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Visual experience regulates the development of long-term synaptic modifications induced by low-frequency stimulation in mouse visual cortex

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ABSTRACT

Manipulation of visual experience can considerably modify visual responses of visual cortical neurons even in adulthood in the mouse, although the modification is less profound than that observed during the critical period. Our previous studies demonstrated that low-frequency (2 Hz) stimulation for 15 min applied to layer 4 induces T-type Ca^{2+} channel-dependent long-term potentiation (LTP) at excitatory synapses in layer 2/3 neurons of visual cortex during the critical period. In this study, we investigated whether low-frequency stimulation could induce synaptic plasticity in adult mice. We found that 2 Hz stimulation induced LTP of extracellular field potentials evoked by stimulation of layer 4 in layer 2/3 in adulthood as during the critical period. LTP in adulthood was blocked by L-type, but not T-type, Ca^{2+} channel antagonists, whereas LTP during the critical period was blocked by T-type, but not L-type, Ca^{2+} channel antagonists. This developmental change in LTP was prevented by dark rearing. Under pharmacological blockade of $GABA_A$ receptors, T-type Ca^{2+} channel-dependent LTP occurred, whereas L-type Ca^{2+} channel-dependent LTP did not occur. These results suggest that different forms of synaptic plasticity can contribute separately to experience-dependent modification of visual responses during the critical period and in adulthood.

1. Introduction

Neurons respond selectively to the features of visual stimulation in the primary visual cortex (Hubel, 1982). This response selectivity is refined depending on the activity of visual cortical neurons produced by visual inputs during a restricted postnatal period called a critical period (Wiesel, 1982). Ocular dominance plasticity has been used to study the mechanisms underlying the experience-dependent developmental process. After monocular deprivation during the critical period, visual cortical neurons undergo depression and potentiation of their visual responses to stimulation of the deprived and nondeprived eyes, respectively (Frenkel and Bear, 2004). It has been suggested that *N*-methyl-D-aspartate (NMDA) receptor-dependent long-term depression (LTD) mediates the depression of deprived eye responses following monocular deprivation (Heynen et al., 2003; Crozier et al., 2007; Yoon et al., 2009). On the other hand, we suggested that T-type Ca^{2+} channel-dependent long-term potentiation (LTP) mediates the potentiation of nondeprived eye responses following monocular deprivation (Yoshimura et al., 2008). This form of LTP is induced in layer 2/3 neurons by 2 Hz stimulation for 15 min and requires the activation of postsynaptic Ni^{2+}-sensitive T-type Ca^{2+} channels for induction (Komatsu and Iwakiri, 1992; Yoshimura et al., 2008). This LTP occurs only during the critical period in normally reared cats and rats (Komatsu et al., 1988; Ohmura et al., 2003), while

Abbreviations: FP, field potential; GABA, γ-aminobutyric acid; LTP, long-term potentiation; LTD, long-term depression; NMDA, *N*-methyl-D-aspartate; PD, postnatal day; ACSF, artificial cerebrospinal fluid; DL-APV, DL-2-amino-5-phosphonovaleric acid; TNFα, tumor necrosis factor-α.

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it occurs even in adulthood when rats are kept in darkness from birth (Yoshimura et al., 2008). Similar changes, depending on age and visual experience, have been observed in ocular dominance plasticity in rat and cat visual cortex (Cynader and Mitchell, 1980; Fagiolini et al., 1994; Hubel and Wiesel, 1970; Mower et al., 1981).

Mice have been often used in recent studies on ocular dominance plasticity, because new genetic technology has enabled specific visualization of particular types of neurons and specific manipulations of particular signaling molecules in these animals. Studies using mice consistently demonstrated that monocular deprivation produces ocular dominance shifts even in adulthood and the modification is ascribed to the potentiation of nondeprived eye responses without substantial depression of deprived eye responses, which is different from the modification during the critical period (Hofer et al., 2009; Sato and Stryker, 2008; Sawtell et al., 2003). Thus, in this study, we investigated whether low-frequency stimulation could produce any synaptic modification in adult mouse visual cortex. We found that 2 Hz stimulation produces LTP of extracellular field potentials (FPs) evoked in layer 2/3 by stimulation of layer 4 in adulthood as well as during the critical period, and that the induction of LTP requires the activation of L-type Ca^{2+} channels in adulthood, while it requires the activation of T-type Ca^{2+} channels during the critical period. The developmental conversion of Ca^{2+} channel subtypes required for LTP induction was prevented by dark rearing.

2. Materials and methods

All of the experiments conducted in this study were carried out under a protocol approved by the Experimental Animal Care Committee of National Institute for Physiological Sciences and Research Institute of Environmental Medicine, Nagoya University.

2.1. Experimental animals and slice preparations

We used C57BL/6J mice at postnatal day (PD) 23–30 during the critical period of ocular dominance plasticity (Gordon and Stryker, 1996) and in adulthood (PD60-90). In the experiments to study the effect of visual deprivation on LTP, mice were kept in complete darkness from birth to adulthood (PD60-90). Coronal slices of primary visual cortex (300 μm thick) were prepared from mice under deep anesthesia with isoflurane, and they were recovered and maintained in an interface-type chamber perfused with an artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 3 KCl, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 1.2 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose at 33 °C. The recording experiments were conducted in the same type of chamber as used for recovery and maintenance, which was perfused with the ACSF at 33 °C. In the experiments in which inhibition was pharmacologically blocked, slices were perfused with a modified ACSF, in which the concentration of divalent cations was increased to avoid excessive excitability. The composition of the modified ACSF was as follows (in mM): 117 NaCl, 3 KCl, 2.6 $MgCl_2$, 4.8 $CaCl_2$, 1.2 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose.

2.2. Analysis of LTP

As described previously (Sugimura et al., 2015), two pairs of bipolar stimulating tungsten electrodes (diameter, 100 μm; interpolar distance, 200 μm) were placed in layer 4 of the primary visual cortex, separated from each other by about 0.4 mm (Fig. 1A). A surgical cut was imposed in layers 4–5 to ensure that separate groups of presynaptic fibers were activated. One electrode was used to test the effect of conditioning stimulation and the other served as a control. Test stimulation was applied alternately to the electrodes at intervals of 5 s. As a conditioning stimulation to induce LTP, 2 Hz stimulation was applied for 15 min. The intensity of test stimulation was adjusted to values between those eliciting half and one fourth of the maximal responses. In standard 2 Hz stimulation, the intensity was about twice the intensity eliciting half the maximal response. In some of the experiments, 2 Hz stimulation was applied at a weak intensity, which was the same as that used for test stimulation. The FPs evoked by electrical stimulation of presynaptic fibers were recorded extracellularly from layer 2/3 using glass microelectrodes filled with ACSF (Fig. 1A).

In the neocortex, pyramidal neurons are distributed rather evenly in layers 2–6, and their soma and dendrites are present in an intermingled spatial arrangement at every site in these layers. Accordingly, the excitatory postsynaptic potential and spike components of FPs are usually not separated. The FPs recorded from layer 2/3, which are usually composed of an initial positive component and a following negative component, may reflect mainly the excitatory synaptic activity including excitatory postsynaptic potential and spike components in pyramidal neurons. The amplitude of FPs was determined by the difference between the initial positive peak and the following negative peak. This amplitude was used for the assessment of LTP, because LTP of FPs assessed by this amplitude was similar in time course and magnitude to LTP of intracellular excitatory postsynaptic potentials assessed by their initial slope in our previous experiments, in which 2 Hz stimulation was used as a conditioning stimulation in rat visual cortex during the critical period (Yoshimura et al., 2008). However, the amplitude of FPs measured at the initial positive peak as well as the following negative peak can be affected by the strength of inhibitory synapses (Bear et al., 1992; Yoshimura et al., 2003). To discriminate between the modification at excitatory and inhibitory synapses, we conducted experiments under pharmacological blockade of γ-aminobutyric acid $(GABA)_A$ receptors. In this case, the intensity of 2 Hz stimulation was determined as it was in normal ACSF. However, we used a very weak intensity for the test stimulation to reduce late responses following the initial negative peak, which appeared due to the absence of inhibition, and the amplitude of test responses was determined from the initial response. The usage of very weak test stimulation may make the contribution of spike components to FPs almost negligible.

2.3. Data analysis and chemical compounds

Data was expressed as means ± SEM and statistical analyses were performed using Kruskal-Wallis test, followed by Dunn's multiple comparison test. *P* values of less than 0.05 were considered significant. The drugs employed were obtained from the following sources: DL-2-amino-5-phosphonovaleric acid (DL-APV) and SR 95531 from Tocris (Bristol, UK); nimodipine from Sigma (St. Louis, MO, USA) and ML 218 from Almone Labs (Jerusalem, Israel). The dose of chemical compounds used was 100 μM (DL-APV), 20 μM (SR 95531), 20 μM (nimodipine), 50 μM ($NiCl_2$) and 10 μM (ML218).

3. Results

3.1. Low-frequency stimulation-induced LTP of FPs depends on T–type, but not L-type, Ca^{2+} channels during the critical period

We analyzed FPs evoked in layer 2/3 by layer 4 stimulation in the mouse visual cortex (Fig. 1A). In normal ACSF, 2 Hz stimulation for 15 min induced LTP of FPs at PD23-30 during the critical period, when it was applied at intensities about twice the intensity of stimulation evoking half the maximal responses (Fig. 1B), which was consistent with our previous studies using mice (Sugimura et al., 2015; Takeda-Uchimura et al., 2015). When 2 Hz stimulation was applied at a weak intensity, which was the same as that used for the