Pergamon

PII: S0306-4522(99)00167-0

Neuroscience Vol. 93, No. 2, pp. 469–477, 1999

0306-4522/99 $20.00+0.00

LONG-TERM SYNAPTIC CHANGES INDUCED BY INTRACELLULAR TETANIZATION OF CA3 PYRAMIDAL NEURONS IN HIPPOCAMPAL SLICES FROM JUVENILE RATS

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Abstract—Minimal excitatory postsynaptic potentials were evoked in CA3 pyramidal neurons by activation of the mossy fibres in hippocampal slices from seven- to 16-day-old rats. Conditioning intracellular depolarizing pulses were delivered as 50- or 100-Hz bursts. A statistically significant depression and potentiation was induced in four and five of 13 cases, respectively. The initial state of the synapses influenced the effect: the amplitude changes correlated with the pretetanic paired-pulse facilitation ratio. Afferent (mossy fibre) tetanization produced a significant depression in four of six inputs, and no significant changes in two inputs. Quantal content decreased or increased following induction of the depression or potentiation, respectively, whereas no significant changes in quantal size were observed. Compatible with presynaptic maintenance mechanisms of both depression and potentiation, changes in the mean quantal content were associated with modifications in the paired-pulse facilitation ratios, coefficient of variation of response amplitudes and number of response failures. Cases were encountered when apparently "presynaptically silent" synapses were converted into functional synapses during potentiation or when effective synapses became "presynaptically silent" when depression was induced, suggesting respective changes in the probability of transmitter release.

It is concluded that, in juvenile rats, it is possible to induce lasting potentiation at the mossy fibre–CA3 synapses by purely postsynaptic stimulation, while afferent tetanization is accompanied by long-lasting depression. The data support the existence not only of a presynaptically induced, but also a postsynaptically induced form of long-term potentiation in the mossy fibre–CA3 synapse. Despite a postsynaptic induction mechanism, maintenance of both potentiation and depression is likely to occur presynaptically. © 1999 IBRO. Published by Elsevier Science Ltd.

Key words: long-term depression, long-term potentiation, postnatal development, excitatory postsynaptic potentials, quantal analysis, paired-pulse facilitation.

Two experimental models are widely used to study memory mechanisms in the mammalian brain: long-term potentiation (LTP) and long-term depression (LTD).[2,9,38] However, the mechanisms of the latter are not well characterized. It has recently been found[7,21] that, before the second postnatal week, a novel *N*-methyl-D-aspartate (NMDA)-independent form of LTD can be induced at mossy fibre–CA3 synapses by a short (1 s), high-frequency (100 Hz) afferent tetanus (AT). Postsynaptic induction mechanisms have been suggested, since the LTD could not be induced when neurons were voltage clamped during the tetanus or when intracellular Ca^{2+} was chelated.[21]

The first aim of the present work was to further verify whether the induction of this form of LTD was postsynaptic. To this end, we used intracellular tetanus (IT), which is known to induce robust long-lasting modifications in other synapses.[11,33,55,56,60,62] This conditioning paradigm has the advantage of excluding the possibility of tetanus-induced changes in excitability of presynaptic fibres[40] and/or in polysynaptic pathways.[41] The second aim was to clarify the maintenance mechanisms of this type of LTD. To this end, we have used approaches based on quantal analysis[59] and on presynaptic paired-pulse facilitation (PPF) changes.[34,48,50,61] Part of this work has been published in abstract form.[8]

EXPERIMENTAL PROCEDURES

Preparation and recordings

Hippocampal slices were prepared from seven- to 16-day-old Wistar rats (University of Trieste, Italy) previously anaesthetized with intraperitoneal injection of urethane (2 g/kg). Transverse slices (500–600 μm thick) were cut with a vibroslicer and maintained at room temperature (22–24°C) in oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 130, KCl 3.5, $CaCl_2$ 2, $MgCl_2$ 1.3, NaH_2PO_4 1.25, $NaHCO_3$ 26, glucose 11, L-glutamine 2, saturated with 95% O_2/5% CO_2. After incubation in ACSF for at least 1 h, one individual slice was transferred to a submerged recording chamber superfused continuously at 32–33°C with oxygenated ACSF at a rate of 3 ml/min.[21] α-3-(2-Carboxy-piperazin-4-yl)-propyl-1-phosphonic acid (20 μM) and picrotoxin (50 μM) were added to the ACSF to block NMDA and $GABA_A$ receptors, respectively, and tetrodotoxin (2–10 nM) was added to diminish polysynaptic activation and prevent interictal discharges. Minimal excitatory postsynaptic potentials (EPSPs) with occasional failures (Fig. 1B, C) were recorded in the whole-cell configuration (current-clamp mode) following low-frequency (1/15 s) testing stimulation. The recording pipette contained (in mM): potassium gluconate 135, KCl 5, $MgCl_2$ 3, K_2ATP 2, HEPES 10, and was corrected to 290 mOsm with sucrose. The pH was adjusted to 7.3 with KOH. After response stabilization and 25–30 min baseline testing, IT was delivered. IT consisted of 10–30 trains of 100–200 depolarizing pulses, each of 5 or 10 ms duration, at a frequency of 50 or 100 Hz, repeated at 1- or 10-s intervals. The current (typically between 0.4 and 1.1 nA) was suprathreshold for spike initiation, but not every pulse induced a spike during the high-frequency conditioning

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Abbreviations: ACSF, artificial cerebrospinal fluid; AT, afferent tetanus; EPSP, excitatory postsynaptic potential; EPSP1 and EPSP2, EPSPs evoked by the first and second paired pulses, respectively; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; ICD, intracellular depression; ICP, intracellular potentiation; IT, intracellular tetanus; LTD, long-term depression; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PPF, paired-pulse facilitation.

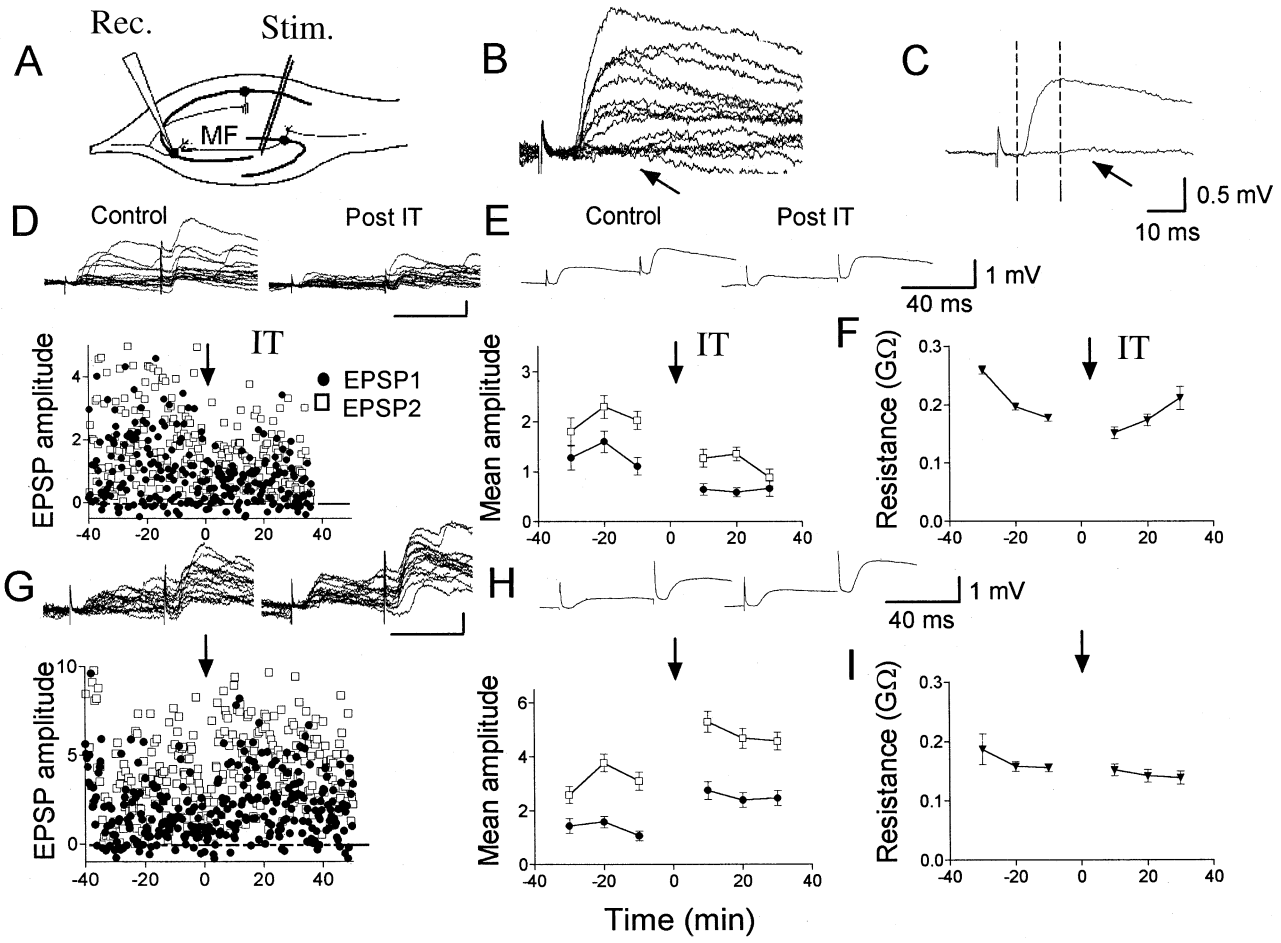

Fig. 1. Minimal EPSPs evoked by mossy fibre stimulation and their changes following IT. (A) Electrode arrangement to stimulate mossy fibres (MF) and to record from CA3 neurons. (B) Superimposed single EPSPs with failures (arrow). (C) The average responses and average failures (arrow). The dashed lines show a window used for amplitude evaluations. (D–F, G–I) Data from experiments with depression and potentiation, respectively, induced by IT. Single (D, G, graphs) and mean (E, H, graphs) amplitudes of the first (EPSP1) and second (EPSP2) responses in the paired-pulse paradigm are plotted as dots and squares, respectively. Fifteen consecutive individual responses (D) and average responses (E) are given above the graphs. The bars represent ±S.E.M. (F, I) Input resistances measured in the same experiments.

train. AT consisted of a 1-s train of 100-Hz stimuli repeated three times at 10-s intervals. Amplitude modifications were calculated by comparing the mean (n=40–100) EPSP amplitudes before and after conditioning IT or AT.

Response measurements

For precise EPSP measurements, we used an approach based on principal component analysis,[26] recently described in detail by Astrelin *et al.*[4] The scores of the first principal component were determined from a window covering the initial slope and the EPSP peak (Fig. 1C, dashed lines). This integral covariance measure was strongly correlated with peak amplitudes, but gives a better signal-to-noise ratio, which is especially important for quantal analysis (Fig. 4). The covariance amplitudes are termed "amplitudes" for simplicity, but note that they are given in conventional units (component scores) in Figs 1–4. Respective waveforms are given in Figs 1–3, with calibrations in mV. The noise was measured for a window of the same duration, but before stimulus artefact. Principal component analysis was also used to separate inputs with different latencies, as described in detail elsewhere.[4] Briefly, we collected responses corresponding to positive values of one component and approximately zero values of another component. For example, to identify the first component (C1 in Fig. 2C), we selected responses corresponding to the 10 largest scores of the first component (dots in the right dashed box in Fig. 2C having the largest x-values) and about $0 \pm 2S_n$ (noise standard deviation) scores of the second component (y-values). To obtain the second component (C2 in Fig. 2C), we selected trials with about zero y-values (left dashed box in Fig. 2C). The algorithm based on the component analysis selected two different waveforms in three of 11 neurons recorded before and after tetanus (either IT or AT, or both). The latency differences between the two components (C1 and C2 in Fig. 2D) strongly suggest that they belong to different inputs.[4] Altogether, we analysed 14 EPSPs or inputs. Similar to the previous finding,[56] the effects of IT and AT were not cell but input specific. Therefore, our data refer to "inputs" rather than "cells".

Failure and quantal analyses

To determine the number of failures (N_0), the latter were selected visually (Fig. 1B, arrow); their repeated averaging tested the absence of non-failures (Fig. 1C). To determine the mean quantal content (m), we used a variant of the "unconstrained" noise deconvolution technique.[3] The algorithm searched for discrete distributions (bars) with coordinates x_i (distance from 0) and P_i (heights). The weighted mean interval between the bars was used to define quantal size $v = \Sigma x_i P_i / \Sigma_i P_i$ and $m = E/v$; $0.15v$ was taken as the intrinsic quantal variance.[3] Because stimuli had to be applied at a low rate (0.07 Hz) to avoid amplitude depression, n was typically small (111 ± 2, mean ± S.E.M., n=54 distributions). To increase n, according to Kullmann and Nicoll,[35] we sometimes combined measurements from both responses in the paired-pulse paradigm (Fig. 4A–C). Computer simulations[3] show that small samples can give reliable estimates provided $v/S_n > 2.5$, where S_n is the noise standard deviation. The mean v/S_n for 54 distributions used for our analysis was 3.9 ± 0.1.

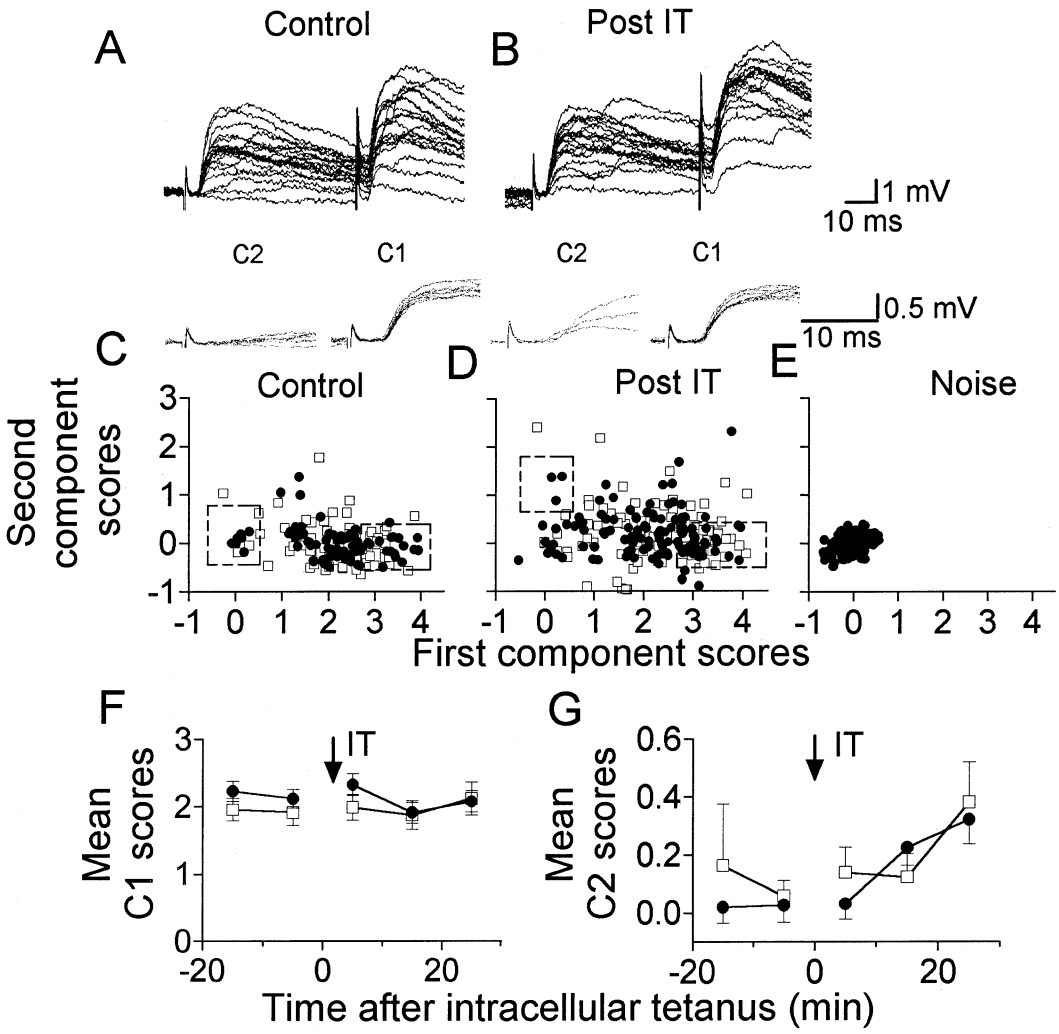

Fig. 2. Component analysis reveals two response components with a selective potentiation of only one component. (A, B) Examples of responses. (C–E) Graphs to plot the scores of the first and second components obtained on the basis of principal component analysis,[26] as described elsewhere,[3] before (C) and after (D) ICP and for the background noise (E). Dots and open squares in the graphs correspond to EPSP1 and EPSP2, respectively. The narrow plot in C (compare with the noise plot in E) suggests only infrequent occurrences of component 2, which never appeared in pure form in EPSP1, as C2 shows. To identify the response components C2 and C1, we selected responses corresponding to the dots within the dashed rectangles, as described in the Experimental Procedures. Before tetanus (C), only response failures (dots in the left dashed triangle, $n=7$) corresponded to pure C2 waveforms (C2 in C), but after IT (C2 in D) large responses appeared ($n=3$). The plot became broader, indicating a larger contribution of the second component. EPSP1 containing the first component with large amplitudes was equally present before and after IT (right dashed boxes in C and D, respectively). Note the latency difference between C1 and C2 (4.1 and 5.8 ms, respectively). (F, G) Changes in the mean scores of the first (F) and second (G) components after IT (arrows). See Fig. 1 for other explanations.

RESULTS

Effects of intracellular tetanization

Minimal EPSPs with occasional response failures (Fig. 1B, C) were evoked by paired stimulation of the mossy fibres (Fig. 1A) before and after IT (Fig. 1D, Control and PostIT).

As shown in the graphs of Fig. 1D and E, delivery of IT induced a persistent reduction of EPSP amplitudes, termed "intracellular depression" (ICD).[33] Unexpectedly, "intracellular potentiation" (ICP)[33] rather than ICD occurred in some experiments (Fig. 1G, H). The amplitude modifications persisted throughout the recording period (30–60 min post-tetanus) and did not correlate with the postnatal age ($r=0.02$, $P>0.95$, $n=12$). The membrane input resistance did not change (Fig. 1I) or increased after IT (Fig. 1F), without correlation with EPSP amplitude modifications ($r=0.17$, $P>0.4$, $n=12$).

For a more precise study, we used a novel algorithm[4] based on principal component analysis.[26] The algorithm selected two different waveforms in three of 11 recorded neurons. Figure 2 illustrates responses from an experiment with IT which induced only a small ICP. Figure 2C and D (graphs) plot the component scores before and after IT, respectively. Response superpositions from different parts of the plots (see Experimental Procedures) represent two components with different latencies (C1 and C2 in Fig. 2D). Comparison of the superpositions (Fig. 2C, D) and the graphs which plotted the changes of the two component scores (Fig. 2F and G, respectively) shows that the first component was not significantly modified by IT. The amplitude of the longer-latency second component was not significantly different from zero before IT in response to the first pulse (Fig. 2G, dots), so that an attempt to separate it in the pure form produced only failures (Fig. 2C, waveforms C2). The second component appeared only after IT (Fig. 2D, waveforms C2), when it showed a slowly developing ICP also observed in one more

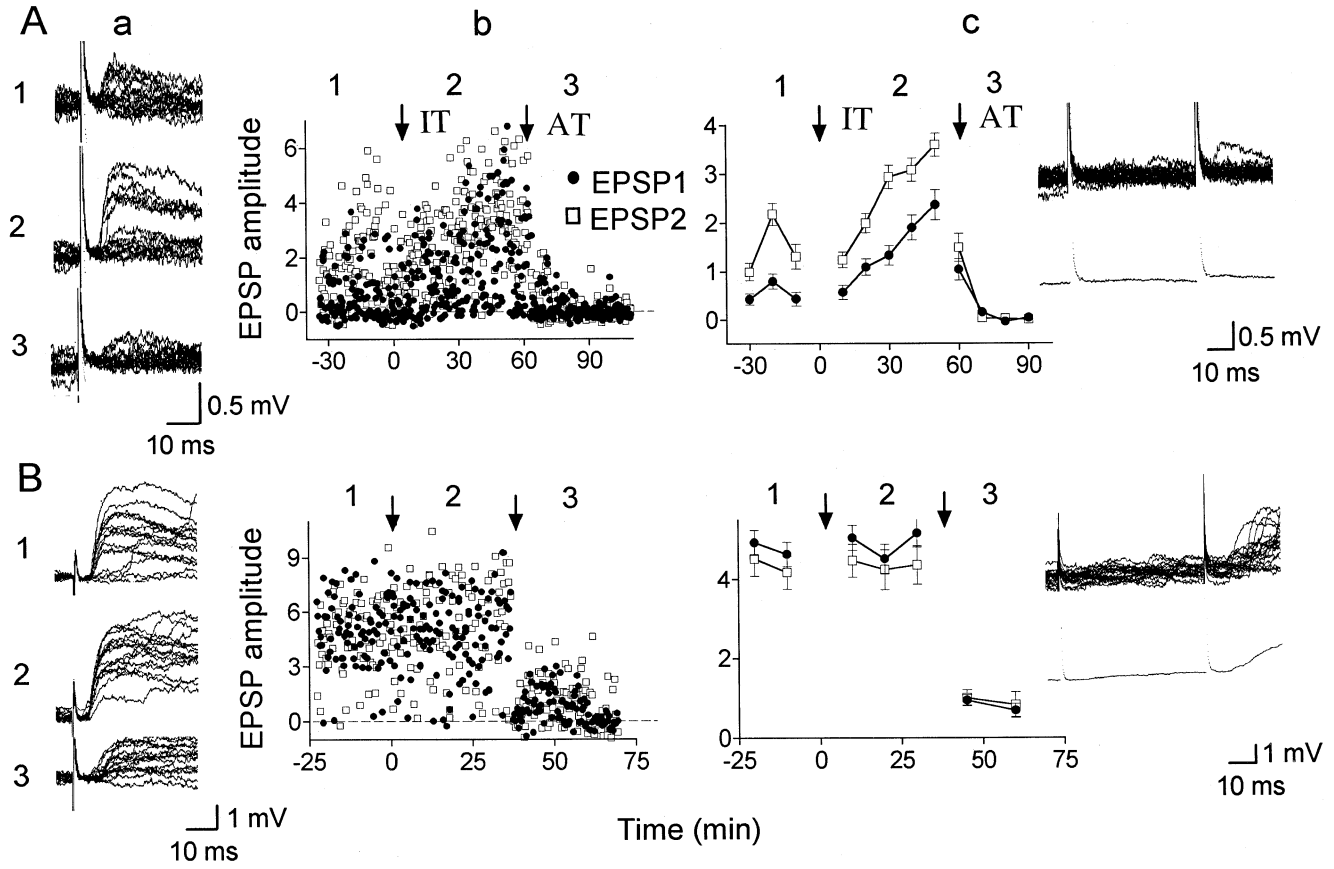

Fig. 3. Depression of minimal EPSPs following AT of the mossy fibres in two experiments (A, B) with different effects of IT. Examples of superimposed EPSPs (a) evoked by the first pulses in the paired-pulse paradigm (EPSP1), and plots of single (b) and mean (c) EPSP amplitudes before IT (1), after IT (2) and after AT (3). Waveforms in c show single and averaged EPSPs after full LTD development. Note a strong depression in both experiments, despite different effects of IT. The delayed depression could be so strong that EPSP1 disappeared almost completely (waveforms in c). Responses to the second pulse (EPSP2) appeared occasionally due to PPF ("presynaptically silent" synapses).

case (see Fig. 3C, IT). Therefore, Fig. 2C and D illustrate modifications of virtually "silent" synapses.[13,24,37,57,65] Occasional occurrences of the large scores of this component, especially in response to the second pulse before IT (Fig. 2A, squares), indicate that it was "presynaptically"[31,51,57,58] rather than "postsynaptically" silent.[13,24,37]

Altogether, in experiments with IT we analysed responses from 13 inputs. A response depression after IT was observed in six of 13 inputs, being negligible (85–97% relative to baseline) in two inputs and statistically significant (39–68%, $P<0.05$, *t*-test) in four inputs. IT induced EPSP amplitude increases (ICP) in seven of 13 inputs, five of which were statistically significant ($P<0.05$). In one of these inputs, responses to the first pulse (EPSP1) significantly different from the baseline appeared only after IT (Fig. 2C, G); in four other inputs, EPSP1 amplitude increased after IT (Fig. 1H, Fig. 3Ac). The magnitude of the increase was 29–324% above the baseline.

Effects of afferent tetanization

Afferent tetanus induced a strong (at least 68%) LTD in four of six inputs tested, irrespective of the IT effects (Fig. 3A, B). A lasting potentiation was never observed. In two inputs, delayed LTD (Fig. 3Ab, Bb, dots) was so strong that the respective inputs could be considered as "silent".[13,24,37,57,65] However, similar to the above cases with IT, occasional occurrences of responses to the second pulse (Fig. 2Ac, Bc, waveforms; Fig. 3Ab3, Bb3, squares) indicated that they were "presynaptically"[31,51,57,58] rather than "postsynaptically" silent.[13,24,37]

Maintenance mechanisms of the post-tetanic modifications

To study the underlying maintenance mechanisms, a variant[3] of the deconvolution technique[46] was applied, and quantal sizes (*v*), together with quantal contents (*m*), were calculated (Fig. 4). EPSP amplitudes fluctuated[29] (Fig. 1B), so that the amplitude distributions were peaky (Fig. 4A). The deconvolution solution typically contained approximately equidistant components (Fig. 4A–C, bars), with their mean distances approximately the same before and after IT. Accordingly, the weighted average distance between the peaks (see Experimental Procedures), which was taken as an estimation of the quantal efficacy (*v* in Fig. 4A–C, insets), did not change significantly following IT, whereas the estimated quantal content (*m*) decreased (Fig. 4Ab, Cc) or increased (Fig. 4Bb, Cb) in parallel with EPSP amplitude modifications.

Figure 5 summarizes the deconvolution analysis. Changes in *m* without significant shifts in *v* (Fig. 5A–C), and the correlations between changes in *m* and EPSP amplitudes (Fig. 5D), are consistent with a presynaptic site of expression of the EPSP amplitude modifications.

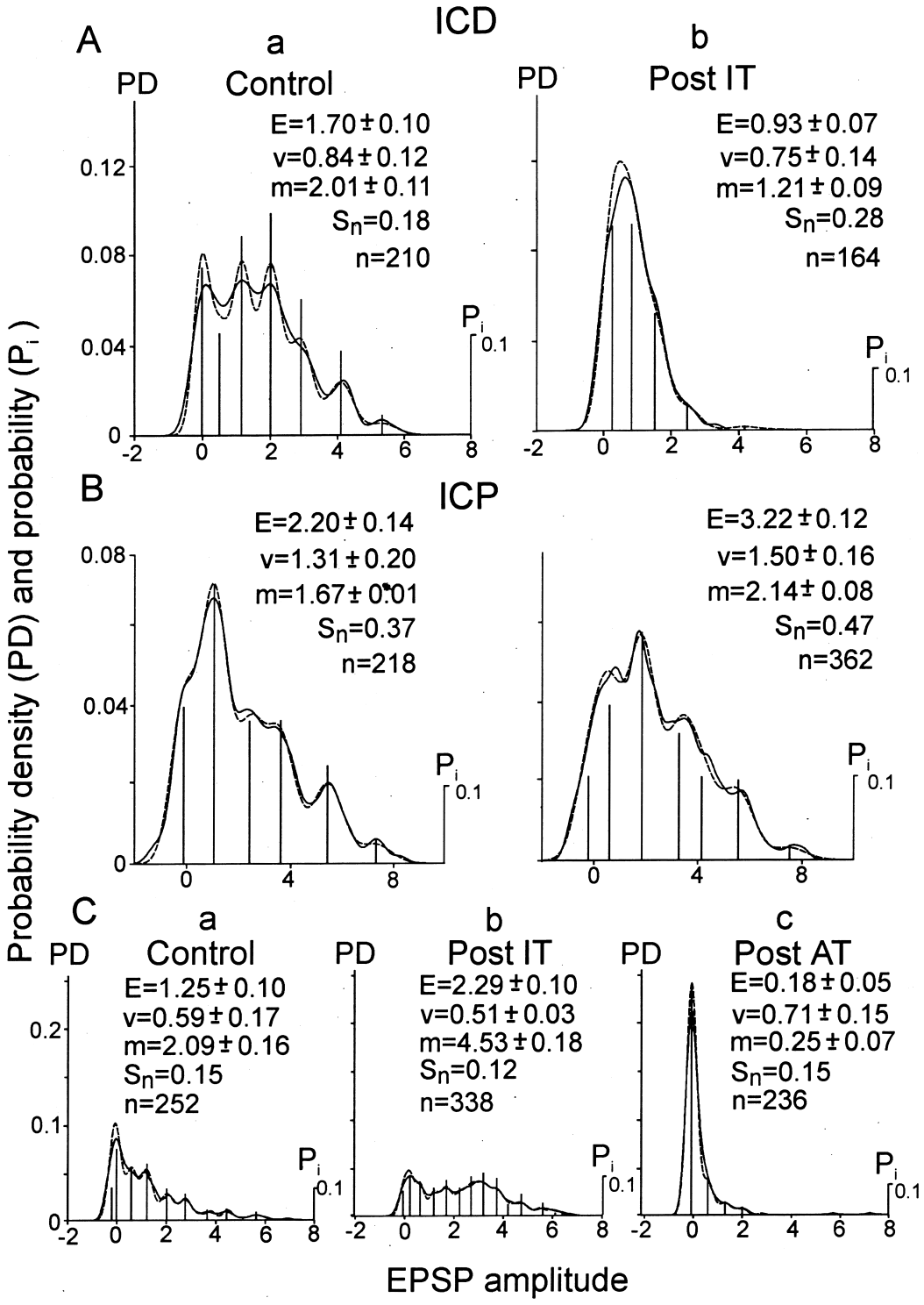

Fig. 4. Quantal (noise deconvolution) analysis of the long-lasting potentiation and depression. Results of three experiments are shown. ICD (A) and ICP (B) were induced by postsynaptic depolarizing pulses (IT). In C, LTD (c) was induced by AT following a potentiation (b) induced by IT. Deconvolved components are shown by bars; experimental and predicted distributions are shown by dashed and continuous curves, respectively. The left (probability density, PD) and the right (probability, P_i) ordinates refer to the curves and bars, respectively. The insets give the mean amplitude (E) of EPSP1, estimated quantal size (v), mean quantal content (m), noise standard deviation (S_n) and sample size (n). Note the lack of significant changes in v with large decreases (Ab, Cc) or increases (Bb, Cb) in m.

The presynaptic involvement was further supported by three additional approaches (Fig. 5E–I). The failure rate correlated with amplitude changes (Fig. 5E). Although the variance method[14,59] is strongly affected by non-uniformity of v across release sites and by some other factors,[14] we applied its simplest variant. The respective strong correlation (Fig. 5F) was also consistent with presynaptic mechanisms of both potentiation[69] (but see Ref. 71) and depression.[10]

The presynaptic nature of ICP, ICD and LTD predicts changes in presynaptic PPF. Accordingly, PPF tended to

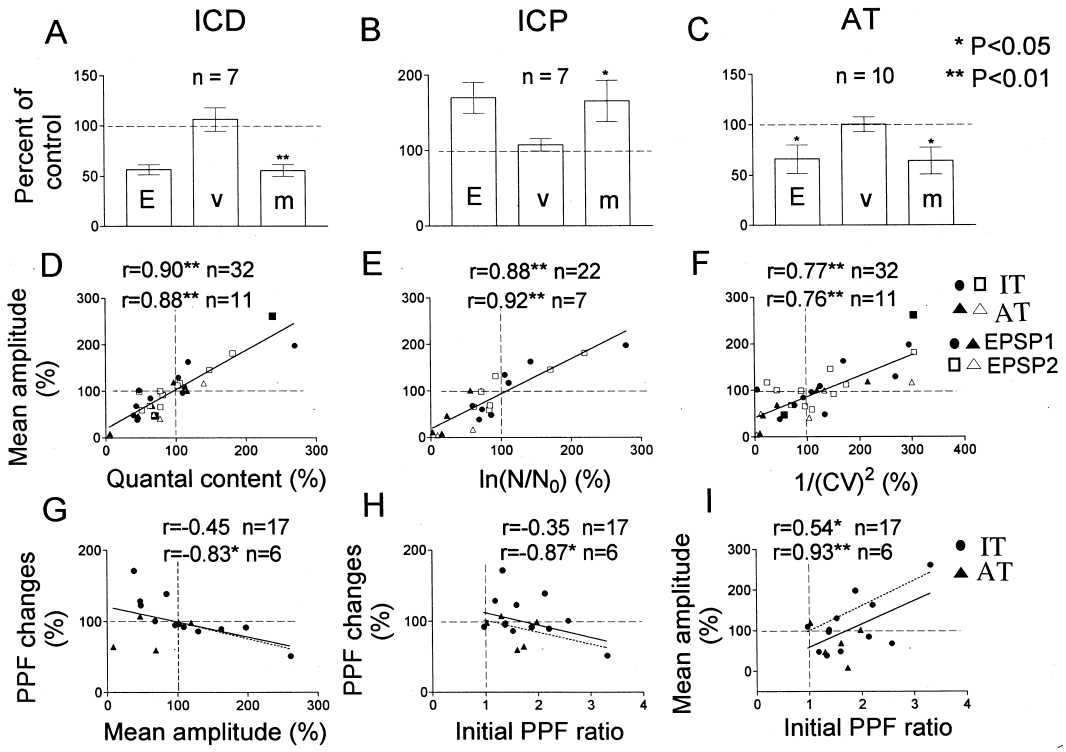

Fig. 5. Summary of the deconvolution analysis (A–D), and additional approaches based on the analyses of failures (E), variances (F) and PPF changes (G–I). (A–C) Averaged changes in the mean amplitude (*E*), quantal size (*v*) and quantal content (*m*). Cases with statistically significant ICD (A) and ICP (B) and all trials of AT (C) are included, except one input with virtually complete LTD (Fig. 3Ac). All data are expressed as percentage of the post-tetanic values relative to the respective pretetanic controls (±S.E.M.). Data for EPSP1 and EPSP2 were either used separately (dots and squares in D–F) or pooled (two large filled squares in D and F). Two cases with small numbers of response amplitudes (<16) above the noise level were excluded. Significance levels (*P*) for changes in *m* (A–C) and the amplitude (C) are given (*t*-test for correlated samples). Changes in *v* were not statistically significant ($P>0.1$). (D–F) Relative mean EPSP amplitudes plotted against *m* (D), logarithm of the inverse failure rate (N/N_0; E) and inverse square of the coefficient of variation corrected for the background noise variance (F). Mean post-tetanic EPSP amplitudes were divided by mean pretetanic amplitudes so that values >100% and <100% refer to potentiation and depression, respectively. Dots and squares represent data for IT (EPSP1 and EPSP2, respectively). Filled and open triangles represent data for AT (EPSP1 and EPSP2, respectively). The sample size (*n*) was smaller for the graph in E, because cases with $N_0<4$ were excluded. Linear regression lines are shown. (G, H) Changes in the PPF ratios plotted against the changes in the EPSP amplitudes (G) and initial PPF ratios (H). The PPF change was expressed as the ratio of the post-tetanic PPF to the pretetanic one, so that 100% signifies no PPF changes. Dots and triangles represent data from experiments with IT and AT, respectively. Continuous regression lines correspond to all data; dashed lines correspond to cases with >100% relative amplitude after IT. (I) Relative EPSP amplitudes plotted against the initial PPF ratios.

decrease with LTP and to increase with LTD, so that the summary plot (Fig. 5G) showed a moderate correlation. It was statistically significant ($r=-0.81$, $n=12$, $P<0.01$) for cases with IT (Fig. 5G, dots) and for the inputs which showed amplitude increases after IT (Fig. 5G, $r=-0.83$). A further analysis (Fig. 5H) revealed a correlation between the initial (pretetanic) PPF ratio and PPF changes,[31,48,50] but it was statistically significant only for the inputs with amplitude increases after tetanus (Fig. 5H, $r=0.87$, dashed line). Correlation between the initial PPF and amplitude modifications[31,56] appeared to be the highest (Fig. 5I). The respective coefficient of correlation was statistically significant for all cases with IT ($r=0.62$, $n=12$, $P<0.05$) and especially high for cases with response potentiation ($r=0.93$; Fig. 5I, dots and dashed line).

DISCUSSION

The present study clearly shows that, in hippocampal slices from juvenile animals, in the absence of synaptic stimulation, both LTP and LTD can be induced in CA3 neurons by an IT. Several observations appear noteworthy: (i) during the first two postnatal weeks, at the mossy fibre–CA3 synapse, when AT induces only LTD, mechanisms of LTP are already present; (ii) a postsynaptic activation can induce LTP-like modifications in the synapse, where LTP has often been considered to be of purely presynaptic origin (for reviews see Refs 43 and 74, but also see Ref. 28); (iii) the same postsynaptic activation could induce either potentiation or depression, and the direction of synaptic modifications tended to depend on the initial release probability; (iv) in spite of a postsynaptic site of induction, both ICP and ICD appear to be maintained by presynaptic mechanisms.

Synaptic modifications induced by intracellular tetanization

LTP-like changes following intracellular tetanus in the absence of the presynaptic stimulation were first described by Kuhnt,[32] with recordings of large EPSPs from CA1 pyramidal neurons. Subsequently, the effect of IT was reproduced with recordings of minimal or large EPSPs by different groups in different CNS structures.[1,11,33,54–56,62] It is worth noting that, although the induction protocol varied between different groups and preparations, it always consisted of multiple and relatively short (5–100 ms) depolarization pulses. The duration and the number of the pulses seem to be critical for LTP induction. Thus, a small number (10–30) of longer (0.5–3 s) pulses can produce LTP in CA1 pyramidal neurons only when paired with the afferent volley.[19,30,47] They were ineffective

without pairings in this[20,30] and other structures,[17,23,45,73] or they induced short-term (minutes or less) potentiation or depression[39,63,64,67,68] (ICP or ICD, respectively, in our terminology). Only few reports described a robust but decremental (during 30–40 min) form of ICP[36] and non-decremental LTD.[5] According to Kullmann *et al*.,[36] a rise in intracellular Ca^{2+} through the activation of voltage-gated Ca^{2+} channels would be responsible for ICP. The observation that the decremental ICP was NMDA independent makes unlikely the possibility that this phenomenon arises from occasional pairings of postsynaptic depolarization with spontaneous presynaptic transmitter release[33,58] (but see Refs 18 and 53).

Short intracellular pulses would facilitate Ca^{2+} influx even in the absence of presynaptic activation, because in these conditions inactivation of voltage-gated Ca^{2+} channels would be minimized. In fact, intracellular depolarization is known to produce a rise in intracellular Ca^{2+} concentration (e.g., Ref. 5) and this may be sufficient to induce long-term changes in synaptic strength. The demonstration that LTP can be induced by light-evoked release of caged Ca^{2+}, and hence by a purely postsynaptic manipulation,[42] strongly favours this hypothesis. Preliminary data show that, in neocortical neurons, IT with short pulses does lead to substantial Ca^{2+}, comparable in magnitude to that occurring during plasticity-inducing AT.[54]

At the mossy fibre–CA3 synapse, long-term potentiation mechanisms are already present during the second postnatal week

Induction of LTP-like modifications at the mossy fibre–CA3 synapse was unexpected in view of previous data showing that only LTD could be induced by AT in this pathway during the first two postnatal weeks.[7] Our findings indicate that the mechanisms for LTP induction are already present during the second postnatal week, but AT is not adequate to activate them. This situation is similar to that observed in the visual cortex of mature rats (four to six weeks) under conditions of preserved $GABA_A$ inhibition, where IT was able to induce ICP in inputs with a high PPF ratio,[56] whereas AT failed to produce LTP and at best induced LTD (Volgushev and Voronin, unpublished observation). Other studies performed on the visual cortex of young rats demonstrated that LTP can be more reliably induced under conditions presumably favourable for sufficiently strong Ca^{2+} influx in the postsynaptic neuron (see Ref. 2 for references and discussion). Taken together, all the above considerations suggest that bursts of short intracellular pulses, in juvenile CA3 neurons, are more effective in increasing intracellular Ca^{2+} concentration as compared to AT, and that is probably the reason why, unlike AT, IT is able to induce LTP.

Effects of intracellular potentiation confirm the existence of two forms of long-term potentiation at the mossy fibre–CA3 synapse

There is general agreement that, at the mossy fibre–CA3 synapse, LTP is NMDA independent.[22,28,74] However, there was a lasting discussion whether or not mossy fibre LTP requires postsynaptic depolarization associated with Ca^{2+} influx into postsynaptic neurons.[28,43,74] Previous attempts to induce mossy fibre LTP by purely postsynaptic depolarization have been unsuccessful.[17,27] In contrast, LTP could be induced by pairing the postsynaptic depolarization with a weak presynaptic train.[27] Recent data suggest the existence of two forms of LTP with either pre- or postsynaptic induction, depending on whether the induction protocol favours Ca^{2+} influx into the pre- or postsynaptic compartment.[53] In keeping with these studies on adult neurons,[53] the present data strongly support the existence of a form of LTP with postsynaptic induction. The failure of purely postsynaptic induction in previous publications[17,27] can be accounted for by using a small number of long postsynaptic pulses which could inactivate voltage-gated Ca^{2+} channels.

Synaptic modifications depend on the initial release probability

The present data show that either potentiation or depression can be induced by IT, and that the direction of the synaptic changes tended to depend on the initial state of the synapse. Hence, synapses with high initial PPF ratio and therefore low release probability[75] tend to be depressed or resistant to modifications. These data, as well as those obtained in the visual cortex,[56] challenge the assumption that the direction of synaptic modifications is related exclusively to different levels of intracellular Ca^{2+} concentration.[2,52] Therefore, the disposition of synaptic connections to undergo potentiation or depression depends on the initial release probability (also see Ref. 10). A dependence of the direction of post-tetanic modifications on whether the input had already been potentiated or not has also been noted in the hippocampus[6,15,66] and in other systems.[72] In the present study, the dependence of the ICP magnitude on the initial PPF ratio was especially strong and was similar to the analogous dependence revealed for LTP in the CA1 area.[31,50]

Maintenance mechanisms of long-term synaptic modifications

The correlation of the initial PPF ratios with the magnitude and direction of synaptic modifications is compatible with the assumption that maintenance mechanisms of both ICP and ICD are presynaptic in origin, made here on the basis of several approaches, including inspection of post-tetanic changes in the PPF ratio, failure analysis, variance analysis and quantal (noise deconvolution) analysis.

The expression of the AT-induced LTD during a critical postnatal period is also consistent with presynaptic mechanisms.[7] Presynaptic mechanisms have been suggested for LTD induced by longer (minutes) low-frequency afferent stimulation in CA1[10,16,70] (but see Refs 44 and 49), in CA3[12] and in the neocortex.[51] In contrast, in the cerebellum, LTD is believed to depend on decreased receptor sensitivity to glutamate.[25] In principle, an apparent diminution in *m* could be accounted for by switching off receptors, rendering respective sites "silent".[13,24,37] However, we consider this explanation unlikely for our case because: (i) the lack of changes in *v* requires selective all-or-none modifications at some release sites without essential changes at other sites; (ii) the interaction between PPF and depression (Fig. 5G–I) indicates changes in presynaptic release probability; (iii) with almost complete depression (Fig. 3), infrequent large responses to the second pulse occurred.

Similar to previous data obtained in experiments with IT,[33,55,56,62] the present data provide further evidence for the role of a retrograde messenger in use-dependent synaptic modifications. The induction protocol did not involve any

direct activation of presynaptic axons, but induced modifications that are likely to be presynaptically located.

CONCLUSIONS

Previous studies have shown that, at early developmental stages, in the CA3 hippocampal region, only LTD is inducible by afferent stimulation of the mossy fibres, whereas the same high-frequency train induces LTP after the second postnatal week. Here, we have demonstrated that, during development, not only LTD but also LTP can be induced at mossy fibre synapses when high-frequency short intracellular pulses are used rather than AT. We have shown that the initial state of the synapses influences the direction and magnitude of the post-tetanic modifications, so that the magnitude of LTP is strongly correlated with the initial (pretetanic) PPF ratio. In spite of a postsynaptic induction, our results are compatible with presynaptic mechanisms underlying the maintenance of both potentiation and depression induced by intracellular tetanization.

Acknowledgements—We thank Dr A. Nistri for helpful comments and W. W. Anderson for providing the acquisition program. This work was supported by grants from the Ministero dell'Università e Ricerca Scientifica to E.C., and also from the Russian Foundation for Basic Research, Volkswagen Foundation and INTAS to L.L.V.; N.B. was a recipient of a fellowship from NOVARTIS Pharma.

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(*Accepted* 17 *March* 1999)