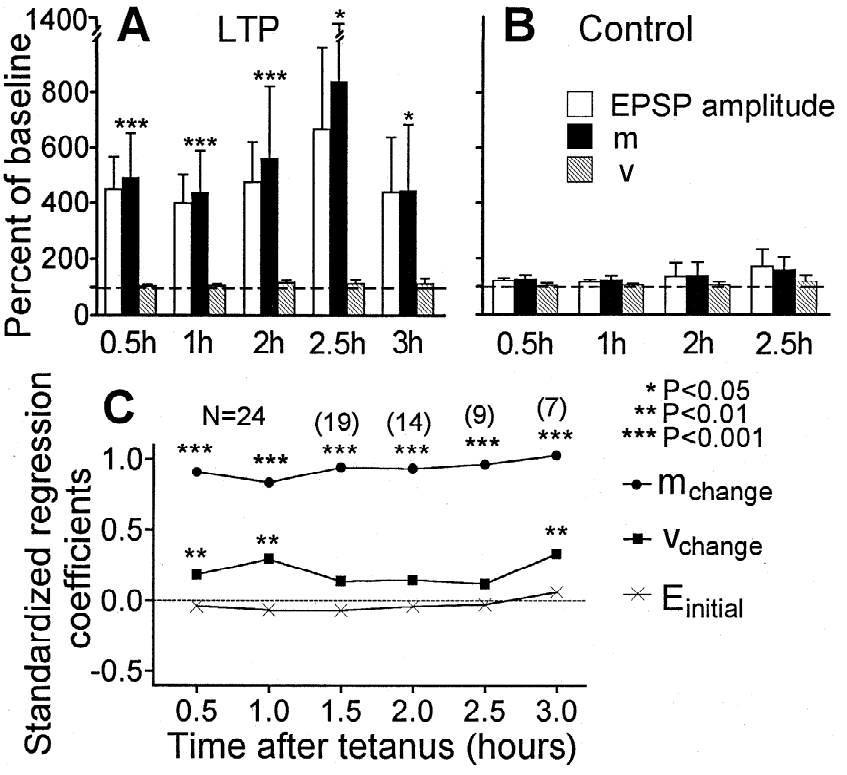

Fig. 8. Summary plots of changes in the quantal parameters and multiple correlation analysis. Relative EPSP amplitude (open bars), quantal content (*m*, black bars) and quantal size (*v*, hatched bars) are calculated for the experimental (A) and control (B) groups. Note large increases in the quantal content similar for all post-tetanic periods (A) and no significant changes in the control group (B). The multiple correlation analysis (C) investigates relations between the dependent variable, LTP magnitude, and the following three independent variables: changes in the mean quantal content (dots), changes in quantal size (squares) and pretetanic EPSP amplitude (crosses). Asterisks mark time periods when the respective coefficients of multiple regression (b_i, see Materials and methods) were significant at the significance levels as shown. Note that the contribution of *m* changes in LTP magnitude was strong and highly significant over all post-tetanic periods. The contribution of changes in *v* was small and not significant for half of the regions analyzed.

major contribution in LTP from the changes in *m* (Fig. 8C, dots). The contribution was highly significant and did not differ for different LTP periods. The contribution from the changes in *v* was essentially smaller (Fig. 8C, squares) and was not statistically significant for some periods. It was significant for the periods of 0.5, 1 and 3 h post-tetanus. However, as mentioned above, this contribution was mainly due to the fact that cases with a relatively small LTP magnitudes (<300%) tended to contain a large proportion of *v* decreases rather than increases in *v* after tetanus. Fig. 8C (squares) shows no signs of the larger contribution of *v* changes in the late LTP maintenance as it would be expected from the hypothesis described in the Introduction. The pretetanic EPSP amplitude (Fig. 8C, crosses) was found to have no influence on the LTP magnitude at any period.

4. Discussion

4.1. Increased quantal content during LTP

The present study demonstrates significant increases in *m* following LTP induction. The result is consistent with presynaptic modifications and confirms previous observations of our and other groups made for LTP1 (see Introduction). We used here several new approaches as compared to previous studies from this and other groups (for reviews, see [51,83,84]). The approaches were aimed at increasing reliability of the quantal analysis and facilitating comparison of the present results with previous ones.

First, we blocked $GABA_A$ inhibition. The block had not been used previously in this group but was used routinely by several others (see Introduction for references). It decreases the spontaneous noise, increases the signal-to-noise ratio and diminishes influences of possible changes in the inhibitory inputs on EPSP amplitude measurements. It should be mentioned that the inhibition blockade has disadvantages; the most serious one is the risk of spontaneous seizures, which can change the state of the preparation and even lead to a slow onset potentiation [24]. By our experience, the cut between CA3 and CA1 is not always sufficient to prevent spontaneous seizures. We used, therefore, a small amount of TTX to decrease the excitability of the preparation [29] in addition to the cut. An input nonspecific ‘delayed LTP’ has been described under picrotoxin however under different recording conditions [24]. Nevertheless this phenomenon could contribute to

LTP time-course in our experiments and might express itself in a delayed potentiation in rare cases (Fig. 3A). However, a major contribution under our conditions is unlikely because our control experiments without tetanus did not show any delayed potentiation. The difference from the above cited observations on the 'delayed LTP' [24] could be due to methodological differences, specifically to the lower neuronal excitability under our conditions due to addition of TTX to the perfusion solution. An additional complication from the inhibition blockade is a possible contamination from polysynaptic activity, which can influence the determination of quantal parameters, especially when peak amplitude measurements are used. Different waveforms for different amplitude range that have been noted by some authors [76,77] could indicate polysynaptic contributions. Our amplitude measurements (see Materials and methods), which used information predominantly from the initial EPSP slope, should be less sensitive to the polysynaptic contamination as compared to the conventional peak amplitude measures. In addition, our data of the component analysis reported elsewhere [2] suggest that essential polysynaptic contribution is unlikely under conditions of our experiments.

The second new approach was the whole cell recording in the current clamp mode. This approach gives better signal-to-noise ratios as compared to both the sharp electrode intracellular recordings [22,48,82–92] and the whole cell recordings in the voltage clamp mode [6,42,57,74,76–78] used in previous LTP studies. In addition, the current clamp mode makes the EPSP amplitudes independent of the variations of the access resistance [33], which is important for statistical analysis of lasting recordings.

The third modification is the response measurement using the covariance amplitudes based on the PCA [2]. The covariance amplitudes utilize more information about the waveform and can be more reliable compared to the conventional electrophysiological measures [12].

Fourth, we used a new variant of the deconvolution procedure [1]. In distinction from several previous models used for the quantal analysis of LTP, the present model was not restricted to any particular type of the release process [65,83] and assumed neither binomial release nor equal effects of a single quantum for different release sites. In this respect it was similar to approaches used by two other groups [42,77,78]. However, our computer simulations [1] indicated that the algorithm could give a better resolution, especially at small samples. We note that in distinction from some publications on quantal analysis [10,74] but similar to many others (for review see [83,84]; see also [46,47,76–78]) we used a traditional model assuming a moderate ($0.2v$ in our case) intrinsic quantal variation (S_v, see Materials and methods) and made no suggestion about the number of presynaptic fibers or release sites activated. The alternative 'monoquantal' mode [23,74] suggests activation of a single release site with a large S_v (about $0.5v$). We prefer the former model by several reasons. In practice, statistical tests of these models (one mode with large S_v or several modes with a smaller S_v) give usually an essential preference to the latter [77,78], especially when the noise level is small enough. The unimodal model assumes that regular peaks in the amplitude distributions of hippocampal responses represent sampling artifacts. However, it had been shown to be unlikely in many cases by others [33,44,46–48,50,75–78] and seems unlikely for our cases in view of approximately equal distances between major modes in the amplitude distributions calculated for several consecutive regions during lasting recordings of the same cell. Evaluations of S_v from evoked responses gave moderate values (about $0.2–03v$ or less) for various central synapses including hippocampal synapses in mature animals [21,33,40,86,94]. However, as mentioned above (see Materials and methods) we cannot exclude appearance of larger S_v under other conditions [23,28,52,59,69]. Altogether the problem of the intrinsic quantal variance is closely related to several other unresolved questions like subsynaptic receptors saturation, multiquantal release from single release sites and multiquantal spontaneous release [4,18,20,23].

Fifth, in distinction from many previous related works we kept to the low frequency testing stimulation (6- or 8-s interstimulus interval). The testing frequency of about 1 Hz used by some authors is favorable for inducing short-term frequency depression or even long-term depression and therefore may influence pretetanic state of the preparation, e.g. release probabilities [80] and LTP mechanisms in general. The low frequency testing protocol has disadvantage in diminishing sample sizes. It is especially unfortunate for the whole cell recordings which are associated with a rather short (about 20 min) 'time window' [6,42,57] after which LTP induction is practically impossible (but see [14]). This phenomenon might explain why LTP was induced only in about half of our experiments. As a result, the general output of potentiated neurons was significantly lower than in previous sharp microelectrode recordings [88–92] (but see [48]). Therefore, in many cases we had to use small sample sizes (typically 120) for pretetanic regions. In previous works [1,92] we paid a special attention to the problem of small samples (like 100). Computer simulations showed that our present algorithm [1] usually gave reliable solutions as an average from several (e.g. 10) histograms even when each histogram contained about 100 measurements when the estimated v/Sn ratio was large enough and the number of effective release sites was small (≤ 10). The v/Sn ratio appeared to be much more important for the proper estimation of v than the sample size so that with v comparable to Sn even 'very large' samples (like 1000 or 10000) were not helpful. Here, the mean v/Sn ratio was high enough (see Materials and methods).

At last, according to the major aim of the present study, the important difference from previous studies was that we

evaluated changes in the quantal parameters not only for the early (LTP1) but also for later (LTP2) periods. We note that the major conclusion of the present study (stability of v over different periods following LTP induction) is supported by data from several post-tetanic regions and therefore from larger sample sizes as compared to the confirmational result on the changes in quantal parameters for LTP1.

The basic method of the present study, that is the noise deconvolution analysis, has limitations inherent to any methodology that attempts to solve a typical 'inverse problem' having in general terms no unique solution. However, the reliability of determination of v is confirmed by similarities between v values estimated from the same samples for EPSP1 and EPSP2 as well as from successive small ($n=100$) samples (as shown in Fig. 4B) or from respective large and small samples (data not shown). Several additional arguments, specifically based on extensive computer simulations, support the validity of the analysis of peaky amplitude histograms [1,9,33,40,78,92]. The general conclusion from the deconvolution analysis is supported by changes in the failure rate and by the variance analysis that represent approaches independent of the deconvolution analysis [65,83, but see [19] for reservations related to the variance analysis]. We applied the simplest variant of the variance method. Similar to the deconvolution analysis the results were consistent with the presynaptic mechanisms for both studied LTP phases (LTP1 and LTP2).

4.2. LTP is accompanied by changes in both the number of failures and mean amplitude of nonfailures

It has been reported [10,74] that LTP is accompanied by decreases in the number of failures without increases in the mean amplitude of nonfailures. These observations are compatible with lack of changes in receptor sensitivity and in the number of release sites and attributed LTP induction exclusively to increases in P_r. Because this observation was in variance with many previous publications (see [15,83,84]) and some more recent ones [30,65,77,78] we tested it for LTP1 and LTP2 periods. In agreement with most other publications, the number of failures decreased whereas the mean amplitude of nonfailures increased in a large majority of our present experiments. Both changes in the failure rate and in the mean amplitudes of successes did not significantly differ for LTP1 and LTP2, which is compatible with similarity of their maintenance mechanisms (see below).

In view of our data on small post-tetanic changes in v, the invariance of the nonfailure amplitudes is easy to understand by suggesting that only one release site had been activated in the above publications [10,74]. However, it demands to postulate a very large Sv, which seems to us unlikely, at least for our case of hippocampal slices from adult animals as discussed above. In the frame of the model with several release sites, one possible reason for the invariance of the mean amplitude of nonfailures following LTP induction is simultaneous decrease in v. Decreases in apparent v had been found in some cases, especially when LTP was small. Our measurements showed that they could not be accounted for by changes in the membrane resistance in the present study. However, voltage clamp recordings used by others [10,74] are very sensitive to changes in access resistance [33]. More 'physiological' explanations of the decreases in v could be based, firstly, on addition of new release sites with smaller v as compared to previously active release sites and, secondly, on superposition of processes related to either long-term depression or short-term low-frequency depression [46,48,80,83].

4.3. Changes in quantal content are correlated with LTP magnitude and time course

The present study confirmed previously described strong correlation between changes in m and LTP magnitude during initial 30–50 min [50,85,88–90] and expanded this finding to the later LTP periods (up to 3 h post-tetanus). Similar correlations for LTP1 and LTP2 periods were illustrated by the comparison of LTP and m time courses. The correlation strengthens the conclusion that the mechanisms responsible for LTP1 maintenance include a presynaptic locus and is compatible with the suggestion that expression mechanisms of LTP1 and LTP2 are similar (see below).

4.4. Changes in quantal size are small and not correlated with LTP magnitude or time course

In contrast to the changes in m, the general changes in v were small and did not correlate with LTP magnitude and time courses. On average, they could explain at best $<20\%$ of LTP magnitude and could probably contribute essentially only when LTP magnitude was small as it has been previously found for the early LTP1 [88–90] (see also [22]).

Under the present conditions we did not find large (e.g. twofold) increases in v reported in some cases in previous publications [50,77,78,84,85]. However, we did encounter such large changes in v in our preliminary quantal analysis of separate EPSP components [2]. It has been hypothesized that such doubling of v could be due to synchronized release of two quanta [85]. Independently whether this hypothesis is correct or not, it should be stressed that even these large changes in v could not explain the major part of LTP so that increases in m remained the major explanation.

4.5. Implications for mechanisms of LTP2 phase

The major finding of the present study is that m

increased during the periods corresponding to both LTP1 and LTP2. The changes in *m* are traditionally interpreted as an indication of presynaptic location of underlying mechanisms. This interpretation is further supported by several additional observations: decreases in the coefficient of variation and in the number of failures, and especially in the PPF ratio under similar condition [72]. These findings were unexpected from the above hypothesis [8,16] suggesting that pre- and postsynaptic mechanisms are responsible for LTP1 and LTP2, respectively.

However, as discussed previously [38,83,84,89] and stressed by others [32] the results of the quantal analysis became more complicated if new synapses appear or previously 'silent' synapses become functional. This is compatible with increases in the mean amplitudes of nonfailures and the number of peaks reported here. As discussed elsewhere [38,72,93] the apparent changes in the classical parameters of transmitter release could be not only due to presynaptic increase in P_r in synapses with very low initial P_r but also due to expression of new postsynaptic receptors or combined pre- and postsynaptic rearrangements, i.e. formation of spinules and new transmission zones [18,26,79]. The latter possibility is attractive for the explanation of LTP2 because the expected time delay for spinule formation (up to 30–40 min) is similar to the period of LTP2 development (see [85]). Previously noted delayed (15–45 min) appearances of additional EPSP components [2] are compatible with combined pre- and postsynaptic rearrangements.

The expression of new effective transmission zones gives a reasonable interpretation to both the previously reported observations on the delayed increase in glutamate sensitivity [16] and to the increased quantal content during LTP2. This hypothesis is supported by LTP-related structural changes [26] see also [18,85] and found strong confirmations in the studies of LTP induced by application of cyclic adenosine 3′,5′-monophosphate (c-AMP) [9,11]. However, it is not clear whether mechanisms of the c-AMP-induced potentiation are the same as those of the conventional tetanus-induced LTP and if they are similar to what LTP phase they could correspond.

In principle, quantal analysis data can be explained suggesting a purely postsynaptic mechanism [41]: expression of the AMPA type glutamate receptors in previously 'postsynaptically silent' synapses with only NMDA receptors active before LTP induction [17,32,54,58]. However, this 'purely postsynaptic' hypothesis cannot easily explain several other lines of evidence in favour of strong involvement of presynaptic mechanisms of the maintenance of both LTP1 and LTP2. (i) PPF decrease correlated with LTP magnitude [39,70,72]. (ii) Dependence of LTP magnitude on initial PPF ratio and P_r [38,49,72]. We note that changes in PPF can be absent when LTP is small [22] or composite responses are recorded (e.g. [3]). In the latter case, responses mediated by different fibers could express different degrees of LTP and PPF changes. (iii) Changes in presynaptic vesicle turnover at individual synapses [55,67,97]. (iv) Fast ($<$10 min) increase in phosphorylation of a presynaptic protein correlated to LTP magnitude [63]. (v) Direct or indirect measurements of increased glutamate release during LTP [8,13] (but see [53,64]). (vi) Occurrence of silent synapses with functional AMPARs, i.e. 'presynaptically silent' synapses [13,25,37,66] and their potentiation [7,93]. Therefore we favour a scheme according to which combined pre- and postsynaptic changes including insertion of new receptors support the maintenance of late LTP2/LTP3 phases [8,18,23,38,62,72,85]. Crosstalk between pre- and postsynaptic elements [51] is crucial for such coordinated (matched) pre- and postsynaptic modifications. Therefore, as it has been already emphasized by other authors [51] this surpasses the controversy of whether late LTP maintenance is pre- or postsynaptic.

As has been mentioned [83,84,89] in the case of appearance of new synapses or transmission zones, the lack of essential changes in *v* reported here indicates that the number and the efficacy of the receptors in the new transmission sites are approximately similar (or only slightly larger) than those in the previously effective sites.

In conclusion, we performed lasting (up to 3.5 h) recordings of minimal EPSPs from the hippocampal CA1 region following LTP induction. Persistent increases in the mean quantal content with only small changes in quantal efficacy are compatible with involvement of presynaptic mechanisms in the maintenance of both very early ($<$0.5 h) and later (up to 3 h) LTP phases, i.e. LTP1 and LTP2. The presynaptic mechanism may include changes in release probability and in the number of effective transmission zones due to pre- or postsynaptic rearrangements or both. In case of expression of previously 'postsynaptically silent' synapses or appearance of new transmission zones, the present data suggest that the efficacy of the new receptors is approximately similar to that in the previously active sites.

Acknowledgements

We thank Drs. A.M. Kleschevnikov and C. Hatton for helpful comments and C. Hatton also for improving the English. This work was supported by grants from Volkswagen-Stiftung, RFBR, INTAS and Wellcome Trust. Part of M. Sokolov's work was supported by Alexander von Humboldt Stiftung.

References

[1] A.V. Astrelin, M.V. Sokolov, T. Behnisch, K.G. Reymann, L.L. Voronin, Noise deconvolution based on L1-metrics and decomposition of discrete distributions of postsynaptic responses, J. Neurosci. Meth. 71 (1997) 17–27.

[2] A.V. Astrelin, M.V. Sokolov, T. Behnisch, K.G. Reymann, L.L. Voronin, Principal components analysis of minimal excitatory postsynaptic potentials, J. Neurosci. Meth. 79 (1998) 169–186.
[3] F. Asztely, M.Y. Xiao, B. Gustafsson, Long-term potentiation and paired-pulse facilitation in the hippocampal CA1 region, NeuroReport 7 (1996) 1609–1612.
[4] C. Auger, A. Marty, Quantal currents at single-site central synapses, J. Physiol. Lond. 5 (2000) 3–11.
[5] K. Backhaus, B. Erichson, W. Plinke, C. Schuchard-Ficher, R. Weiber, Multivariate Analysemethoden, Springer, Berlin, 1980.
[6] J.M. Bekkers, C.F. Stevens, Presynaptic mechanism for long-term potentiation in the hippocampus, Nature 346 (1990) 724–729.
[7] N. Berretta, A.V. Rossokhin, E. Cherubini, A.V. Astrelin, L.L. Voronin, Long-term synaptic changes induced by intracellular tetanization of CA3 pyramidal neurons in the hippocampal slices from juvenile rats, Neuroscience 93 (1999) 469–477.
[8] T.V.P. Bliss, G.L. Collingridge, A synaptic model for memory: long-term potentiation in the hippocampus, Nature 361 (1993) 31–39.
[9] V.Y. Bolshakov, H. Golan, E.R. Kandel, S.A. Siegelbaum, Recruitment of new sites of synaptic transmission during the cAMP-dependent late phase of LTP at CA3–CA1 synapses in the hippocampus, Neuron 19 (1997) 35–651.
[10] V.Y. Bolshakov, S.A. Siegelbaum, Regulation of hippocampal transmitter release during development and long-term potentiation, Science 269 (1995) 1730–1734.
[11] O. Bozdagi, W. Shan, H. Tanaka, D.L. Benson, G.W. Huntley, Increasing numbers of synaptic puncta during late-phase LTP: *N*-cadherin is synthesized, recruited to synaptic sites, and required for potentiation, Neuron 28 (2000) 245–259.
[12] R.M. Chapman, J.W. McCrary, EP component identification and measurements by principal components analysis, Brain Cognit. 27 (1995) 288–310.
[13] S. Choi, J. Klingauf, R.W. Tsien, Postfusional regulation of cleft glutamate concentration during LTP at 'silent synapses', Nat. Neurosci. 3 (2000) 330–336.
[14] K.A. Clark, G.L. Collingridge, Synaptic potentiation of dual-component excitatory postsynaptic currents in the rat hippocampus, J. Physiol. Lond. 482 (1995) 39–52.
[15] G.L. Collingridge, A question of reliability, Nature 371 (1994) 652–653.
[16] S.N. Davies, R.A.J. Lester, K.G. Reymann, G.L. Collingridge, Temporally distinct pre- and postsynaptic mechanisms maintain long-term potentiation, Nature 338 (1989) 500–503.
[17] G.M. Durand, Y. Kovalchuk, A. Konnerth, Long-term potentiation and functional synapse induction in developing hippocampus, Nature 381 (1996) 71–75.
[18] F.A. Edwards, Anatomy and electrophysiology of fast central synapses lead to a structural model for long-term potentiation, Physiol. Rev. 75 (1995) 759–787.
[19] D.S. Faber, H. Korn, Application of the coefficient of variation methods for analyzing synaptic plasticity, Biophys. J. 60 (1991) 1288–1294.
[20] S.A. Fedulova, D.V. Vasilyev, E.V. Isaeva, S.G. Romanyuk, N.S. Veselovsky, Possibility of multiquantal transmission at single inhibitory synapse in cultured rat hippocampal neurons, Neuroscience 92 (1999) 1217–1230.
[21] L. Forti, M. Bossi, A. Bergamaschi, A. Villa, A. Malgaroli, Loose-patch recordings of single quanta at individual hippocampal synapses, Nature 388 (1997) 874–878.
[22] T.C. Foster, B.L. McNaughton, Long-term enhancement of CA1 synaptic transmission is due to increased quantal size, not quantal content, Hippocampus 1 (1991) 79–91.
[23] M. Frerking, M. Wilson, Saturation of postsynaptic receptors at central synapses, Curr. Opin. Neurobiol. 6 (1996) 395–403.
[24] U. Frey, Cellular mechanisms of long-term potentiation: late maintenance, in: J.W. Donahoe (Ed.), Neural Network Models of Complex Behavioral Foundation, Elsevier, 1997, pp. 105–128.
[25] S. Gasparini, C. Saviane, L.L. Voronin, E. Cherubini, Silent synapses in the developing hippocampus: Lack of functional AMPA receptors or low probability of glutamate release?, Proc. Nat. Acad. Sci. USA 97 (17) (2000) 9741–9746.
[26] Y. Geinisman, L. de Toledo-Morell, F. Morell, R.E. Heller, M. Rossi, R.F. Parshall, Structural synaptic correlate of long-term potentiation: formation of axospinous synapses with multiple, completely partitioned transmission zones, Hippocampus 3 (1993) 435–446.
[27] E.M. Glaser, D.S. Ruchkin, Principles of Neurobiological Signal Analysis, Academic, New York, 1976.
[28] E. Hanse, B. Gustafsson, Quantal variability at glutamatergic synapses in area CA1 of the rat neonatal hippocampus, J. Physiol. Lond. 531 (2001) 467–480.
[29] J.E. Huetter, R.W. Baughman, The pharmacology of synapses formed by identified corticocollicular neurons in primary cultures of rat visual cortex, J. Neurosci. 8 (1988) 160–175.
[30] J.T.R. Isaac, G.O. Hjelmstad, R.A. Nicoll, R.C. Malenka, Long-term potentiation at single fiber inputs to hippocampal CA1 pyramidal cells, Proc. Natl. Acad. Sci. USA 93 (1996) 8710–8715.
[31] J.T.R. Isaac, A. Luthi, M.J. Palmer, W.W. Anderson, T.A. Benke, G.L. Collingridge, An investigation of the expression mechanism of LTP of AMPA receptor-mediated synaptic transmission at hippocampal CA1 synapses using failures analysis and dendritic recordings, Neuropharmacology 37 (1998) 1399–1410.
[32] J.T.R. Isaac, R.A. Nicoll, R.C. Malenka, Evidence for silent synapses: implications for the expression of LTP, Neuron 15 (1995) 427–434.
[33] J.J.B. Jack, A.U. Larkman, G. Major, K. Stratford, Quantal analysis of the synaptic excitation of CA1 hippocampal pyramidal cells, Adv. Second Messenger Phosphoprotein Res. 29 (1994) 275–299.
[34] J.E. Jackson, A User's Guide To Principal Components, Wiley, New York, 1991.
[35] A.M. Kasyanov, V.V. Maximov, A.L. Byzov, N. Berretta, M.V. Sokolov, S. Gasparini, E. Cherubini, K. Reymann, L.L. Voronin, Intrasynaptic ephaptic feedback alters amplitude-voltage relations of mossy fibre responses in rat CA3 hippocampal neurones, Neuroscience 101 (2000) 323–336.
[36] B. Katz, The Release of Neural Transmitter Substance. Charles C. Thomas, Springfield, IL, 1969.
[37] F. Kimura, Y. Otsu, T. Tsumoto, Presynaptically silent synapses: spontaneously active terminals without stimulus-evoked release demonstrated in cortical autapases, J. Neurophysiol. 77 (1997) 2805–2815.
[38] A.M. Kleschevnikov, M. Sokolov, G.S. Dawe, J.D. Stephenson, L.L. Voronin, Changes in paired-pulse facilitation correlated with induction of long-term potentiation in area CA1 of rat hippocampal slices, Neuroscience 76 (1997) 829–843.
[39] U. Kuhnt, L.L. Voronin, Interaction between paired-pulse facilitation and long-term potentiation in area CA1 of guinea pig hippocampal slices: application of quantal analysis, Neuroscience 62 (1994) 391–397.
[40] D.M. Kullmann, Quantal variability of excitatory transmission in the hippocampus: implications for the opening probability of fast glutamate-gated channels, Proc. Roy. Soc., Lond. B253 (1993) 107–116.
[41] D.M. Kullmann, Amplitude fluctuations of dual component EPSCs in hippocampal pyramidal cells: implications for long-term potentiation, Neuron 12 (1994) 1111–1120.
[42] D.M. Kullmann, R.A. Nicoll, Long-term potentiation is associated with increases in quantal content and quantal amplitude, Nature 357 (1992) 240–244.
[43] D.M. Kullmann, S.A. Siegelbaum, The site of expression of NMDA receptor dependent LTP: new fuel for an old fire, Neuron 15 (1995) 997–1002.
[44] A. Larkman, T. Hannay, K. Stratford, J. Jack, Presynaptic release probability influences the locus of long-term potentiation, Nature 360 (1992) 70–73.

[45] A.U. Larkman, J.J.B. Jack, Synaptic plasticity: hippocampal LTP, Curr. Opin. Neurobiol. 5 (1995) 324–334.
[46] A. Larkman, J. Jack, K. Stratford, Quantal analysis of excitatory synapses in rat hippocampal CA1 in vitro during low-frequency depression, J. Physiol. Lond. 502 (1997) 457–471.
[47] A.U. Larkman, J.J. Jack, K.J. Stratford, Assessment of the reliability or amplitude histograms from excitatory synapses in rat hippocampal CA1 in vitro, J. Physiol. 505 (1997) 443–456.
[48] A. Larkman, K. Stratford, J. Jack, Quantal analysis of excitatory synaptic action and depression in hippocampal slices, Nature 350 (1991) 344–347.
[49] S. Li, R. Anwyl, M.J. Rowan, A persistent reduction in short-term facilitation accompanies long-term potentiation in the CA1 area in the intact hippocampus, Neuroscience 100 (2000) 213–220.
[50] D. Liao, A. Jones, R. Malinow, Direct measurement of quantal changes underlying long-term potentiation in CA1 hippocampus, Neuron 9 (1992) 1089–1097.
[51] J.E. Lisman, K.M. Harris, Quantal analysis and synaptic anatomy—integrating two views of hippocampal plasticity, Trends Neurosci. 16 (1993) 141–147.
[52] G. Liu, S. Choi, R.W. Tsien, Variability of neurotransmitter concentration and nonsaturation of postsynaptic AMPA receptors at synapses in hippocampal cultures and slices, Neuron 22 (1999) 395–409.
[53] C. Luscher, R.C. Malenka, R.A. Nicoll, Monitoring glutamate release during LTP with glial transporter currents, Neuron 21 (2) (1998) 435–441.
[54] R.C. Malenka, R.A. Nicoll, Long-term potentiation—a decade of progress?, Science 285 (1999) 1870–1874.
[55] A. Malgaroli, A.E. Ting, B. Wendland, A. Bergamaschi, A. Villa, R.W. Tsien, R.H. Scheller, Presynaptic component of long-term potentiation visualized at individual hippocampal synapses, Science 268 (1995) 1624–1628.
[56] R. Malinow, Transmission between pairs of hippocampal slice neurons: quantal levels, oscillations, and LTP, Science 252 (1991) 722–724.
[57] R. Malinow, R.W. Tsien, Presynaptic enhancement shown by whole-cell recording of long-term potentiation in hippocampal slices, Nature 346 (1990) 177–180.
[58] R. Malinow, Z.F. Mainen, Y. Hayashi, LTP-mechanisms: from silence to four-lane traffic, Curr. Opin. Neurobiol. 10 (2000) 352–357.
[59] A.K. McAllister, C.F. Stevens, Nonsaturation of AMPA and NMDA receptors at hippocampal synapses, Proc. Natl. Acad. Sci. USA 97 (11) (2000) 6173–6178.
[60] B.L. McNaughton, The mechanism of expression of long-term enhancement of hippocampal synapses: current issues and theoretical implications, Annu. Rev. Physiol. 55 (1993) 375–396.
[61] H. Matthies, In search of cellular mechanisms of memory, Progr. Neurobiol. 32 (1989) 277–349.
[62] A. Nayak, D.J. Zastrow, R. Lickteig, N.R. Zahniser, M.D. Browning, Maintenance of late-phase LTP is accompanied by PKA-dependent increase in AMPA receptor synthesis, Nature 394 (1998) 680–683.
[63] A.B. Oestreicher, P.N.E. de Graan, W.H. Gispen, J. Verhaagen, L.H. Schrama, B-50, the growth associated protein-43: modulation of cell morphology and communication in the nervous system, Prog. Neurobiol. 53 (1997) 627–686.
[64] S. Otani, Y. Ben-Ari, Biochemical correlates of long-term potentiation in hippocampal synapses, Int. Rev. Neurobiol. 35 (1993) 1–41.
[65] S. Redman, Quantal analysis of synaptic potentials in neurons of the central nervous system, Physiol. Rev. 70 (1990) 165–198.
[66] J.J. Renger, C. Egles, G. Liu, A developmental switch in neurotransmitter flux enhances synaptic efficacy by affecting AMPA receptor activation, Neuron 29 (2001) 469–484.
[67] T.A. Ryan, N.E. Ziv, S.J. Smith, Potentiation of evoked vesicle turnover at individually resolved synaptic boutons, Neuron 17 (1996) 125–134.
[68] L. Sachs, Angewandte Statistik. Anwendung Statistischer Methoden, Springer, Berlin, 1984.
[69] B.R. Sastry, L.S. Bhagavatula, Quantal release of transmitter at a central synapse, Neuroscience 75 (1996) 987–992.
[70] P.E. Schulz, E.P. Cook, D. Johnson, Changes in paired-pulse facilitation suggest presynaptic involvement in long-term potentiation, J. Neurosci. 14 (1994) 5325–5337.
[71] M.V. Sokolov, A.M. Kleschevnikov, I.T. Bayazitov, A.V. Astrelin, L.L. Voronin, Quantal analysis of a late phase of hippocampal long-term potentiation (LTP): whole cell and sharp electrode recordings, Eur. J. Neurosci. 12 (Suppl. 11) (2000) 5.
[72] M.V. Sokolov, A.V. Rossokhin, A.V. Astrelin, T. Behnisch, K.G. Reymann, L.L. Voronin, Interaction between paired-pulse facilitation and long-term potentiation of minimal EPSPs in rat hippocampal slices: a patch clamp study, Neuroscience 85 (1998) 1–13.
[73] U. Staubli, J. Ambros-Ingerson, G. Lynch, Receptor changes and LTP: An analysis using aniracetam, a drug that reversibly modified glutamate (AMPA) receptors, Hippocampus 2 (1992) 49–58.
[74] C.F. Stevens, Y. Wang, Changes in reliability of synaptic function as a mechanism for plasticity, Nature 371 (1994) 704–707.
[75] K.J. Stratford, J.J. Jack, A.U. Larkman, Calibration of an autocorrelation-based method for determining amplitude histogram reliability and quantal size, J. Physiol. Lond. 505 (1997) 425–442.
[76] C. Stricker, A.I. Cowan, A.C. Field, S.J. Redman, Analysis of NMDA-independent long-term potentiation induced at CA3–CA1 synapses in rat hippocampus in vitro, J. Physiol. Lond. 520 (1999) 513–525.
[77] C. Stricker, A.C. Field, S. Redman, Statistical analysis of amplitude fluctuations in EPSCs evoked in rat CA1 pyramidal neurones in vitro, J. Physiol. Lond. 490 (1996) 419–441.
[78] C. Stricker, A.C. Field, S. Redman, Changes in quantal parameters of EPSCs in rat CA1 neurones in vitro after the induction of long-term potentiation, J. Physiol. Lond. 490 (1996) 443–454.
[79] N. Toni, P.A. Buchs, I. Nikonenko, P. Povilaitite, L. Parisi, D. Muller, Remodeling of synaptic membranes after induction of long-term potentiation, J. Neurosci. 21 (16) (2001) 6245–6251.
[80] N. Torii, T. Tsumoto, L. Uno, A.V. Astrelin, L.L. Voronin, Quantal analysis suggests presynaptic involvement in expression of neocortical short- and long-term depression, Neuroscience 79 (1997) 317–321.
[81] L.L. Voronin, Long-term potentiation in the hippocampus, Neuroscience 10 (1983) 1051–1069.
[82] L.L. Voronin, Quantal analysis of long-term potentiation, in: H.L. Haas, G. Buzsaki (Eds.), Synaptic Plasticity in the Hippocampus, Springer, Berlin, 1988, pp. 27–30.
[83] L.L. Voronin, On the quantal analysis of hippocampal long-term potentiation and related phenomena of synaptic plasticity, Neuroscience 56 (1993) 275–304.
[84] L.L. Voronin, Quantal analysis of hippocampal long-term potentiation, Rev. Neurosci. 5 (1994) 141–170.
[85] L.L. Voronin, A. Byzov, A. Kleschevnikov, M. Kozhemyakin, U. Kuhnt, M. Volgushev, Neurophysiological analysis of long-term potentiation in mammalian brain, Behav. Brain Res. 66 (1995) 45–52.
[86] L.L. Voronin, N.V. Ivanov, U. Kuhnt, Estimate of the variance of the quantal size in excitatory synapses of area CA1 of hippocampal slices, Neurophysiology 25 (1993) 10–17.
[87] L.L. Voronin, U. Kuhnt, Long-term potentiation affects facilitation ratio of EPSPs recorded from CA1 pyramidal cells in the guinea pig hippocampal slice, Neurosci. Res. Commun. 6 (1990) 149–155.
[88] L.L. Voronin, U. Kuhnt, A.G. Gusev, Changes in quantal parameters of CA1 synaptic transmission are dependent on the magnitude of long-term potentiation, Neurosci. Res. Commun. 9 (1991) 1–7.
[89] L.L. Voronin, U. Kuhnt, A.G. Gusev, Analysis of fluctuations of 'minimal' excitatory postsynaptic potentials during long-term potentiation in guinea pig hippocampal slices, Exp. Brain Res. 89 (1992) 288–299.

[90] L.L. Voronin, U. Kuhnt, A.G. Gusev, G. Hess, Quantal analysis of long-term potentiation of 'minimal' excitatory postsynaptic potentials in guinea pig hippocampal slices: binomial approach, Exp. Brain Res. 89 (1992) 275–287.
[91] L.L. Voronin, U. Kuhnt, G. Hess, Quantal analysis of long-term post-tetanic modifications of minimal excitatory postsynaptic potentials of in vitro hippocampal slices, Neurophysiology 22 (1990) 561–570.
[92] L.L. Voronin, U. Kuhnt, G. Hess, A. Gusev, V. Roschin, Quantal parameters of 'minimal' excitatory postsynaptic potentials in guinea pig hippocampal slices: binomial approach, Exp. Brain Res. 89 (1992) 248–264.
[93] L.L. Voronin, M. Volgushev, M. Chistiakova, U. Kuhnt, W. Singer, Involvement of silent synapses in the induction of LTP and LTD in hippocampal and neocortical neurones, Neuroscience 74 (1996) 323–330.
[94] L.M. Wahl, K.J. Stratford, A.U. Larkman, J.J.B. Jack, The variance of the successive peaks in synaptic amplitude histograms: effect of inter-site difference in quantal size, Proc. R. Soc. Lond. B262 (1995) 77–85.
[95] M.J. Wall, M.M. Usowicz, Development of the quantal properties of evoked and spontaneous synaptic currents at a brain synapse, Nature Neurosci. 1 (1998) 675–683.
[96] B. Walmsley, Quantal analysis of synaptic transmission, in: D.I. Wallis (Ed.), Electrophysiology: A Practical Approach, IRL Press, Oxford, 1993, pp. 110–141.
[97] S.S. Zakharenko, L. Zablow, S.A. Siegelbaum, Visualization of changes in presynaptic function during long-term synaptic plasticity, Nature Neurosci. 4 (2001) 711–717.