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

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ABSTRACT

Supragranular long-term potentiation (LTP) and depression (LTD) are continuously induced in the pathway from layer 4 during the critical period in the rodent primary visual cortex, which limits the supragranular long-term synaptic plasticity from being a synaptic model for the mechanism of ocular dominance (OD) plasticity. The results of the present study demonstrate that the pulse duration of extracellular stimulation to evoke a field potential (FP) is critical to induction of LTP and LTD in this pathway. LTP and LTD were induced in the pathway from layer 4 to layer 2/3 in slices from 3-week-old rats when FPs were evoked by 0.1- and 0.2-ms pulses. LTP and LTD were induced in slices from 5-week-old rats when evoked by stimulation with a 0.2-ms pulse, but not by stimulation with a 0.1-ms pulse. Both the inhibitory component of FP and the IPSP/EPSP amplitude ratio evoked by stimulation with a 0.1-ms pulse were greater than the values elicited by a 0.2-ms pulse. Stimulation with a 0.1-ms pulse at various intensities that showed the similar inhibitory FP component with the 0.2-ms pulse induced both LTD and LTP in 5-week-old rats. Thus, extracellular stimulation with shorter-duration pulses at higher intensity resulted in greater inhibition than that observed with longer-duration pulses at low intensity. This increased inhibition might be involved in the age-dependent decline of synaptic plasticity during the critical period. These results provide an alternative synaptic model for the mechanism of OD plasticity.
Key words: LTP, LTD, inhibitory circuit, recruitment, current pulse
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 (postnatal week 5 in rats) (Kirkwood and Bear 1994a; Kirkwood et al. 1995). However, LTP is consistently induced in the pathway from layer 4 to layer 2/3 during the CP. The different age profiles in the induction of LTP between layer 4 and white matter stimulation could result from 1) bypassing some inhibitory circuits to supragranular layers with stimulation of the middle cortical layer, which are recruited by deep layer stimulation (Aizenman et al. 1996; Rozas et al. 2001) and/or 2) late maturation of intracortical inhibitory circuits (Huang et al. 1999; Morales et al. 2002).

In contrast, although homosynaptic long-term depression (LTD) has been proposed as a synaptic mechanism for OD plasticity after monocular deprivation (Bear and Rittenhouse 1999; Heynen et al. 2003), a corresponding in vitro synaptic model is not yet available because supragranular LTD is continuously induced in rat visual cortical slices during the CP (Jiang et al. 2007; Kirkwood and Bear 1994b; Kirkwood et al. 1997; Sermasi et al. 1999). Our previous
study showed a decline in LTP in the pathway from layer 4 to layer 2/3 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 pulses (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.
METHODS

Coronal sections containing the visual cortex were prepared as described previously (Kim et al. 2006) from Sprague-Dawley rats of either sex (postnatal 3 to 5 weeks, Orientbio Inc., Korea) that were housed under standard conditions (23 ± 1°C, 12/12 h light/dark cycle). Animal care and surgical procedures were approved by the Ethics Committee of the Catholic University of Korea and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Brains were quickly isolated after anesthetization with chloral hydrate (400 mg/kg, ip) and were immediately submerged in ice-cold dissection buffer (in mM: 125 NaCl, 2.5 KCl, 1 CaCl₂, 2 MgSO₄, 1.25 NaH₂PO₄, 25 NaHCO₃, and 10 D-glucose) that was oxygenated with 95% O₂/5% CO₂. Slices (400 μm thick) were cut using a vibratome (Campden, UK), allowed to recover for 40 min at 37°C in a submerging chamber, and then were maintained at room temperature until used in recording experiments. The slices were transferred to the recording chamber and perfused continuously (1.5-2 ml/min) with artificial cerebrospinal fluid (ACSF, in mM: 125 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgSO₄, 1.25 NaH₂PO₄, 25 NaHCO₃, and 10 D-glucose) that was saturated with 95% O₂/5% CO₂ at 32-33 °C.

The primary visual cortex was identified according to a rat brain atlas (Paxinos and Watson 1997). Recording electrodes, pulled from glass pipettes, were filled with ACSF (1-3 MΩ) and positioned in layer 2/3. Field potential (FP) was evoked by a brief square current pulse
(0.05-0.4 ms) to the underlying layer 4 at a site in the middle of the cortex, using a concentric bipolar stimulating electrode (100 μm in diameter, SNE-100, David Kopf, CA, USA). The amplitude of the negative FP peak was used as a measure of the evoked population excitatory synaptic current. The baseline response was obtained at 30-s intervals for 10 min with a stimulus intensity that yielded a half-maximal FP amplitude (Kirkwood and Bear 1994a). Either theta-burst stimulation (TBS, five bursts at 5 Hz of ten pulses at 100 Hz) applied five times at 10-s intervals or low-frequency stimulation (LFS, 900 pulses at 1 Hz) was applied to induce either LTP or LTD, respectively, at the test stimulus intensity and pulse duration. The FP was recorded for 50 min 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-week-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, CA, USA), 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 picrotoxin (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 picrotoxin (5 μM) was measured at the first peak, approximately 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, OR, USA).

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, USA). Patch electrodes (4-6 MΩ) were pulled from borosilicate glass and filled with a solution containing (in mM) 130 K-gluconate, 10 KCl, 3 Mg-ATP, 10 Na₂-phosphocreatine, 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-WI fitted with a 40×/0.80NA 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 D-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 picrotoxin, were purchased from Sigma (St. Louis,
MO, USA). Command generation, data acquisition and analyses were performed using pClamp 9.2 Suite software (Axon Instruments, Foster City, CA, USA). 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 mean ± 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 the Methods section. Under this experimental condition, the LTP in layer 2/3 induced by TBS of layer 4 in slices from 3-week-old rats (127.6 ± 3.1%, $n = 8$, $P < 0.001$) declined in 5-week-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 approximately 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-week-old (134.5 ± 3.6%, $n = 5$, $P < 0.001$) and 5-week-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-week-old rats (77.9 ± 2.0%, $n = 8$, $P < 0.001$) and declined in 5-week-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-week-old (79.4 ± 1.8%, $n = 5$, $P < 0.001$) and 5-week-old rats (77.0 ± 3.6%, $n = 7$, $P < 0.001$). Using 0.1-ms pulses in slices from 4-week-old rats, both TBS and LFS resulted in moderate induction of LTP (110.2 ± 6.9%, $n = 6$, $P = 0.201$)
and LTD (91.9 ± 3.5%, n = 8, P < 0.05), respectively. The induction of LTP and LTD in 3-week-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 ~1400 μm to ~1500 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 Fig. S1), 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. Thus, 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.

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-week-old rats (Fig. 1C). Whereas, in 5-week-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-week-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 (GABA) 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 GABAA 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-week-old rats (Fig. 2A). However, although the stimulus-response
plots in the presence of picrotoxin for the two stimuli were similar in 5-week-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-week-old rats. However, in 5-week-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 tissue (Butson and McIntyre 2005), the two stimuli may spatially activate different numbers of axons and neurons in layer 4, thereby evoking different levels of postsynaptic depolarization in individual neurons in layer 2/3. Thus, we examined the amplitude of FPs resulting from stimulation, as the recording pipette was moved horizontally through layer 2/3, which spatially represents the extent of stimulation. However, in slices from 5-week-old rats, the peak values and lateral profiles in FP amplitude for the two stimuli were not different ($n = 7$, data not shown). This result indicates that the slice volumes stimulated by the two stimuli are equal.

Next, we directly investigated differences in the inhibitory component between the two stimuli in individual cells using a whole-cell patch-clamp recording. Because FP responses
comprise an array of EPSPs with different recruitment of excitatory and inhibitory inputs in individual cells, we measured IPSP amplitude at various stimulus intensities (96 – 205 μA for 0.1-ms pulse; 52 – 114 μA for 0.2-ms pulse) evoking the same range of EPSP amplitudes (5 – 30 mV) and analyzed the pooled data. As shown in Fig 3A, the slope of linear fit of IPSP to EPSP with the 0.1-ms pulse (0.81 ± 0.09, n = 13) was not significantly different from that obtained for 0.2-ms pulse stimulation in 5-week-old rats (0.74 ± 0.08, P = 0.070). However, 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 one-way repeated measures ANOVA) were generated in 5-week-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 LTP and 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 LTP and LTD to decline as inhibitory intracortical circuits reach maturation at 5 weeks 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-week-old rats (Jiang et al. 2007). Thus, we addressed this issue by induction of LTP 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-week-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 if LTD and LTP were induced with the 0.1-ms stimulus at these intensities in slices from 5-week-old rats. TBS with the 0.1-ms stimulus that evoked 75% of the maximal FP amplitude induced LTP (122.8 ± 3.4%, 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%, n = 6, P < 0.01) in 5-week-old rats (Fig. 4). Induction of LTP and LTD was blocked by addition of D-AP5 (50 μM) to the bath (n = 5, each). These results indicate that NMDAR-dependent LTP 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 plasticity gate. However, age-independent induction of LTP 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-week-old rats. This finding implies that LTD induction may require an optimal 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 level 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 RIβ (Hensch et al. 1998b) and PKA RIIβ (Fischer et al. 2004), reported conflicting results between OD plasticity \textit{in vivo} and LTP/LTD \textit{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 which 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 upon 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 layer-specific different mechanisms
might be one explanation for the dissociation between LTP/LTD \textit{in vitro} and OD plasticity \textit{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 IPSC and IPSP/EPSP ratios are nearly doubled at this week compared to 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 \textit{in vitro} model of OD plasticity.
GRANTS

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FOOTNOTES

1 The online version of this article contains supplemental Fig. S1.
REFERENCES


FIGURE LEGENDS

FIG. 1. Age-dependent decline in LTP and LTD in the visual cortex. Extracellular stimulation was applied to layer 4 and the resulting FP in layer 2/3 was recorded in slices from 3- (A) and 5-week-old rats (B). LTP was induced by TBS with either a 0.1- (closed circles) or a 0.2-ms (open circles) pulse duration stimulus. LTD was induced by LFS with either a 0.1- (closed triangles) or 0.2-ms (open triangles) pulse duration stimulus. Insets show averaged representative traces recorded during the period indicated. The arrow indicates the application of TBS for 1 min or of LFS for 15 min. The right panels summarize the FP response resulting from TBS or LFS with either a 0.1- or 0.2-ms pulse duration stimulus. C. Average FP responses resulting from TBS (closed circles) and LFS (open triangles) with stimuli pulse durations ranging from 0.05 to 0.4 ms in 3- and 5-week-old rats. The numbers of experiments are indicated in parentheses.
FIG. 2. Increase in the FP inhibitory component evoked by 0.1- and 0.2-ms stimulation with age. FPs were recorded in layer 2/3 by stimulation of layer 4. Stimulus intensity-FP amplitude curves were obtained in slices from 3- (A) and 5-week-old rats (B).

Stimulus currents were applied with 0.1- (closed symbols) or 0.2-ms (open symbols) pulse durations in normal ACSF (circles) and in the presence of picrotoxin (squares, 5 μM). The arrows between the lines indicate the difference in FP amplitude between the two solutions at intensities that evoked half-maximal FP amplitudes in normal ACSF, i.e., inhibitory components. Insets show representative FP traces at 60% maximal stimulus intensities with 0.1- (solid lines) or 0.2-ms (dotted lines) pulse duration. Experiments were performed in 6 to 7 slices in each group. *, $P < 0.05$ and **, $P < 0.01$ vs. 0.1-ms stimulation.
FIG. 3. Increase in IPSP amplitude with age and stimulus intensity. A. EPSP and IPSP were recorded in layer 2/3 by stimulation of layer 4. EPSP-IPSP plots were obtained from 3- (squares) and 5-week-old rats (circles). Stimulus currents were applied with 0.1- (closed symbols) or 0.2-ms (open symbols) pulse duration in normal ACSF for EPSP and in the presence of D-AP5 (50 μM) and 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 (solid line) or 0.2 ms (dashed line), obtained from a cell. *, P < 0.05 and **, P < 0.01 between the two stimuli. B. IPSP plots for individual pyramidal cells from 5-week-old rats and the IPSP/EPSP amplitude ratio (I/E ratio, open circle) at stimulus intensities that evoked a 25-mV EPSP with varying pulse durations (n = 12). Stimulus intensities for various pulse durations were also plotted (closed triangle). 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 vs. 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-week-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 show average traces recorded during the period indicated. The number of experiments ranged from 5 to 6 in each group. Right panels show both the average and individual FP responses. **, $P < 0.01$, vs. the baseline response.
Supplemental Fig. S1

**Current source density (CSD) profiles of visual cortical slices with mid-cortical stimulation revealed that the stimulation site involves layer 4 in both 3- and 5-week-old rats.**

To confirm that mid-cortical stimulation actually drove layer 4 to the layer 2/3 circuit, CSD analysis was performed in both 3- and 5-week-old rats. The CSD profile was calculated as described by Mitzdorf (1985) using a spatial differentiation grid of 200 µm, but the direction of sink and source was inverted to directly compare our data to that of Aizenman et al. (1996).

The thickness of the cortex in 3- and 5-week-old rats was 1420 ± 12 µm \((n = 5)\) and 1510 ± 19 µm \((n = 5)\), respectively, and the stimulation position was 696 ± 14 µm and 730 ± 14 µm from the pial surface, respectively. The main sink was positioned at 340 ± 24 µm and 390 ± 37 µm and the peak time of the main sink was 3.90 ± 0.18 ms and 3.98 ± 0.16 ms, respectively.

There were no significant differences in the CSD profiles between both ages. Thus layer 4 and the upper layer 5 were the main stimulation sites for both ages.

**References**

