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INTERACTION BETWEEN INTRACELLULAR TETANIZATION AND PAIRING-INDUCED LONG-TERM SYNAPTIC PLASTICITY IN THE RAT VISUAL CORTEX

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Abstract—Long-term changes in synaptic transmission in slices of rat visual cortex were induced either by pairing the excitatory postsynaptic potentials with postsynaptic depolarization or by intracellular tetanization without synaptic stimulation. Changes in the excitatory postsynaptic potential amplitude induced by any of the protocols applied in isolation persisted for longer than 1 h. Pairing-induced long-term potentiation was input specific. We studied the interaction between intracellular tetanization and pairing-induced plasticity by applying the two protocols one after the other at 10-min intervals. The pairing procedure applied after intracellular tetanization did not lead to any further potentiation, but to a depotentiation of the potentiated inputs. A second pairing protocol applied 10 min later led to further depotentiation, while previously unaffected inputs became weakly depressed. If intracellular tetanization was applied after the pairing procedure, the synaptic responses did not change immediately, but a slow return of the excitatory postsynaptic potential amplitude to the control level could be observed. Therefore, intracellular tetanization is not capable of inducing further potentiation after pairing, and pairing cannot further potentiate the inputs which have already been potentiated by intracellular tetanization. The maintenance of long-term potentiation induced by any of the protocols was impaired by successive application of another procedure.

These results suggest a similarity of the mechanisms of synaptic changes induced by the two protocols and demonstrate that the direction of synaptic gain change depends on the history of the synapse. © 1999 IBRO. Published by Elsevier Science Ltd.

Key words: synaptic plasticity, long-term potentiation, long-term depression, intracellular tetanization, visual cortex, rat.

Multiple forms of synaptic plasticity can be induced in both the neocortex and the hippocampus with various induction protocols (for reviews, see Refs 3, 12 and 17). Of particular interest is the interaction between forms of plasticity induced with conventional protocols such as a pairing procedure or afferent tetanization, and purely postsynaptic challenges applied without activation of the presynaptic fibres.[6,10,13,14,19] In the hippocampus, plastic changes induced by purely postsynaptic treatments were shown to occlude tetanus-induced long-term potentiation (LTP).[10,13] The aim of our study was to reveal the interaction between the mechanisms of synaptic changes induced by a pairing procedure and by intracellular tetanization, and to investigate the possible dependence of an effect of plasticity-inducing challenge on the previous changes at the neocortical synapses.

EXPERIMENTAL PROCEDURES

Slices of the visual cortex of three- to six-week-old Wistar rats were prepared using conventional methods.[20] The rats were anaesthetized with ether and decapitated, and slices of the visual cortex were prepared in an ice-cold perfusion medium containing (in mM): 125 NaCl, 2.5 KCl, 1.5 $MgSO_4$, 2 $CaCl_2$, 1.25 Na_2HPO_4, 25 $NaHCO_3$ and 25 D-glucose (pH 7.4), and bubbled with 95% O_2/5% CO_2. Intracellular recordings from layer 2/3 pyramidal cells were obtained with sharp microelectrodes (2.5 M potassium acetate or 1.2 M potassium acetate with 1% biocytin) under submerged conditions at 30°C. Two inputs to a cell were activated with stimuli applied at 0.05–0.08 Hz through two bipolar stimulation electrodes located 0.5–1.5 mm below (layer 4) and lateral (layers 2/3) to the recording site. After amplification (Axoclamp 2A), data were fed into a computer (PC-486; Digidata-1200; PClamp software, Axon Instruments) for subsequent off-line analysis. Intracellular tetanization consisted of three trains (one per minute) of 10 bursts (one per second) of 20 depolarizing pulses (0.5–1.3 nA, 10 ms, 50 Hz). The current amplitude was set to evoke spikes in the first three to seven pulses in each burst. No synaptic stimuli were applied during intracellular tetanization. The intracellular tetanization can be considered as a purely postsynaptic challenge,[19,20] because spiking of a single layer 2/3 pyramidal cell cannot lead to generation of action potentials in other cells and thus cannot activate polysynaptic pathways.[18] Autaptic synapses are unlikely to contribute substantially to our test responses. Spontaneous synaptic events, even if present during the tetanization, could not contribute much to plasticity changes, because their occurrence would be unlikely to be synchronized with postsynaptic spiking. The pairing procedure consisted of 10 trains (one/4 s) of five subthreshold synaptic stimuli applied at 10 Hz combined with depolarizing current steps evoking spikes.

Response amplitudes were measured as the difference between average voltage in two windows of 1–3 ms duration. The first window was positioned between the stimulus artefact and the beginning of the averaged response. The second window was positioned over the last third of the initial slope of the averaged excitatory postsynaptic potential (EPSP) immediately before the peak, or covering the peak. The position and width of the second window were adjusted so that it did not include a rapid falling slope, which was evident in some responses and could indicate a contribution of inhibition. The position and width of the windows were kept constant while processing data from one experiment.

For statistical evaluation of summary data, the response amplitudes were averaged over 1-min periods, normalized to the control (16 min before the first pairing or tetanization) and then averaged across the cells. The number of data points subjected to a statistical test was thus equal to the duration of a period of analysis in minutes. The control period always consisted of 16 data points. ANOVA and non-parametric Mann–Whitney test (statistical software package SPSS 8.0) were used. For all comparisons described below, results of the two tests were similar in terms of the significance of differences.

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Abbreviations: EPSP, excitatory postsynaptic potential; LTP, long-term potentiation.

RESULTS

A pairing procedure or intracellular tetanization induces long-term changes in synaptic transmission

Pairing the EPSP with spikes evoked by depolarizing current steps led to potentiation of synaptic transmission. Typically, the EPSP amplitude increased immediately after the pairing procedure and remained increased for the length of the recording period (55 min in Fig. 1A). Summary data show that pairing-induced LTP was input specific and occurred only in the paired inputs (Fig. 1B, circles), but not in the control inputs (Fig. 1B, asterisks). During the period from 25 to 55 min after the pairing procedure, the mean EPSP amplitudes in the paired inputs ($n = 12$) were significantly higher than in control inputs ($n = 9$) or before pairing ($P < 0.001$ in both cases; here and in all cases below: ANOVA and Mann–Whitney test). The mean response amplitudes, measured during the same period in the inputs which were not subjected to the pairing procedure, were not significantly different from the responses before pairing ($P > 0.1$).

Intracellular tetanization led to long-term changes in synaptic transmission in 34 of 43 inputs. When the EPSP amplitude increased or decreased during the first 10–15 min after intracellular tetanization, it remained potentiated or depressed over the whole recording period. Twenty inputs were potentiated and 14 depressed; sometimes potentiation and depression occurred simultaneously in two different inputs to the same cell. The direction and magnitude of the amplitude change depended on the initial properties of an input, and were correlated with the paired-pulse facilitation ratio expressed during the control period. Intracellular tetanization typically led to potentiation of inputs with initially high paired-pulse facilitation. The degree of potentiation was higher in inputs with higher initial paired-pulse facilitation ratio (data not shown here; see Ref. 20 for details). To justify comparison with the data obtained in the following series of experiments (see below), we selected 12 of 20 potentiated inputs. These inputs were selected to match the paired-pulse facilitation ratios of the inputs described in the following section (ranges from 1.02 to 2.60 and from 0.89 to 2.56; means 1.59 ± 0.20 and 1.43 ± 0.16, respectively), and intracellular tetanization in the two groups led, on average, to an initial potentiation of similar magnitude and time-course. Figure 1D shows the time-course of the EPSP amplitude changes in these 12 selected inputs and in nine inputs which did not change after intracellular tetanization. The mean EPSP amplitudes measured 25–50 min after the intracellular tetanization in these 12 inputs were significantly higher than in the inputs which expressed no changes ($n = 9$) or during the period before intracellular tetanization ($P < 0.001$ in both cases).

To study the interaction between plasticity induced by intracellular tetanization and by pairing, we applied these two protocols one after the other.

Intracellular tetanization prevents potentiation by pairing

In the first series of experiments, intracellular tetanization was followed by the pairing procedure. Since intracellular tetanization could lead to changes in either of the two inputs to a cell, pairing was applied to both inputs at 10-min intervals. Thus, 10 min after intracellular tetanization, one of the inputs was subjected to the pairing procedure, and 10 min later pairing was applied to the other input. In the inputs which already expressed potentiation after intracellular tetanization, the pairing procedure failed to induce any further increase in the response amplitude (Fig. 2A, B). In fact, pairing led to a depotentiation which developed further after the second pairing procedure. The EPSP amplitudes returned to the control level, and during the period from 35 to 60 min after the intracellular tetanization, the mean amplitudes of responses in these inputs ($n = 12$) were no longer different from the responses in control inputs (asterisks in Fig. 1B, D; in both cases $n = 9$, $P > 0.1$). Interestingly, even in the inputs which did not change or were depressed by intracellular tetanization, pairing failed to induce any potentiation, but the second pairing procedure led to a weak, slowly developing depression (Fig. 2C). Therefore, intracellular tetanization applied shortly before the pairing procedure effectively prevented potentiation of synaptic transmission. The effect of pairing in this situation was a depotentiation or a depression.

Pairing prevents the effects of intracellular tetanization

In the second series of experiments, we reversed the sequence of the plasticity-inducing protocols. Pairing of one input to a cell was followed first by pairing of the other synaptic input and then by intracellular tetanization at 10-min intervals. The first pairing led to an input-specific potentiation of the EPSP amplitude (Fig. 3A, B). The successive pairing of the other input did not lead to potentiation, but rather to a decrease in the response amplitude in both inputs: a weak homosynaptic depression and some heterosynaptic depotentiation. Intracellular tetanization applied subsequently did not lead to immediate changes in any of the inputs, but the EPSP amplitude slowly returned to control levels in both previously potentiated (Fig. 3B) and previously depressed (Fig. 3D) inputs. An increase in the mean EPSP amplitudes during the period 25–45 min after the first pairing was significantly smaller in these inputs ($n = 13$) than during the same period after the pairing procedure alone ($n = 12$) ($P < 0.001$). During the period 40–60 min after the first pairing, mean EPSP amplitudes were no longer different from those of the control inputs (asterisks in Fig. 1B; $n = 9$, $P > 0.1$). Therefore, intracellular tetanization applied shortly after a pairing procedure failed to induce further long-term changes in synaptic transmission and prevented maintenance of the changes induced by the pairing procedure.

DISCUSSION

Mutual occlusion between the pairing procedure and intracellular tetanization

The present data demonstrate mutual interaction between the pairing procedure and a purely postsynaptic challenge, an intracellular tetanization. On the one hand, application of any of these protocols effectively prevented further potentiation by the other protocol. On the other hand, the procedure applied later prevented the maintenance of the synaptic gain changes evoked earlier. Thus, the synaptic changes were long lasting only if the pairing or intracellular tetanization was applied in isolation. This mutual interaction suggests a similarity of the mechanisms of synaptic changes induced by the two challenges. It has been reported that, in the hippocampus,

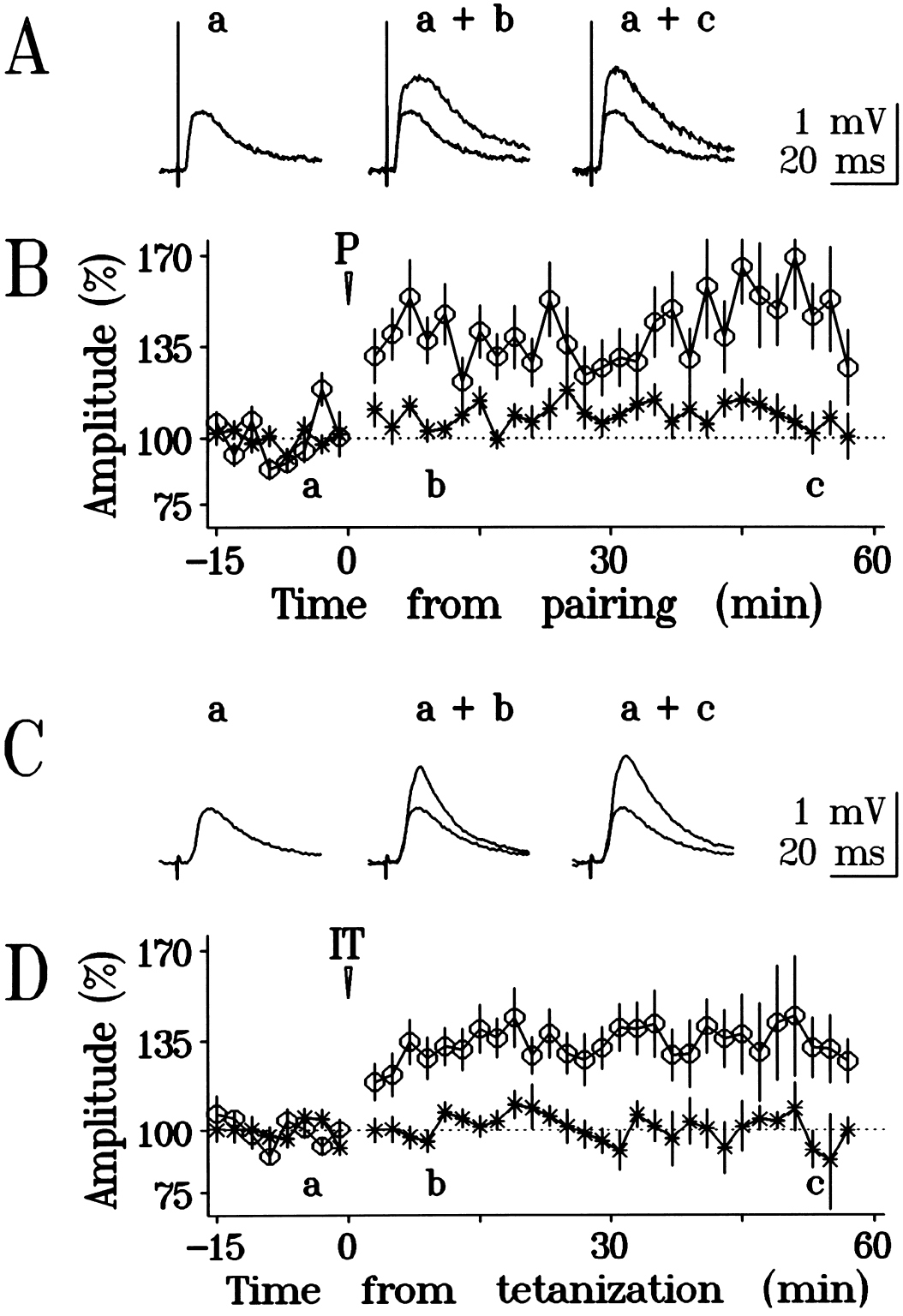

Fig. 1. LTP induced by pairing (A, B) and by intracellular tetanization (C, D). (A) Sample EPSP recorded during the control period (a) and its superposition with the EPSPs recorded 10 min (a + b) and 55 min (a + c) after the pairing procedure, as indicated in B. Each trace is an average of 25 consecutive responses. (B) Summary of the EPSP amplitude changes in the inputs subjected to the pairing procedure (circles, $n = 12$) and in control inputs (asterisks, $n = 9$). In this and the following figures, response amplitudes were averaged over 2-min periods, normalized to the control (16 min before pairing) and then averaged across the cells. The arrowhead marked P indicates the onset of the pairing procedure. (C, D) Sample EPSP (C) and summary of the EPSP amplitude changes in the inputs which were potentiated (D, circles, $n = 12$) or not changed (D, asterisks, $n = 9$) after intracellular tetanization (the arrowhead marked IT).

potentiation induced by intracellular tetanization[10] or by raising intracellular Ca^{2+} concentration with the help of light-sensitive calcium cages[13] occludes tetanus-induced potentiation. These data, together with the results of the present study, lend further support to the notion that a purely postsynaptic challenge is capable of activating intracellular cascades which have common elements with those involved in pairing- or tetanus-induced plastic changes.

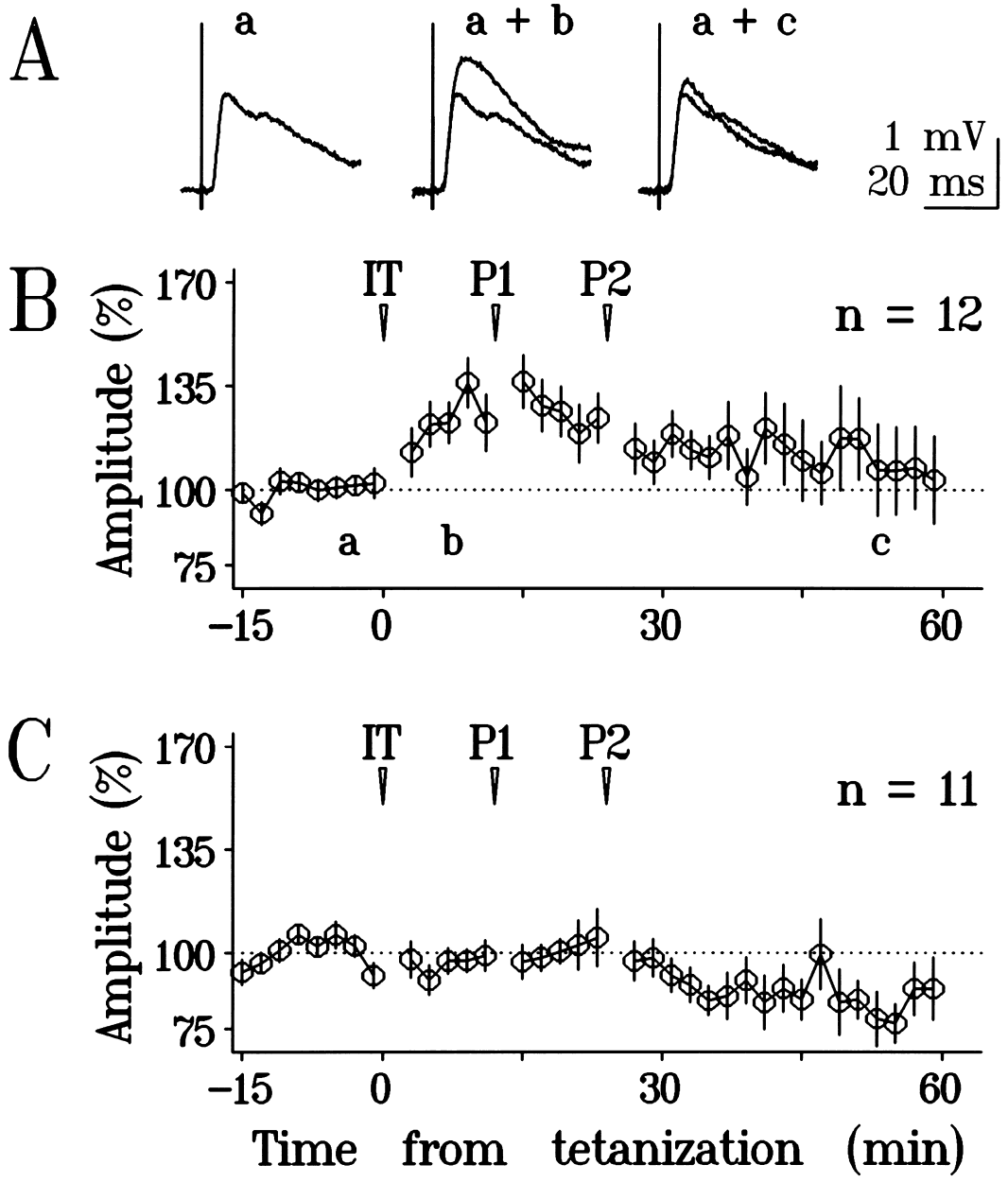

Fig. 2. Intracellular tetanization prevents potentiation by pairing. Sample EPSP (A) and summary of the EPSP amplitude changes (B, C) induced by intracellular tetanization (the arrowhead marked IT), followed by the pairing procedure applied to one (P1) and then the other (P2) input to a cell. (B) Summary data for the inputs which were potentiated after intracellular tetanization. (C) Summary data for the inputs which were depressed or did not change after intracellular tetanization.

Dependence of synaptic changes on the stimulation context

Several lines of evidence indicate that, when a number of plasticity-inducing challenges is applied, both the sequence and timing of their delivery are important variables for determining the resulting synaptic change.

In the CA1 area of the hippocampus, a temporal loss of ability of the pathway to undergo LTP has been observed after application of a weak tetanus which induced decremental short-term potentiation,[8] or a brief episode of low-frequency stimulation that induced decremental short-term depression.[16] Susceptibility to LTP could be regulated by metabotropic glutamate receptors, either in a switch-like manner[4] or in a modulatory mode,[7] the latter possibility appearing more likely, since even after saturating potentiation the ability for additional potentiation is shown to recover gradually and spontaneously within 3–4 h.[9] Our data demonstrate that, also at the neocortical synapses, application of the pairing procedure or intracellular tetanization prevents induction of further potentiation with a subsequently applied protocol.

Induction of LTP is also accompanied by increased sensitivity of the mechanisms responsible for down-regulation of the synaptic gain.[15,16,21] Of relevance here also are data on over-tetanization,[2,5] which demonstrate that increasing the number of theta-bursts in the tetanus above a certain amount does not lead to a stronger potentiation, but to depotentiation of the synaptic gain in the hippocampus. It has been reported recently that, when the mechanisms responsible for potentiation are blocked, a pairing protocol leads to a depression

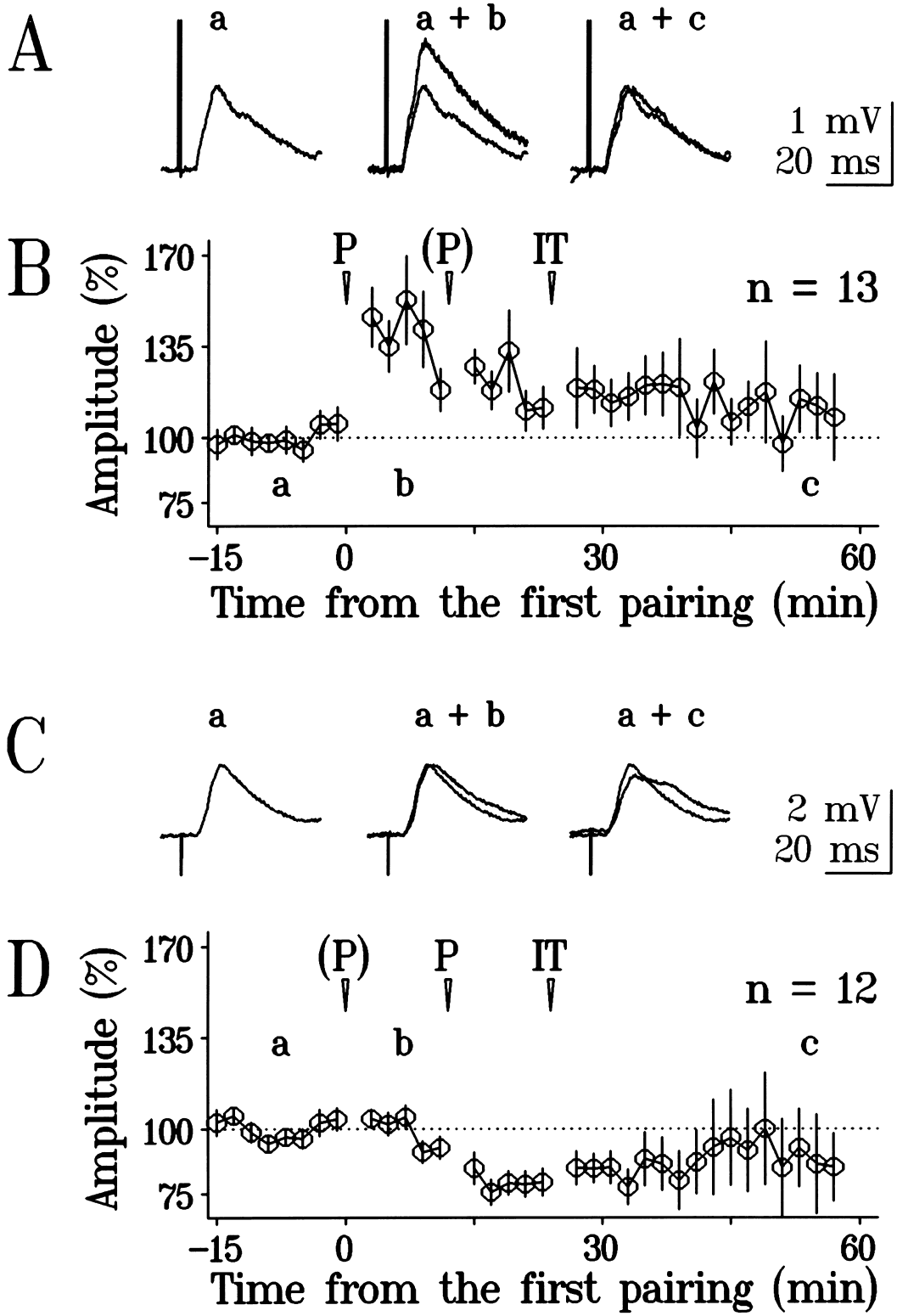

Fig. 3. Intracellular tetanization fails to induce long-term changes in synaptic transmission when applied after the pairing procedure. (A, B) Sample EPSP (A) and summary of the EPSP amplitude changes (B) in the inputs subjected to the pairing procedure (the arrowhead marked "P"), followed by the pairing procedure applied to another input [marked as "(P)"] and intracellular tetanization (the arrowhead marked IT). (C, D) Sample EPSP (C) and summary of the EPSP amplitude changes (D) after the pairing procedure applied to another input to a cell [the arrowhead marked "(P)"], followed by the pairing procedure (marked "P") and intracellular tetanization (IT).

rather than to a potentiation of synaptic transmission in the dentate gyrus.[22] Our results also show that, in the neocortex under certain conditions, the pairing procedure can lead to depression. These data support the conjecture that the mechanisms of both potentiation and depression are normally activated by pairing, and that the final result depends on the balance between the two mechanisms. The possibility to influence the direction of the synaptic gain change and thus

to switch between potentiation and depression has also been demonstrated in the CA1 region of the hippocampus and in the amygdala. Neveu and Zucker[13] reported that, in CA1 cells, raising the intracellular Ca^{2+} concentration by photolysis of pre-loaded light-sensitive cages can lead to either potentiation or depression, the direction of the change being unpredictable in the naive synapses. However, the same procedure led to potentiation when applied after induction of long-term depression, and to depression if applied to pre-potentiated synapses. In the amygdala, low-frequency stimulation, which usually induces persistent response enhancement, led to depression when applied after recovery from short-term potentiation induced with a brief, high-frequency tetanus.[11] These data, together with our results, demonstrate that the balance between potentiation and depression can be shifted not only by biochemical interventions to the intracellular milieu,[22] but also by induction of the synaptic changes.

CONCLUSIONS

Two conclusions can be drawn from the present data. First, the mutual interaction between the pairing procedure and a purely postsynaptic challenge, intracellular tetanization, suggests that intracellular cascades which mediate plastic changes induced by the two protocols have common elements. Second, the dependence of synaptic changes on the preceding stimulation indicates that a plasticity-inducing challenge not only modifies the synaptic gain, but also shifts the balance between the potentiation and depression mechanisms, thus modulating the predispositions of the inputs to undergo potentiation or depression[20] and influencing the direction of the future changes.[1]

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