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QUANTAL ANALYSIS OF LONG-TERM POTENTIATION OF COMBINED NEURONAL POSTSYNAPTIC POTENTIALS ON HIPPOCAMPAL SLICES IN VITRO

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Excitatory postsynaptic potentials (EPSP) were recorded from 14 neurons in guinea pig hippocampal slices (area CA1) after stimulating the stratum radiatum (Schaffer collaterals) and stratum oriens. An increase occurring in EPSP amplitude in 7 units (9 pathways) recorded 15-45 min after tetanic stimulation of Schaffer collaterals is viewed as long-term potentiation (LTP). Statistical analysis conducted according to two sets of quantal theory (histogram and variance methods) showed an increase in mean quantal content (m) during LTP. An increase in quantal size, found only when using the histogram method, did not correlate with LTP level. This increase is thought to be associated with the considerably greater sensitivity of the histogram method to noise level in comparison with the variance method, the latter being more reliable with signals of high noise level. The increase found in m using both methods matches findings previously obtained for the whole brain; it also points to presynaptic location of mechanisms responsible for raised synaptic efficacy during LTP.

INTRODUCTION

The phenomenon of long-term potentiation (LTP), viewed as a model for memory and associative training [5, 6, 18, 24, 26], has received considerable attention in recent years. Findings from intracellular research into in vivo and in vitro preparations have shown that increased efficacy of excitatory synapses underlies this phenomenon. The question of whether this rise should be related to pre- or postsynaptic mechanisms remains debatable, however. Applying quantal theory of synaptic transmission is one approach to finding an answer [4, 5, 10, 12], giving mean amplitude of excitatory postsynaptic potentials (EPSP) as

$$E = m \cdot v, \quad (1)$$

where m stands for mean quantal content (mean number of quanta or "packets" of transmitter released due to the arrival of a single presynaptic impulse) and v is quantal size (the shift in membrane potential produced by the action of a single quantum). Taking n as the number of transmitter release sites [19] and p as the probability of release at an individual release site – and consequently of release of one quantum – it then follows that

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$$m = n \cdot p, \quad (2)$$

and the number of EPSP resulting from the release of varying numbers of quanta may be calculated using a binomial distribution equation with parameters p and n. We have already used quantal theory [5, 6, 25, 26] for analyzing mechanisms of hippocampal LTP by recording threshold-level EPSP (those produced by near-threshold microstimulation of afferent fibers) in the CA3 region of unanesthetized rabbits. This analysis induced a rise in m without any substantial change in v which, according to quantal theory, denotes a presynaptic location of LTP mechanisms. Difficulties experienced in intracellular recording during experiments on intact animals did not allow for research into a sufficiently large number of neurons and recording of neuronal activity for a spell of over 15 min following tetanization. This was why we used hippocampal slices in this research, allowing us to carry out more prolonged and stable intracellular recording. Our first step consisted of analyzing "combined" EPSP with an amplitude of over 1 mV, recorded by intracellular techniques from area CA1 units during adequate stimulation of afferent fibers.

METHODS

The techniques of stimulation, recording, and analysis used were similar to those described previously [6, 8, 9, 19]. Guinea pig hippocampal slices served as experimental material. The (glass) recording microelectrode was inserted into the pyramidal layer of CA1. The principal (tungsten) electrode for applying test stimuli, with glass insulation and a resistance of 0.1-1.0 MΩ, was introduced into the radial layer for stimulating Schaffer's collaterals; an additional stimulating electrode was inserted into the stratum oriens in a portion of the experiments. The microelectrodes used for intracellular recording were filled with 4 M potassium acetate; resistance 20-100 MΩ. An extra electrode for extracellular recording (2 M NaCl; 1-10 MΩ) was employed for controlling stimulus intensity and monitoring the condition of slices according to field potentials. Trials consisted of single stimuli applied every 8-10 sec. Stimulus intensity and duration (of 1-8 V and 0.02-0.1 msec, respectively) were chosen so as to induce intracellular EPSP measuring from 1 to 5 mV in amplitude. Tetanization was produced by means of the principal (test) electrode and took the form of 10 stimulus trains (at the rate of 100/sec; duration of train 200 msec; interval between trains 8-10 sec).

A modified version of the two standard techniques (histogram and variance methods) for calculating quantal parameters [4, 5] was used, which was fully described in our recent study [7]. Parameters obtained by applying the two methods will be indexed as 1 and 2, respectively. The first (i.e., histogram) method was automated, using a special program allowing for the selection of a theoretical distribution corresponding as closely as possible to that applying in our experiments; this followed the line described previously [2, 5, 7]. Within the context of the second (variance) method, adjustments were made [9] to the equation under which parameter p is determined as the ratio between mean EPSP amplitude (E) and peak level [3]. Having calculated p_2, mean quantal content was determined as $m_2 = E^2(1 - p_2)/(S^2 - S_n^2)$ where S^2 stands for scatter in amplitude of response and S_n^2 for scatter of noise. Values v and n were then determined from Eqs. (1) and (2), respectively. Potentials were recorded on disk using a laboratory computer and underwent processing subsequently. Amplitudes of single responses were measured from peak EPSP using a special program. Following other workers [21], we did not measure noise systematically, although we did use S_n values established from the best match between theoretical and experimentally obtained distributions. These values (averaging 118 ± 69 μV – standard deviations given here and subsequently) usually fell below those corresponding to reality (of about 200 μV on average) as measured in several instances. Statistical processing after calculating quantal parameters was performed using standard programs for PC's.

RESULTS

Recording began not sooner than 20 min after piercing of the neuronal membrane. We investigated cells with no background firing and with an absolute membrane potential level of not less than 60 mV, changing by not more than 1-3 mV compared with the control value over the entire recording period (see Fig. 1e). Activity was recorded from 14 units in controls (not less than 99 stimuli to at least one test pathway) and at intervals varying between 15 and 45 min after tetanization. A significant increase in E ($P < 0.01$; Student's t-test) was noted after tetanization in 7 cells (9 different pathways); we shall regard this as LTP (Fig. 1a-d).

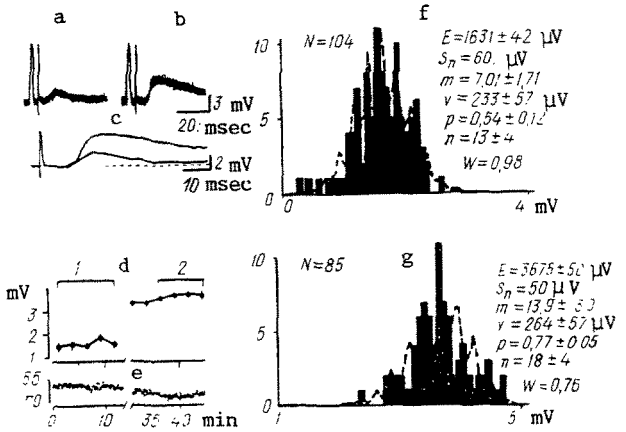

Fig. 1. Sample EPSP in a hippocampal neuron and statistical analysis of these by production of histograms; a and b) superposition of single responses before (a) and after (b) tetanization. Calibrating impulse of 10 mV amplitude given at initial portion of trace; c) superposition of averaged responses before and after tetanization; averaging of 104 and 85 responses respectively (N for f and g); d) change, with time, of mean EPSP amplitude during the same experiment. Abscissa: time after start of recording, min; ordinate: amplitude calculated for 20 successive EPSP, mV (vertical strokes denote standard error), quasi-steady state segments marked before (1) and after (2) tetanization; e) changes in membrane potential (ordinate, mV) during experimentation. Abscissa: same as d; f and g) experimentally and theoretically (i.e., computerized) obtained amplitude distributions (filled columns and dashed lines respectively). Abscissa: EPSP amplitude, mV; ordinate; number of events. Parameters calculated by producing histograms, estimation of standard error, and calculating highest probability of null hypothesis (χ^2 test; W).

In all instances, LTP of EPSP recorded by intracellular techniques was accompanied by a rise in amplitude together with increased field potential readings. The value of LTP (i.e., the relationship between mean EPSP amplitudes before and after tetanization) was between 114 and 225% for all neurons. Mean LTP level (see value E in Fig. 5) equalled 158 ± 36%.

The relationship between mean amplitudes, taken from either 10 or 20 successive measurements, and serial number of the test stimulus was plotted for the purposes of detailed statistical work (see Fig. 1d and Fig. 2a). Control quasi-steady state segments (segment 1 in Fig. 1d and Fig. 2a) were determined [5, 12], as well as segments for the time spell 15-45 min after tetanization (segment 2, same diagrams). Segments within which mean amplitudes did not differ significantly ($P > 0.05$; Student's t-test) were considered quasi-steady state. Each such segment covered an average of 92 measurements (varying between 60 and 140 from one unit to the next). Mean amplitude level for segments 1 measured 3.21 ± 1.60 mV (4.84 ± 1.82 mV for segments 2); N = 9.

Quantal parameters m and v and binomial parameters n and p were calculated following the two methods described. Those calculated by two different methods may differ noticeably from each other in some instances, but remained fairly close on average (see Fig. 3 and Table 1). A significant correlation was seen between equivalent parameters obtained using different methods (Fig. 3).

The rise in m proved to be the most easily reproducible change occurring post-tetanization. It manifested in the context of method 1 as a marked rightwards shift in amplitude distribution (Fig. 1g) compared with that found in controls prior to tetanization (Fig. 1f) with no significant increase in the distance between theoretical distribution peaks. A rise in m_1 was observed in 7 out of 9 cases, with mean value of m_1 at 131 ± 39% versus control level (see Fig. 5, m_1; a significant difference from controls, taken as 100% — Wilcoxon's paired t-test; $P < 0.04$). The rise occurring in m_2 was even more significant ($P < 0.008$);

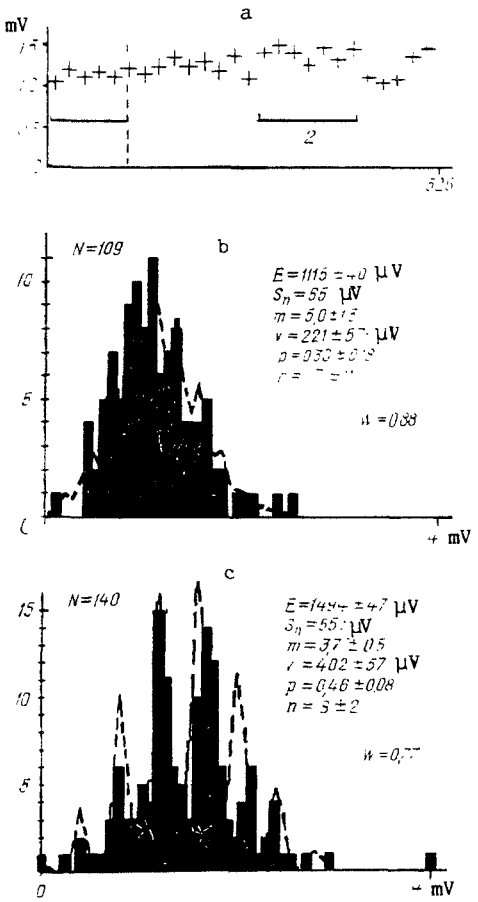

Fig. 2. Case of the maximal post-tetanic increase in quantal size. a) Dynamics of mean EPSP amplitude (N = 20) during experimentation; b, c) distribution of amplitude before (b) and after (c) tetanization. Notation for a is the same as for Fig. 1d and for b and c — same as for Fig. 1f and g.

this was seen in 9 out of a total of 9 instances, giving a mean percentage of 155 ± 55% (Fig. 5, m_2; see also Table 1). A significant correlation was found between percentage amplitudes of EPSP and m as determined using the two methods (Fig. 4). Equivalent correlation coefficients calculated for m_1 ($r = 0.87$) and m_2 ($r = 0.82$) were likewise highly significant ($P < 0.003$).

At the same time, it may be seen from Fig. 4a that most values are situated leftwards of the line bisecting the right-angle, indicating a rise in v. In two cases (see triangles to right of ordinate) data obtained using method 1 could be used so as to treat LTP as resulting solely from the rise in v. Figure 2b and c shows examples of equivalent distributions. This increase was only significant for v_1, however ($P < 0.02$, Wilcoxon's paired t-test); it did not exceed standard error level for v_2 (see Fig. 5 and Table 1). In addition, no correlation was observed between the rise in EPSP amplitude and relative level of v_1 ($r = -0.2$, $P > 0.6$). Neither changes in v_2 as a result of tetanization ($r = 0.1$, $P > 0.7$), nor total changes in v_1 and v_2 correlated with LTP level (see Fig. 4b).

Mean levels of binomial parameters p and n, taken as a percentage of controls, equalled 120 ± 21% and 107 ± 40% post-tetanization for method 1 and 105 ± 7% and 147 ± 51% for method 2. Differences from control levels were significant for p_1 ($P < 0.05$) and n_2 ($P < 0.01$, Wilcoxon's paired/two-tailed t-test).

DISCUSSION

By using surviving hippocampal slices we were able to continue quantal analysis of LTP for a longer period post-tetanization than had previously been possible [5, 6, 25, 26]. Techniques of analysis were refined [7, 9] and their potential use under conditions approaching those applying in our experiments have been extensively analyzed in our earlier publication [7]. The automated histogram method serves, at the same time, to test for matching between a binomial model and experimentally obtained distributions. The program used produced binomial distributions not differing significantly from those obtained experimentally according to the χ^2 test (see W values, Figs. 1 and 2) in all instances. It is true that this match does not prove the exclusivity of the binomial model [13, 16]. Nonetheless, the fit with the model obtained (as well as the aforementioned correlation between parameters

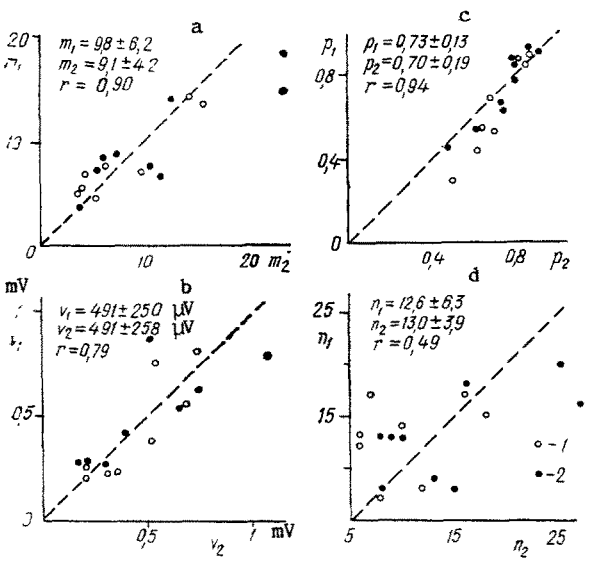

Fig. 3. Correlation between quantal parameters m (a), v (b), p (c), and n (d) calculated by applying two different methods. Abscissa: parameters calculated by histogram method; ordinate: same, but using variance method. Dashed line denotes lines bisecting right angle. Shown are mean levels ± standard deviation and correlation coefficients (r); 1) pre- and 2) post-tetanization.

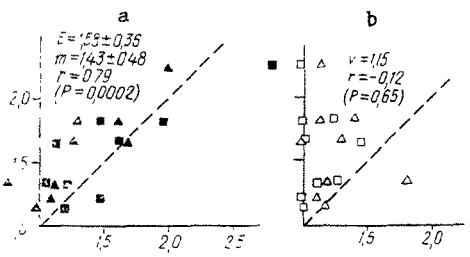

Fig. 4

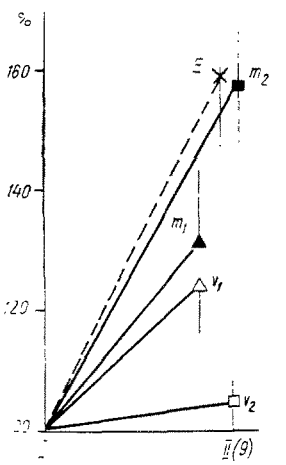

Fig. 5

Fig. 4. Long-term potentiation (ordinate — mean amplitude post-tetanization expressed as percent of controls) plotted against change in m (abscissa; a) and v (abscissa; b) as obtained using two different methods. Triangles indicate data obtained by applying method 1, squares — by method 2. P) Significance level of correlation coefficients (r). Remaining notation same as in Fig. 3.

Fig. 5. Post-tetanic changes in mean EPSP amplitude (E), mean quantal content (m_1 and m_2), and quantal size (v_1 and v_2). Values calculated for control section pre-tetanization (taken as 100% — point I on horizontal axis); values calculated for post-tetanic sections (point II on horizontal axis) expressed as percentages of control levels and in the form of mean ± standard error. Number of measurements made given in brackets. See text for detailed explanations.

TABLE 1. Quantal EPSP Parameters (mean ± standard deviation) in Hippocampal Neuronal EPSP Pre- and Post-tetanization (I) and (II) Respectively

Method	Section	m	v, μV	p	n
Histogram	I	8,2±3,7	424±248	0,65±0,22	12,9±3,8
	II	10,0±4,7*	551±266*	0,74±0,17*	13,1±4,3
Variation coefficient	I	7,8±4,7	450±204	0,71±0,13	10,4±4,6
	II	11,6±7,1**	528±292	0,75±0,13	14,6±7,1**

Note. Significant increase compared with control (pre-tetanization) level (Wilcoxon's paired t-test) marked by a single ($P < 0.05$) and double ($P < 0.01$) asterisk, respectively. See text for detailed explanations.

calculated by different methods) would justify applying the binomial model as a first approximation for our purposes, as in many instances described in the literature [4, 12, 21]. The correlation found could not be explained by the fact that the value S_n ascertained within the context of method 1 was used in method 2, since special calculations showed that S_n exerted only very minor influence (of less than 5-10%) on level of m_2. Attempts to employ other, more complex models have been reported in the literature [1, 20], as noted in our recent study [7]. As requirements regarding size of sample and noise level would thereby be raised considerably, however, use of such models would be rendered impracticable in many cases.

The post-tetanic changes in basic quantal parameters (m and v) calculated by using the two methods were qualitatively similar, with a rise in m being mainly observed, correlating with LTP level. This resembles the finding obtained previously during experiments on intact animals [5, 6, 25, 26]. The increase in m, indicating as it does a presynaptic location of LTP mechanisms, also corresponds to findings obtained by the majority of other workers who had applied quantal analysis to study long-term post-tetanic changes in vitro at peripheral synapses [3, 14, 22], long-lasting facilitation in mollusk central neurons [17], and lastly long-term changes in the EPSP of neurons within hippocampal area CA3 after administering phorbol esters [27]. These data also fit in with findings pointing to increased transmitter (glutamate and aspartate) releases after tetanization [15, 23]. The question of what gives rise to changes in m (whether increase in p or n or, the most likely hypothesis, in both these parameters) remains unanswered; application of the two methods resulted in somewhat conflicting data, while results from our computerized experiments [7] showed that employing these techniques for small samples does not produce sufficiently reliable estimates of binomial parameters and of n in particular.

No significant changes were found in v applying the coefficient of variation method. The histogram method did reveal a rise in v, which, however, did not correlate with the level of LTP. The histogram method was found to be more sensitive to noise level than the variance technique in our recent study [7]. The latter had proved more reliable under our own experimental conditions, producing fairly good estimates of v and m even at $S_n > v$ [7], in contrast with the former, giving v_1 values several times the true value of v. The possibility should therefore not be excluded that estimates of v_1 are substantially reduced in some cases (such as that illustrated in Fig. 2b). Measurement of amplitude according to peak levels could also produce a deterioration in quantal pattern of distribution to discoordinated peaking of different quanta. This factor could not be said to exert a significant influence due to the laminar organization of afferent pathways, limited dependence of v on electrotonic distance from recording site [20], and extensive duration of the lead front of hippocampal EPSP (of 6-15 msec) compared with the discoordination anticipated in transmitter release (measurable in fractions of msec). In view of the above considerations, we nonetheless used measurements similar to calculations of EPSP area in subsequent studies, as well as other approaches improving signal–noise ratio, in addition to further techniques of quantal analysis.

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