Age-Dependent Decline in Supragranular Long-Term Synaptic Plasticity by Increased Inhibition During the Critical Period in the Rat Primary Visual Cortex

Hyun-Jong Jang,1 Kwang-Hyun Cho,1,2 Hyun-Sok Kim,1 Sang June Hahn,1 Myung-Suk Kim,1 and Duck-Joo Rhie1,2

1Department of Physiology, College of Medicine, and 2Catholic Neuroscience Center, The Catholic University of Korea, Seoul, South Korea

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INTRODUCTION

Long-term potentiation (LTP) has been extensively studied as a synaptic model of ocular dominance (OD) plasticity in supragranular layers by stimulation of underlying white matter during the critical period (CP) of the primary visual cortex in rodents (Hensch et al. 1998a; Huang et al. 1999; Vetencourt et al. 2008). The role of LTP in OD plasticity was first investigated because the robust induction of LTP by stimulation of the white matter disappeared at the end of the CP (Kim et al. 2006). We have found that LTD also declined in 5-week-old rats in the present study.

The decline of OD plasticity has been related to the maturation of intracortical inhibition (Fagiolini and Hensch 2000; Huang et al. 1999). Recently, Vetencourt et al. (2008) reported that reduced intracortical inhibition without changes in excitation can cause changes in OD plasticity and synaptic plasticity pattern in adult rats. Those reports showed that changes in plasticity can be attributed to the changes in inhibitory influence. Because duration and intensity of the current pulse for extracellular stimulation is critical in the activation of a different set of neural substrates, we hypothesized that our stimulation with a short-duration (0.1 ms), high-intensity current pulse recruits more inhibitory circuits than does stimulation with long-duration (0.2 ms), low-intensity pulse (Kirkwood et al. 1995; Morales et al. 2002). In the present study, we found that both N-methyl-d-aspartate receptor (NMDAR)-dependent LTP and LTD in the pathway from layer 4 to layer 2/3 decline at the end of the CP, during which time more inhibitory circuits were recruited by extracellular stimulation with short-duration, high-intensity currents. Thus the present study provides an alternative in vitro synaptic model for the mechanism of OD plasticity.
were pulled from borosilicate glass and filled with a solution containing whole cell patch-clamp recording technique with a bridge amplifier. MalFP responses in normal ACSF. Stimulus intensity-FP amplitude was fitted with a 40/0.80 NA water-immersion objective, Olympus, neurons in layer 2/3 of the primary visual cortex were identified using evoked with 20% of maximal stimulus intensity in the presence of stimulus, because the synaptic response showed multiple peaks. Stimulation was applied to induce either LTP or LTD, respectively, at the test injection of layer 4 with age, current source density (CSD) analysis was applied after the application of the conditioning stimulus. To verify stimulation of layer 4 with age, current source density (CSD) analysis was performed in slices from 3- and 5-wk-old rats (Aizenman et al. 1996). The signals were amplified 1000-fold, filtered between 0.1 and 3 kHz, digitized at 10 kHz (Digidata 1200A, Axon Instruments), and then saved to a Pentium PC using either the LTP Program (v2.3, www.ltp-program.com) (Anderson and Collingridge 2001) or pClamp 9.0 (Axon Instruments).

To detect differences in FP responses between stimulation with 0.1- and 0.2-ms pulses, we compared FP amplitude evoked by stimuli of various intensities. The FP response in the presence of picrotinin (5 μM) was also measured after preincubation for 30 min. The maximal negative FP values were analyzed, except that the amplitude of FPs evoked with 20% of maximal stimulus intensity in the presence of picrotinin (5 μM) was measured at the first peak, ~4 ms after the stimulus, because the synaptic response showed multiple peaks. Stimulus intensities were normalized to the intensities that evoked maximal FP responses in normal ACSF. Stimulus intensity-FP amplitude curves were fitted with a sigmoid function using the IgorPro v5.0 program (WaveMetrics).

In some experiments, EPSP and IPSP were recorded using the whole cell patch-clamp recording technique with a bridge amplifier (BVC-700A, Dagan, Minneapolis, MN). Patch electrodes (4–6 MΩ) were pulled from borosilicate glass and filled with a solution containing (in mM) 130 K-gluculate, 10 KCl, 3 Mg-ATP, 10 Na₃-phospho-creatin, 0.3 Na₃-GTP, and 10 HEPES (pH 7.25/KOH). Pyramidal neurons in layer 2/3 of the primary visual cortex were identified using IR-DIC video-microscopy with an upright microscope (BX51-W1 fitted with a 40×/0.80 NA water-immersion objective, Olympus, Tokyo, Japan), and their regular spiking patterns were confirmed. Typical access resistance was 15–20 MΩ. Membrane potentials were not corrected for ~14-mV junction potential. EPSP was measured at a holding potential of ~80 mV in normal ACSF, and IPSP was subsequently recorded at a holding potential of 0 mV in the presence of α-aminopentanoate (d-AP5, 50 μM) and 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 μM). d-AP5 and DNQX were purchased from Tocris (Bristol, UK). Other chemicals, including picrotinin, were purchased from Sigma (St. Louis, MO). Command generation, data acquisition and analyses were performed using pClamp 9.2 Suite software (Axon Instruments, Foster City, CA). Data were filtered at 5 kHz, sampled at 20 kHz (Digidata 1320A, Axon Instruments), and saved to the hard drive of a Pentium PC.

Data are expressed as means ± SE. Group comparisons were performed using the paired or unpaired two-tailed Student’s t-test unless otherwise specified. One-way repeated-measures ANOVA followed by Tukey’s post hoc test was also used. The level of significance was set at P < 0.05.

RESULTS

Age-dependent decline in supragranular LTP and LTD

Initially, a 0.1-ms current pulse was applied to layer 4 to evoke FP in layer 2/3, according to a previously published method (Kim et al. 2006) and as described in METHODS. Under this experimental condition, the LTP in layer 2/3 induced by TBS of layer 4 in slices from 3-wk-old rats (127.6 ± 3.1%, n = 8, P < 0.001) declined in 5-wk-old rats (102.9 ± 2.0%, n = 9), consistent with the aforementioned study (Fig. 1A). When a 0.2-ms stimulus was applied (Kirkwood et al. 1995; Morales et al. 2002), the stimulus intensity required (125 ± 4 μA, n = 22) to evoke half-maximal FP amplitude was ~50% of the intensity of the 0.1-ms stimulus (267 ± 7 μA, n = 22). FPs evoked in this pathway by a 0.2-ms pulse were potentiated by TBS in both 3-wk-old (134.5 ± 3.6%, n = 5, P < 0.001) and 5-wk-old rats (126.9 ± 2.8%, n = 8, P < 0.001), consistent with previous reports (Kirkwood and Bear 1994a; Kirkwood et al. 1995). Furthermore, with stimulation with 0.1-ms current pulses, the LTD was induced in slices from 3-wk-old rats (77.9 ± 2.0%, n = 8, P < 0.001) and declined in 5-wk-old rats (101.4 ± 2.0%, n = 8; Fig. 1B). However, when we applied 0.2-ms low-intensity currents, LTD was continuously induced in 3-wk-old (79.4 ± 1.8%, n = 5, P < 0.001) and 5-wk-old rats (77.0 ± 3.6%, n = 7, P < 0.001). Using 0.1-ms pulses in slices from 4-wk-old rats, both TBS and LFS resulted in moderate induction of LTD (110.2 ± 6.9%, n = 6, P = 0.201) and LTD (91.9 ± 3.5%, n = 8, P < 0.05), respectively. The induction of LTD and LTD in 3-wk-old rats with a 0.1-ms pulse was blocked by addition of d-AP5 (50 μM) to the bath (99.7 ± 5.9%, n = 4; 100.5 ± 5.9%, n = 8; respectively), indicating NMDAR dependence.

We positioned a stimulating electrode in the middle of the cortex to stimulate layer 4. The depth from the pia increased from ~700 μm at the third week of age to ~730 μm at the fifth week of age with increasing cortical thickness from ~1,400 to ~1,500 μm at the respective ages (n = 5 each). CSD analysis showed similar time and spatial profiles of source and sink along the cortex at both ages (see Supplementary Fig. S11), consistent with middle layer stimulation of a previous report (Aizenman et al. 1996). This result confirmed that FPs were evoked by the stimulation of lower layer 4 and superficial layer 5 in the present study, regardless of age. The age-dependent decline in LTP and LTD in the pathway from layer 4 to layer 2/3 was demonstrated when FPs were evoked by short 0.1-ms pulses.

1 The online version of this article contains supplemental data.
We further examined the effect of various pulse durations on the induction of LTP and LTD. The induction of LTP and LTD was not dependent on pulse duration (0.05–0.4 ms) in 3-wk-old rats (Fig. 1C). Whereas, in 5-wk-old rats, the shorter stimulus pulse (≤0.1 ms) did not induce LTP and LTD, but the longer stimulus pulse (0.2 ms) did. However, in 5-wk-old rats, a 0.4-ms pulse induced only LTP. Because stimulus intensity and duration are critical for generation of action potentials in neural structures, these findings suggest that extracellular stimulation with short-duration, high-intensity pulses might activate a different set of cortical neurons and axons.

Increased recruitment of inhibitory circuits with short-duration, high-intensity pulses

The late development of intracortical inhibition during the CP of the visual cortex significantly affects induction of LTP (Huang et al. 1999; Jiang et al. 2005). IPSPs have a higher threshold than EPSPs in neocortical slices (Ling and Benardo 1995; Rozas et al. 2001). Thus we hypothesized that the short-duration, high-intensity pulse might recruit a greater number of inhibitory circuits to layer 2/3 than the long-duration, low-intensity pulse. Initially, the difference in the FP inhibitory components between the 0.1- and 0.2-ms pulse stimulation was investigated using picrotoxin, a γ-aminobutyric acid-A (GABA_A) receptor antagonist. The inhibitory component was estimated by subtraction of the FP amplitude in normal ACSF from that in the presence of picrotoxin. A low concentration of picrotoxin (5 μM) was used to resolve the difference in the amplitude of the first peak without full-blown epileptiform discharge by complete inhibition of GABA_A receptors. FP amplitude increased with stimulus intensity both in normal ACSF and in the presence of picrotoxin, yielding similar sigmoid-fitted curves between 0.1- and 0.2-ms pulses in 3-wk-old rats (Fig. 2A). However, although the stimulus-response plots in the presence of picrotoxin for the two stimuli were similar in 5-wk-old rats, the FP amplitude evoked by the 0.2-ms pulse was greater than that evoked by the 0.1-ms pulse in normal ACSF (Fig. 2B). Thus we assumed that the inhibitory components induced by the two stimuli were similar in 3-wk-old rats. However, in 5-wk-old rats, the 0.1-ms stimulus recruited a greater inhibitory component than the 0.2-ms stimulus, especially at intensities evoking half-maximal FP amplitudes in normal ACSF (Fig. 2). This result implies that extracellular stimulation with short-duration, high-intensity current pulses preferentially activates GABAergic inhibitory circuits.

Because of capacitive currents to stimulating electrodes, due to the connections between the stimulus isolation unit and the
ever, the IPSP/EPSP ratio resulting from the 0.1-ms pulse was greater than that produced by the 0.2-ms pulse at high stimulus intensities (at 15 – 30 mV EPSP, n = 13, P < 0.001). Thus we investigated differences in the IPSP component among different stimulation parameters (0.05- to 0.4-ms pulse duration) evoking 25-mV EPSPs (Fig. 3B). With decreasing pulse duration, greater stimulus intensity was required to evoke 25-mV EPSPs and greater IPSPs [n = 12, F(3,33) = 26.14, P < 0.001 by 1-way repeated measures ANOVA] were generated in 5-wk-old rats. These results suggest that extracellular stimulation using short-duration, high-intensity pulses results in greater recruitment of inhibitory components than stimulation using long-duration, low-intensity pulses. Thus induction of LTD in layer 2/3 by stimulation of layer 4 with 0.1-ms pulses might recruit more inhibitory components than stimulation with 0.2-ms pulses, which may cause LTD and LTD to decline as inhibitory intracortical circuits reach maturation at 5 wk of age.

**Suppression of NMDAR-dependent LTP and LTD by inhibition**

Because the balance between inhibition and excitation is important in LTP induction (Hensch and Fagiolini 2005; Jiang et al. 2005), increased recruitment of inhibitory circuits may mask induction of synaptic plasticity in layer 2/3 in 5-wk-old rats (Jiang et al. 2007). Thus we addressed this issue by induction of LTD and LTD with stimulation at intensities that resulted in fewer inhibitory components. The ratio of inhibition to excitation (I/E) in half-maximal FP responses evoked by a 0.1-ms stimulus was 0.57 in 5-wk-old rats (Fig. 2). The 0.1-ms stimulus at intensities that evoke 25 and 75% of maximal FP amplitudes showed similar I/E ratios (0.46 and 0.44, respectively) and were similar to the I/E ratio for the 0.2-ms stimulus at an intensity that evoked half-maximal FP amplitudes (0.47).

Therefore we determined whether LTD and LTP could be induced with the 0.1-ms stimulus at these intensities in slices from 5-wk-old rats. TBS with the 0.1-ms stimulus that evoked 75% of the maximal FP amplitude induced LTD (122.8 ± 15.1 mV, n = 5, P < 0.01). LFS with the 0.1-ms stimulus that evoked 25% of the maximal FP amplitude induced LTD (80.7 ± 3.0 mV, n = 6, P < 0.01) in 5-wk-old rats (Fig. 4). Induction of LTD and LTD was blocked by addition of n-AP5 (50 μM) to the bath (n = 5, each). These results indicate that NMDAR-dependent LTD and LTD do not disappear but might be suppressed as inhibitory circuits in the visual cortex reach maturation.

**Discussion**

It has been proposed that the “inhibitory plasticity gate,” which is located in layer 4, filters signals from the thalamus and that layer 4 stimulation escapes the inhibitory gate (Kirkwood and Bear 1994a; Rozas et al. 2001). Under the experimental conditions used in the present study, stimulation of layer 4 might have included the inhibitory gates. However, age-independent induction of LTD and LTD in the present study with a 0.2-ms stimulus (Kirkwood and Bear 1994a), the identical spatial profiles of FPs resulting from the two stimuli, and the CSD profiles that are similar to a previous report (Aizenman et al. 1996), might eliminate this possibility. Thus a novel finding of the present study is that long-term...
synaptic plasticity in intracortical circuits from layer 4 to layer 2/3 in vitro, beyond the inhibitory plasticity gate, declines with age during the CP.

Although it is not possible to activate specific neural elements with extracellular stimulation, we observed more increase in recruitment of inhibitory than excitatory components as a result of extracellular stimulation at the end of the CP. Furthermore, we observed greater recruitment of inhibitory circuits with short-duration, high-intensity stimulation. Postnatal development of inhibitory circuits lags behind formation of excitatory connections in the cerebral cortex (Huang et al. 1999; Morales et al. 2002). Thus late maturation of inhibitory circuits is one of the proposed mechanisms by which long-term synaptic plasticity in layer 2/3 is diminished and the CP of the visual cortex closes. Based on minimal differences in inhibitory components between the 0.1- and 0.2-ms stimuli, induction of long-term synaptic plasticity in the ascending pathway from layer 4 to layer 2/3 appears to be tightly regulated in a narrow window by inhibitory influences, at least during this developmental period. It is of interest to note that 0.4-ms stimulation induced LTP only, but not LTD, in 5-wk-old rats. This finding implies that LTD induction may require an opti-

**FIG. 3.** Increase in inhibitory postsynaptic potential (IPSP) amplitude with age and stimulus intensity. A: excitatory postsynaptic potential (EPSP) and IPSP were recorded in layer 2/3 by stimulation of layer 4. EPSP-IPSP plots were obtained from 3 (■ and □) and 5-wk-old rats (○ and ●). Stimulus currents were applied with 0.1 (■ and ●)- or 0.2-ms (□ and ○) pulse duration in normal ACSF and in the presence of d-amino-5-pentanoate (d-AP5; 50 μM) and 6,7-dinitroquinolinone-2,3-dione (DNQX, 20 μM) for IPSP. Fitted lines show the slope of the EPSP vs. IPSP plot (n = 13). Traces show representative EPSPs and IPSPs with pulse duration of 0.1 (—) or 0.2 ms ( - - -), obtained from a cell. *, P < 0.05 and **, P < 0.01 between the 2 stimuli. B: IPSP plots for individual pyramidal cells from 5-wk-old rats and the IPSP/EPSP amplitude ratio (I/E ratio, ●) at stimulus intensities that evoked a 25-mV EPSP with varying pulse durations (n = 12). Stimulus intensities for various pulse durations were also plotted (●). Traces show representative EPSPs and incremental IPSPs with pulse duration ranging from 0.4 to 0.05 ms, obtained from a cell. *, P < 0.05 versus 0.1-ms pulse in the repeated-measures ANOVA with Tukey’s post hoc test.

**FIG. 4.** Activity-dependent induction of LTD and LTP in layer 2/3 in 5-wk-old rats. A 0.1-ms pulse was used to induce LTD and LTP. A: induction of LTD with LFS at the stimulus intensity that evoked 25% of the maximal FP amplitude. B: induction of LTP with TBS at the stimulus intensity that evoked 75% of the maximal FP amplitude. D-AP5 (50 μM) was applied to the bath solution throughout the experiments. Insets: average traces recorded during the period indicated. The number of experiments ranged from 5 to 6 in each group. Right: both the average and individual FP responses. **, P < 0.01, vs. the baseline response.
mal range of inhibition (Jiang et al. 2007; Morales et al. 2002), which is finely tuned with maturation. Furthermore, our finding that a reduction in the amount of inhibitory components due to certain stimulus intensities might induce long-term synaptic plasticity, as shown in Fig. 4, supports the idea that the ascending inputs to layer 2/3 may mask synaptic plasticity that extends beyond the CP (Jiang et al. 2007; Kirkwood et al. 1997).

Biophysical properties between excitatory and inhibitory axons

Axonal structural features, such as the degree of myelination and diameter, and biophysical properties are not uniform, especially in the cerebral cortex. Thus both the duration and intensity of extracellular stimulation are important in activation of neuronal elements. Stimulus current threshold is inversely proportional to axon diameter (BeMent and Ranck 1969; Rubinstein 1991) and degree of myelination (Ali et al. 1999; Nowak and Bullier 1998). In general, long-range excitatory axons are more myelinated and larger in diameter than local inhibitory axons (Gonchar and Burkhalter 1999; Keller 1995). In this case, local inhibitory fibers, as well as thin unmyelinated local ascending excitatory connections from stellate cells in layer 4C to layer 3 (Peters and Sethares 1996), might need greater extracellular stimulation currents than extrinsic excitatory axons (Swadlow 1998). However, systematic studies of the axonal properties of various neurons in the visual cortex have not been reported to date, to the best of our knowledge, partly due to the complex structure of the neocortex. There are many different levels of axonal myelination even in one type of inhibitory interneurons in the cerebral cortex (Thomson and Bannister 2003). The high-intensity currents used in the present study seemed to activate more axons from inhibitory interneurons during postnatal development, probably due to differences in axonal diameter and degree of myelination.

Functional implications

Homosynaptic LTP/LTD appears to play a role in OD plasticity after monocular deprivation (Bear and Rittenhouse 1999; Kirkwood et al. 1995; Vetencourt et al. 2008). Although overexpression of brain-derived neurotrophic factor (BDNF) evokes early closure of the CP and accelerates the developmental decline in the induction of LTP in the visual cortex (Huang et al. 1999), recent studies with genetically manipulated mice, such as knock-out of BDNF (Bartoletti et al. 2002), GAD65 (Hensch et al. 1998a; but see Choi et al. 2002), metabotropic glutamate receptors (Renger et al. 2002), PKA RIIβ (Hensch et al. 1998b), and PKA RIIβ (Fischer et al. 2004), reported conflicting results between OD plasticity in vivo and LTP/LTD in vitro. Part of the discrepancy may be due to limitations of the synaptic models as well as to differences in the age and species of the experimental animals, and in the experimental protocols (Choi et al. 2002). For example, in a previous study that used high-intensity 0.1-ms stimulation similar to that used in the present study, heterozygous BDNF knock-out mice showed normal OD plasticity but only a transient potentiation in supragranular FP by layer 4 stimulation (Bartoletti et al. 2002). However, the impaired LTP might have been due to presynaptic fatigue (Abidin et al. 2006). Thus further studies of both development of inhibitory circuits during the CP and induction of LTD on layer 4 stimulation using our model may give more insight into the involvement of LTP/LTD in OD plasticity.

An OD shift after monocular deprivation during the CP is more prominent in supragranular and infragranular layers than in layer 4 in mice, which suggests that intracortical connections also are plastic age-dependently, in addition to age-dependent plasticity in the geniculocortical pathway (Gordon and Stryker 1996). LTP and LTD are layer specifically induced by activation of either NMDAR or metabotropic glutamate receptors in rats except in layer 4 (Rao and Daw 2004; Wang and Daw 2003). These differing layer-specific mechanisms might be one explanation for the dissociation between LTP/LTD in vitro and OD plasticity in vivo (Daw et al. 2004). It appears that visual cortical plasticity requires a minimal level of intracortical inhibition or a threshold in the balance between inhibition and excitation (Hensch and Fagiolini 2005; Jiang et al. 2005). Perisomatic innervation of basket cells reaches a plateau in the fifth postnatal week in infragranular layers of the mice visual cortex (Chattopadhyaya et al. 2004). Maximal inhibitory postsynaptic current and IPSP/EPSP ratios are nearly doubled at this week compared with those at the third postnatal week in supragranular layers of the rat visual cortex (Morales et al. 2002). Thus as demonstrated in the present study, adopting an experimental model that changes the balance between inhibition and excitation might explain the recently reported discrepancies between LTP/LTD and OD plasticity.

In conclusion, by recruiting more inhibitory circuits with short-duration, high-intensity stimulation in addition to the development of inhibition in the postnatal period, the present study demonstrated age- and activity-dependent LTP and LTD in the pathway from layer 4 to layer 2/3 during the CP for the rat primary visual cortex, thereby identifying an alternative in vitro model of OD plasticity.

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REFERENCES


DEVELOPMENTAL DECLINE OF SUPRAGRANULAR LTP/LTD


