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RAPID PUBLICATION

Long-Lasting Potentiation and Depression Without Presynaptic Activity

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SUMMARY AND CONCLUSIONS

1. Long-term potentiation (LTP) and long-term depression (LTD) require a rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in the postsynaptic CA1 pyramidal neuron to activate Ca-dependent biochemical processes. This rise in $[Ca^{2+}]_i$ is a necessary trigger for the induction of LTP and LTD, but it is unclear if concurrent presynaptic activity is required for their induction or expression.

2. We used photolysis of the caged-Ca^{2+} compound nitr-5 to elevate postsynaptic $[Ca^{2+}]_i$. Long-lasting potentiation (LLP) and long-lasting depression (LLD) were obtained in the absence of presynaptic activity.

3. Because LLP and LLD share common features with LTP and LTD induced by presynaptic stimulation these results show that a rise in $[Ca^{2+}]_i$ in the postsynaptic CA1 pyramidal neurons is necessary and sufficient for the induction and expression of LTP and LTD, and that concurrent presynaptic activity is not required.

INTRODUCTION

High-frequency stimulations (100 Hz for 1–2 s) induce long-term enhancement of synaptic strength, referred to as long-term potentiation (LTP), in certain synapses of the CNS. On the contrary, low-frequency activation (1 Hz for 10 min) results in a long-lasting decrease in synaptic transmission referred to as long-term depression (LTD). They constitute forms of activity-dependent synaptic plasticity which are widely believed to be involved in learning and memory. In the CA1 area of the hippocampus, these reversible processes require a postsynaptic depolarization, the activation of *N*-methyl-D-aspartate (NMDA) receptors, and an influx of Ca^{2+} (Bliss and Collingridge 1993; Linden and Connor 1995). A rise in postsynaptic $[Ca^{2+}]_i$ is a necessary trigger for the induction of these persistent modifications of synaptic efficacy; postsynaptic injections of Ca^{2+} chelators prevent their induction (Bröcher et al. 1992; Kimura et al. 1990; Lynch et al. 1983; Malenka et al. 1988; Yoshimura et al. 1991).

The locus of expression of LTP is controversial. Substantial evidence exists for both pre- and postsynaptic loci of expression, as well as evidence against one or the other site of expression (Bliss and Collingridge 1993; Larkman and Jack 1995; Nicoll and Malenka 1995). Evidence for the site of expression of LTD is sparse, but the results so far also suggest changes in both transmitter release and postsynaptic sensitivity to transmitter (Bolshakov and Siegelbaum 1994; Larkman et al. 1991; Oliet et al. 1995; Selig et al. 1995; Stevens and Wang 1994).

If LTD and LTP are expressed presynaptically but induced postsynaptically, some sort of retrograde signal must connect the postsynaptic induction process to a presynaptic increase in transmitter release. Two popular candidates for this retrograde messenger are NO and CO gases (Bear and Malenka 1994; Larkman and Jack 1995). Interestingly, evidence exists indicating that these substances have little effect in isolation on presynaptic terminals, but that they alter transmitter release only by acting in concert with appropriate frequencies of presynaptic electrical activity (Arancio et al. 1995; Zhuo et al. 1993, 1994). This has led to the suggestion that induction of long-lasting synaptic plasticity in hippocampal cortex involves two associative sites of concurrence—one postsynaptic site requiring NMDA-receptor activation by glutamate associated with simultaneous postsynaptic depolarization, and one presynaptic locus requiring the action of a retrograde messenger associated with simultaneous presynaptic activity (Hawkins et al. 1993).

If this model of LTP and LTD induction is correct, these long-lasting forms of synaptic plasticity cannot be induced in the complete absence of presynaptic activity. We have now tested this prediction by using the photosensitive Ca^{2+} chelator nitr-5 (Adams et al. 1988) to transiently elevate Ca^{2+} in single CA1 pyramidal cells in hippocampal slices from young rats. We have shown recently that photolysis of nitr-5 can induce long-lasting potentiation or depression (LLP or LLD) in synapses from Schaffer collateral-commissural afferents onto these neurons (Neveu and Zucker 1996). In the present experiments, we test whether this induction of LLP or LLD requires concurrent presynaptic activity.

METHODS

Transverse hippocampal slices (400–600 μm thick) were cut with a vibratome from cerebral hemispheres removed from young (11–22 days) Sprague-Dawley rats that were anesthetized with halothane and decapitated. Slices were placed in a humidified holding chamber for $\geq$1 h and then transferred to the recording chamber as described elsewhere (Neveu and Zucker 1996). The superfusing medium contained the following (in mM): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, and 11 glucose and was saturated with 95% O_2-5% CO_2.

Schaffer collateral-commissural afferents were stimulated at 0.1 Hz by using bipolar, stainless steel electrodes. Extracellular field potentials were recorded in stratum radiatum by using 3–6 MΩ glass electrodes filled with 3 M NaCl. Whole cell patch-clamp recordings were made under current clamp from CA1 pyramidal neurons by using 4–7 MΩ electrodes filled with a standard internal solution consisting of the following (in mM): 117.5 cesium gluconate, 17.5 CsCl, 8 NaCl, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid (HEPES), 0.2 ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 2 Na_2-ATP, and 0.2 guanosine triphosphate (GTP) (pH 7.2 adjusted with CsOH; osmolarity: 290–300 mosM). To control postsynaptic $[Ca^{2+}]_i$, the tips of the electrodes (2–3 mm) were filled with internal solution con-

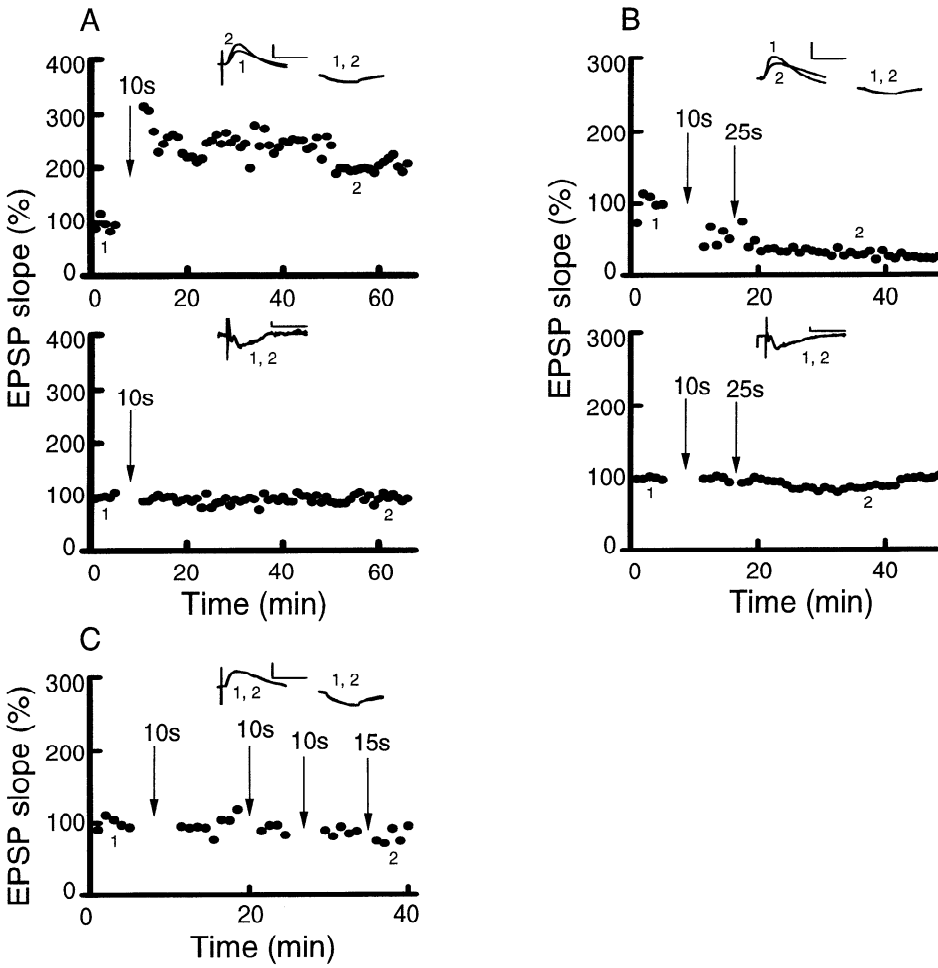

FIG. 1. Photolysis of intracellularly injected nitr-5 can enhance or depress synaptic transmission without presynaptic activity. In *A–C* the graphs of the maximum rising slope of the intracellular excitatory postsynaptic potential (EPSP, *top panel*) recorded in the soma of CA1 pyramidal neurons from 3 slices and the initial maximum rising slope of the simultaneously recorded extracellular EPSP (*A* and *B*, *bottom panels*) in the stratum radiatum are displayed. Each point represents the average of 6 slope measurements to responses to 0.1 Hz stimulation of Schaffer collateral/commissural afferents. *Insets* in each panel show raw and smoothed data traces for the intracellular EPSP and the extracellular EPSP, respectively, taken before and ≥30 min after photolysis of nitr-5. Calibration bars show 10 mV and 50 ms for intracellular EPSPs, and 0.2 mV and 20 ms for extracellular EPSPs. Input resistances of the cells are also displayed as responses to 30-pA hyperpolarizing current. The cells were impaled at the surface of the slice and held at a membrane potential of −80 mV (*A*), −79 mV (*B*), and −83 mV (*C*). At the time marked by the arrows, the slices were exposed to ultraviolet light for the durations indicated in seconds on the graphs. *A* and *B*: the cells were injected with an internal solution containing 5 mM nitr-5 and 2.5 mM $CaCl_2$. Afferent stimulation was suspended 2 min before until 2.5 min after the 1st light exposure. *C*: cell was injected with standard internal solution without nitr-5 and was exposed to ultraviolet light for the durations shown.

taining 5 mM nitr-5 (Calbiochem) and 2.5 mM $CaCl_2$, and backfilled with standard internal solution. Cells were held at their resting potentials (−76 to −86 mV; corrected for a measured −6 mV junction potential) and input resistance monitored as responses to 50-ms hyperpolarizing current steps (0.03 nA). Access resistance ranged from 9 to 60 MΩ, and cells were discarded when their input resistance varied >20%.

Data were collected and analyzed on line (5–10 kHz sampling rate) by using a proprietary AXOBASIC (Axon Instruments) program provided by David Selig. Initial slopes of excitatory postsynaptic potentials (EPSPs) were calculated by using either a least-squares regression or a cubic spline fit. Summary graphs were produced by averaging all responses in each experiment within 60-s time bins, normalizing responses to the average value of all responses during the initial 5–10 min baseline recording, and aligning points with respect to the light exposure. Each point shows the mean ± SE. Student's *t*-tests were used to test for significant differences between groups.

$[Ca^{2+}]_i$ was elevated by nitr-5 photolysis with the use of ultraviolet light from a collimated, continuously run, xenon lamp (150 W CERMAX, ILC Technology, Sunnyvale, CA) fitted with a Uniblitz shutter (Vincent Assoc., Rochester, NY) that had been found to photolyze 12% of Ca-loaded nitr-5 in 1 s. The purity and K_D of each batch of nitr-5 were checked (Zucker 1994). The expected average rise in free $[Ca^{2+}]_i$ upon exposure to light was calculated as described elsewhere (Landó and Zucker 1989; Marrion et al. 1991; Neveu and Zucker 1996). The calculations took into account the measured absorbance of cortical tissue to UV radiation (2.5 mm^{-1} at 350 nm), the buffering effects of nitr-5, its photoproducts, and native cytoplasm, the measured photolysis efficiency of our light source, and the effects of additional exogenous buffering on the measured rate of restoring elevated $[Ca^{2+}]_i$ to resting levels (Regehr and Tank 1992; Spruston et al. 1995; Yuste and Denk 1995). Photolysis was performed within 20 min of breaking into a cell, in order to minimize washout (Kato et al. 1993) but still allow sufficient time for nitr-5 to equilibrate to pipette levels at synapses near the cell body. We selected for analysis only synapses with large and rapidly rising EPSPs that are likely to be generated near the cell body.

RESULTS AND DISCUSSION

Postsynaptically injected nitr-5 was photolyzed to increase the $[Ca^{2+}]_i$ in the impaled CA1 pyramidal neuron. We found previously (Neveu and Zucker 1996) that this procedure induces a LLP or LLD in different cells. These changes in synaptic strength persisted ≥40 min, and appeared to share characteristics and mechanisms with electrically induced synaptic plasticity: LLP was occluded by tetanically induced LTP and blocked by calmodulin inhibition, and LLD was occluded by LTD induced by 1-Hz afferent stimulation for 10 min and blocked by phosphatase inhibition.

In the present experiments, we tested whether or not afferent stimulation is required for induction of LTP or LTD by

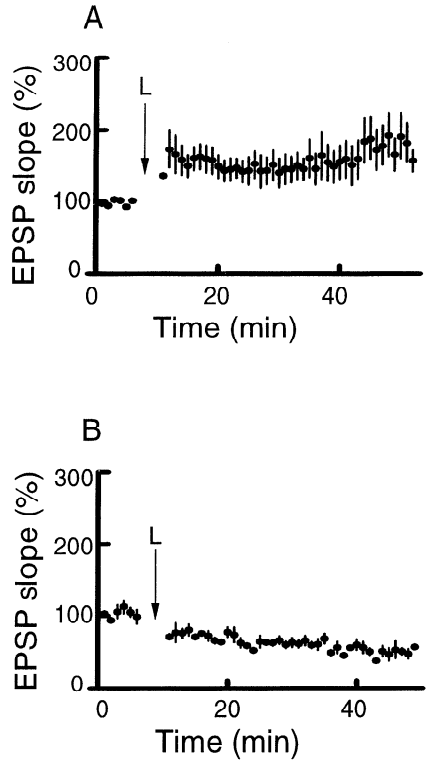

FIG. 2. LLP and LLD do not require presynaptic activity. Summary of experiments in which the photolysis of nitr-5 was carried out during a 5-min pause in afferent stimulation. Long recordings (>40 min) were obtained in 5 cells which showed LLP (*A*) and in 3 which showed LLD (*B*). Nitr-5 was photolyzed at the arrow marked with an L.

a postsynaptic elevation of $[Ca^{2+}]_i$ by suspending afferent stimulation from 2 min before until 2.5 min after the photostimulus. In these conditions, the photolysis of nitr-5 during 10 s still led to either a potentiation (107% after 50 min in the experiment of Fig. 1*A*), or a depression (40% after the 1st light exposure, and 70% after the 2nd light exposure in the experiment of Fig. 1*B*). We estimate that a 10-s photolysis elevated $[Ca^{2+}]_i$ in the recorded neuron to ~0.75 μM for ~15 s; a subsequent 25-s exposure would photolyze all remaining nitr-5 and have a similar effect. Persistent changes in synaptic transmission were only encountered in the recorded cells. The extracellular field potentials, which were always monitored simultaneously with the intracellular EPSP, showed no change during any of the experiments (Fig. 1, *A* and *B*). We also ensured that the light by itself induced no photosensitive reaction in cells not filled with nitr-5 (Fig. 1*C*). Similar LLP [38 ± 15% (SD), $n = 9$] and LLD (32 ± 6% , $n = 8$) were observed in analogous conditions in other slices (Fig. 2, *A* and *B*). The magnitudes of effects in the absence of stimulation are not significantly different ($P > 0.05$, 2-sided *t*-test) from what we observed previously (Neveu and Zucker 1996), when stimulation continued at 0.1 Hz throughout the experiment (LLP: 56 ± 32%, $n = 18$ of 42 cells; LLD: 38 ± 15%, $n = 17$ of 42 cells). Few cells (3 among 20) presented no change in synaptic transmission after light exposure. This, too, is similar to the proportion of nonresponsive cells when stimulation was not suspended (7 of 42, or 17%). These results show that LLP and LLD do not require presynaptic activity for their induction, and that such activity does not significantly affect the frequencies of observing potentiation and depression, nor their magnitudes. This in turn indicates that LLP and LLD, and presumably also LTP and LTD, do not require concurrent presynaptic activity for their induction.

The usual procedures for inducing LTP and LTD naturally involve presynaptic stimulation. Other procedures have been described which generate long-lasting potentiation, such as potassium channel block (Aniksztejn and Ben-Ari 1991), anoxia (Crepel et al. 1993), and exposure to NMDA (Kamiya et al. 1993). Although these treatments presumably all would be effective in the absence of afferent stimulation, the protocols used continued stimulation throughout the induction treatment (except for the NMDA study, Kamiya et al. 1993). However, in none of these studies has it been shown that the long-lasting potentiation is occluded by tetanically induced LTP, or that it shares the pharmacological sensitivities of LTP. Furthermore, in all these studies, the treatment used to induce long-lasting potentiation could act pre- as well as postsynaptically. Only in the present study are LLP and LLD induced strictly by an elevation of postsynaptic $[Ca^{2+}]_i$. And only this way could the effects of presynaptic activity be distinguished clearly from the postsynaptic induction of synaptic plasticity.

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