Induction of LTP and LTD in visual cortex neurones by intracellular tetanization

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Neurones from supragranular layers of rat visual cortex slices were activated by intracellular tetanization (IT) without concomitant presynaptic stimulation. The effect of IT was examined on EPSPs evoked at low stimulation intensity from two subsets of afferents by electrodes positioned in layers II and IV, respectively. In 17 of 23 inputs to 15 cells IT led to changes in EPSP amplitudes which persisted throughout the recording period (from at least 40 min to 3 h). For 10 potentiated inputs (nine cells) and eight depressed inputs (seven cells), EPSP amplitudes measured 30 min after tetanization were 167 ± 14% and 55 ± 14% of the pretetanic controls, respectively. In seven cells both inputs changed, in five cases modifications were of the opposite and in two cases of the same polarity.

Key words: LTP; LTD; Postsynaptic inclusion; Intracellular tetanization; Slices; Rat neocortex

Introduction

Use-dependent modifications of synaptic transmission such as long-term potentiation (LTP) and depression (LTD) have attracted considerable interest as putative mechanisms of learning.1 Normally, LTP and LTD are induced by presynaptic tetanization or conjoint pre- and postsynaptic activation of the recorded neurone. However, long-term2-7 and short-term8-11 synaptic modifications have also been reported after activating the postsynaptic cell alone. In this study we examined whether it is possible to induce LTP and LTD-like phenomena in the visual cortex by solely activating postsynaptic neurones with current injection.

Methods

Slices (350 μm) of the visual cortex of 4–6 week old rats were prepared by conventional methods12 and investigated under submerged conditions at 30°C. Perfusion medium contained (in mM) 124 NaCl, 5 KCl, 2 CaCl2, 1.5 MgSO4, 1.25 NaHPO4, 26 NaHCO3 and 10 D-glucose bubbled with 95% O2 and 5% CO2. Intracellular recordings were obtained with glass microelectrodes (3 M potassium acetate, 60–100 MΩ). Electrical stimuli (1–10 V, 0.04–0.1 ms) were applied as paired pulses (interval 50 ms) through bipolar tungsten electrodes positioned laterally (layers I–II) and below (layer IV) the recording site. Stimulation intensity was set just above threshold for the elicitation of an EPSP to minimize the number of activated presynaptic fibres and was kept constant throughout the experiment. In eight cells responses were evoked from both stimulation sites in alternation, in four cells only responses to stimulation of layer IV and in the remaining three cells only responses to layer II stimulation were recorded. Stimulation frequency was 0.05–0.08 Hz. After control periods of 20–40 min, intracellular tetanization was applied without presynaptic stimulation. Tetani consisted of 2–3 trains (1 min−1) of 10 bursts (1 s−1) of 20 depolarizing pulses (0.5–1.3 nA, 10 ms, 50 Hz). Current was adjusted so that the first 2–5 pulses in a burst evoked spikes. Current-voltage relationships were recorded several times before and after intracellular tetanization by applying de- and hyperpolarizing current steps (450 ms duration, 0.2 Hz).

After amplification (Axoclamp-2A) data were digitized at 10 kHz and fed into a computer (PC-386; Labmaster, TL-1 DMA interface and pCLAMP software, Axon Instruments). EPSP amplitudes (peak values) were averaged over 1 min epochs and plotted against time. Cells without significant drift of EPSP amplitude during the control period and with stable membrane potential (more negative than −70 mV, mean 78.1 ± 1.5 mV) and stable resistance (63 ± 14 MΩ) were subject to analysis. Throughout the text, means are given together with ± s.e.m.

Results

Excitatory postsynaptic potentials (EPSPs) were recorded from 15 layer II–III cells following stimulation of layers II and IV. EPSP amplitudes ranged from 0.5 to 3.1 mV (mean 1.3 ± 0.1 mV, n = 23).

Figure 1 shows the effects of intracellular tetanization (IT) on responses evoked in the same cell from two different sites. During the control period, mean EPSP
amplitudes were stable and fluctuations did not exceed ± 15%. After IT the response to the layer IV stimulus was potentiated (Fig. 1A) and that to the layer II stimulus depressed (Fig. 1B). EPSP amplitudes averaged from 20–40 min after IT were 191% and 42% of the pretetanic values, respectively. Both modifications persisted over the whole recording period (Fig. 1Ad, Bd). The time courses of the amplitude changes depicted in Figure 1 refer only to responses to the first pulse of the pair. The responses to the second pulse showed even larger changes (207% potentiation and 40% depression, Fig. 1Aa–c, Ba–c).

The I/V curves determined for this cell before, 25 and 45 min after IT were nearly identical, indicating that there were no changes in membrane resistance (Fig. 2).

We classified EPSP changes as potentiation or depression if the average response amplitudes measured within intervals of 20 min before and after IT differed significantly (p < 0.05, paired t-test). During the 20 min interval following IT, potentiation occurred in 10 of 23 tested inputs (9 of 15 cells, Fig. 3A, upper plot) and amounted to 163 ± 33% of control (n = 10; range 113–227%). For all but one case, potentiation persisted for 20–40 min after IT (172 ± 39%, n = 9) and for eight cases for 40–60 min (152 ± 41%). Two cells were held for more than 3 h, and potentiation was sustained.

Depression was observed in eight of 23 inputs (seven of 15 cells) and amounted to 63 ± 15% of control (range 22–88%) during the initial 20 min after IT (Fig. 3A, lowermost plot). In seven inputs depression persisted for 20–40 min after IT (48 ± 12% of control) and
lasted throughout the whole recording period which extended from 40 min to 3 h post-tetanus.

In seven of eight cells in which responses were tested from both stimulation sites both inputs were modified. In five cases potentiation of the input from layer IV was accompanied by depression of the layer II input, as shown in Figure 1. In one cell both inputs were potentiated and in one cell both were depressed.

Segregating the data according to stimulation site (Fig. 3B) revealed different susceptibilities of the two inputs to undergo lasting changes. Of 11 tested layer IV inputs seven were potentiated, two were depressed and two were unchanged, so that the net effect was potentiation (Fig. 3B, S1). Of 12 inputs from layer II (Fig. 3B, S2), only three were potentiated while six were depressed and three showed no change.

Discussion

The present results indicate that IT of supragranular neurones can induce strong and long-lasting modifications in synaptic transmission of inputs that were not stimulated during the tetanization. Not stimulating afferents does not necessarily imply that there was no transmitter released during the tetanization. Presynaptic afferents could have been spontaneously active or cells providing excitatory inputs to the tetanized neuron could have been driven by the activation of the latter, and finally, spontaneous release could have been increased by intracellular depolarization. We consider the first possibility as unlikely because spontaneous EPSPs were rare and spontaneous spiking was absent in our preparation. Since excitatory coupling between individual cortical cells is usually weak, it is also unlikely that the few spikes evoked in a single cell by intracellular tetanization activated exactly those cortical cells whose axons terminate on the recorded cell and are excited by the test stimuli (see also Ref. 4). The extent to which the third mechanism caused spontaneous release during IT is unknown. These considerations suggest that modifications of synaptic transmission can be induced by postsynaptic activation alone, without conjunction with spike evoked release from the respective presynaptic terminals.

The modifications observed in the present study closely resemble LTP and LTD as they last more than 30 min, are associated with substantial changes in EPSP amplitude and rising slope and occur in the absence of changes in membrane potential and resistance of the postsynaptic neuron. The induction of LTD and LTP requires a critical increase of intracellular \([Ca^{2+}]\) (for review see Ref. 14). LTD in hippocampus and in neocortex and short-term potentiation in the hippocampus could be induced without presynaptic activation by manipulations which increase intracellular \([Ca^{2+}]\). As our induction protocol is likely to trigger a substantial \(Ca^{2+}\) influx, we propose that the observed modifications are caused by a surge of intracellular \([Ca^{2+}]\).

Our induction protocol caused a long lasting potentiation. Similar results were reported recently for the hippocampus. However, application of long intracellular pulses at low frequency (e.g. 3 s, 0.2 Hz, see Ref. 9) usually evoked only short-term changes (see Introduction). A likely explanation is that our stimulation protocol was more effective in inducing an increase of intracellular \([Ca^{2+}]\), as brief pulses minimized inactivation of voltage gated ionic channels. Thus, \(Ca^{2+}\) influx should have been stronger than with application of long pulses. This interpretation is supported by reports from other structures showing that postsynaptic activation with short and long pulses leads to long and short \(Ca^{2+}\) potentiation, respectively.

An interesting observation was that IT affected different afferents to the same neurone in different ways. Synapses may differ in their predisposition to undergo potentiation or depression. Alternatively, synapses with similar predispositions could be differentially affected because levels of depolarization and resulting \(Ca^{2+}\) increases are likely to differ at different sites of the dendritic tree during application of IT.

The EPSP changes induced by IT were larger than those reported after conventional induction of LTP or LTD. We attribute this to the fact that we used weak test stimuli rather than to peculiarities of IT. The present data show that a given level of postsynaptic activation can lead to modifications of opposite polarity in different inputs. If strong test stimuli are used, which is usually the case in order to obtain large responses, many afferents are coactivated and if modifications are not homogeneous, the net change of the compound EPSP will be smaller than the changes of individual inputs.

Conclusions

The present results suggest that modifications of synaptic transmission that closely resemble LTP and LTD can be induced in the absence of presynaptic activation by tetanizing the postsynaptic neurone. This agrees with the notion that the signals for the induction of LTP and LTD are generated postsynaptically. Because the present results have been obtained under artificial activation conditions, they do not violate the Hebbian conjunction rule because under normal conditions postsynaptic activation is always caused by synaptic activity and synergistic actions among ligand- and voltage-gated conductances are particularly suitable for the induction of a change. As different inputs to the same cell can change in opposite directions, probably because of differences in local activation conditions, there is also no conflict with the evidence for input specificity of synthetically induced modifications.
References


ACKNOWLEDGEMENTS: We thank Dr A. Borroni for critically reading the manuscript and Renate Ruhl-Volsing for the preparation of figures.

Received 4 July 1994;
accepted 7 August 1994