Neuron, Vol. 44, 1145–1158, April 8, 2004 ©2004 Elsevier Inc. DOI 10.1016/j.neuron.2004.11.004

Spike Timing-Dependent LTP/LTD Mediates Visual Experience-Dependent Plasticity in a Developing Retinotectal System

Yangling Mu[1] and Mu-ming Poo[1,*]
[1] Division of Neurobiology
Department of Molecular and Cell Biology
Helen Wills Neuroscience Institute
University of California
Berkeley, California 94720

Summary

Sensory experience plays an instructive role in the development of the nervous system. Here we showed that visual experience can induce persistent modification of developing retinotectal circuits via spike timing-dependent plasticity (STDP). Pairing light stimuli with spiking of the tectal cell induced persistent enhancement or reduction of light-evoked responses, with a dependence on the relative timing between light stimulus and postsynaptic spiking similar to that for STDP. Using precisely timed sequential three-bar stimulation to mimic a moving bar, we showed that spike timing-dependent LTP/LTD can account for the asymmetric modification of the tectal cell receptive field induced by moving bar. Furthermore, selective inhibition of signaling mediated by brain-derived neurotrophic factor and nitric oxide, which are respectively required for light-induced LTP and LTD, interfered with moving bar-induced temporally specific changes in the tectal cell responses. Together, these findings suggest that STDP can mediate sensory experience-dependent circuit refinement in the developing nervous system.

Introduction

Sensory experience is capable of shaping the connectivity of developing neural circuits (Wiesel and Hubel, 1963; Katz and Shatz, 1996), through a process that may be related to activity-dependent persistent synaptic modifications known as long-term potentiation (LTP) and long-term depression (LTD) (Constantine-Paton et al., 1990; Goodman and Shatz, 1993; Fregnac et al., 1994; Zhang and Poo, 2001; Malenka and Bear, 2004). In the developing nervous system, LTP may be causally related to synapse strengthening and stabilization, whereas LTD is related to synapse weakening and elimination. Based on an extended form of Hebb's postulate (Stent, 1973), i.e., correlated pre- and postsynaptic activities strengthen the synapse whereas uncorrelated activities weaken the synapse, computational analyses have successfully predicted experience-dependent refinement of the retinotopic map in the optic tectum and the formation of ocular dominance columns in the primary visual cortex (Willshaw and von der Malsburg, 1976; Miller et al., 1989; Goodhill, 1993). However, although correlated pre/post activities have been shown to induce LTP at many excitatory synapses, it is not clear whether uncorrelated activities cause LTD. Furthermore, there are many forms of LTP/LTD, depending on the stimulation protocol and synapse type, and which forms are most relevant to developmental refinement remains largely unknown.

Recent experimental and theoretical evidence has pointed to the importance of specific temporal order of pre/post spiking for synaptic modification in both developing and adult nervous systems (Dan and Poo, 2004). The synaptic strength can be bidirectionally modified by correlated pre/post spiking within a narrow time window (in the order of tens of milliseconds): pre-before-post (*pre-post*) spiking leads to LTP, whereas *post-pre* spiking leads to LTD. This form of LTP/LTD, now generally referred to as spike timing-dependent plasticity (STDP, Abbott and Nelson, 2000), has been found at excitatory synapses in many systems, including cortical slices (Markram et al., 1997; Egger et al., 1999; Feldman, 2000; Froemke and Dan, 2002), hippocampal slices (Debanne et al., 1998; Nishiyama et al., 2000), cerebellum-like structures (Bell et al., 1997), hippocampal cultures (Bi and Poo, 1998), and developing retinotectal system (Zhang et al., 1998). Compared to the correlation-based Hebb's rule, STDP offers attractive features for neural computation, allowing for self-adjustment of overall synaptic weight in a neuron (Song et al., 2000; van Rossum et al., 2000) and the development of asymmetric and direction-selective receptive fields (Mehta et al., 2000; Rao and Sejnowski, 2001). Precisely timed sensory stimuli are known to drive functional changes of mammalian visual cortex in a manner consistent with STDP mechanisms (Schuett et al., 2001; Yao and Dan, 2001). In *Xenopus* tadpoles, fast-moving bars were observed to induce a persistent asymmetric modification of the tectal neuron's receptive field (RF) along the direction of the movement (Engert et al., 2002). Although spiking of the tectal cell is required for this RF modification, it is not clear whether STDP is involved in this experience-dependent plasticity. In this study, we have investigated specifically whether spike timing-dependent LTP/LTD are induced at retinotectal synapses by the moving bar stimuli and can account for the refinement of the RF properties of the tectal cell by this natural visual experience.

We first confirmed that conditioning with unidirectional moving bar stimuli specifically enhances the tectal cell response to the moving bar in the conditioned direction (Engert et al., 2002). Further analyses showed that this direction selectivity was accompanied by a marked enhancement of the early-phase and a slight reduction of the late-phase compound synaptic currents (CSCs) evoked by the bar stimulus in the tectal cell, suggesting a temporally specific bidirectional modification of synaptic efficacy. In addition, we found that sequential light bar stimuli that mimicked the moving bar stimulus induced LTP/LTD of the early excitatory CSCs with a temporal specificity expected for STDP. We also elucidated the cellular mechanisms underlying light-induced LTP/LTD of tectal responses, in particular, its dependence on NMDA receptor (NMDAR) activation, BDNF signaling, and NO synthesis. Finally, we demonstrated that

*Correspondence: mpoo@uclink.berkeley.edu

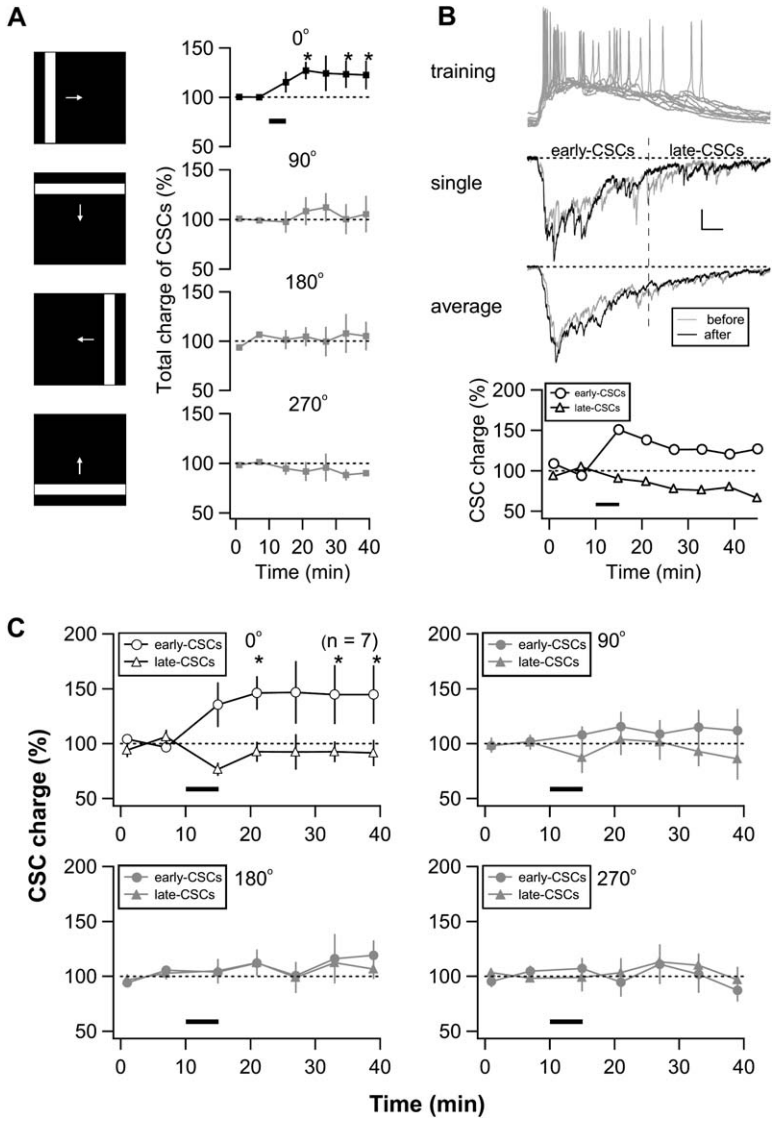

Figure 1. Temporal Asymmetry in the Modification of Compound Synaptic Currents by Conditioning with Moving Bar

(A) Persistent changes of tectal responses induced by conditioning with repetitive unidirectional moving bar. The CSCs ($V_c \sim -45$ mV) evoked by moving bar in each of the four orthogonal directions (left) were monitored (at 0.008 Hz) before and after the cell was conditioned for 5 min by unidirectional moving bar (0.2 Hz, 0°, black bar). The total integrated charge of CSCs for the conditioned direction was significantly enhanced, as compared to the mean value before conditioning (*$p < 0.05$, two-tailed t test). In contrast, no significant change was found for all other three directions. Data from each experiment were normalized to the average before conditioning and are shown in 6 min bins (n = 7 experiments; error bar, SEM).

(B) Moving bar-induced enhancement of early CSCs and reduction of late CSCs. "Training": superimposed samples (ten sweeps) of tectal cell spiking induced by the moving bar. "Single": sample traces of CSCs evoked by the moving bar (in the conditioned direction) before (gray) and after (black) the conditioning. "Average": average of four traces. The dashed line divides the CSCs into early and late CSCs. Scales: 20 pA (or 10 mV), 50 ms. Graph below shows integrated charge for early and late CSCs, normalized to the preconditioning average, from an example experiment (same as that for the traces above).

(C) Asymmetric changes in early and late CSCs. Data from the same set of experiments as in (A). Data showing significant difference in comparison to the pre-training value are marked by asterisk ($p < 0.05$, two-tailed t test; error = SEM).

blocking the induction of spike timing-dependent LTP/LTD at retinotectal synapses by inhibiting BDNF/NO signaling selectively abolished the asymmetric modification of the early- and late-phase CSCs and interfered with direction-selective tectal cell responses induced by moving bar conditioning. Thus, spike timing-dependent LTP/LTD may indeed play a critical role in sensory experience-driven refinement of neural circuits.

Results

Moving Bar-Induced Synaptic Modification Exhibits Temporal Asymmetry

Repetitive application of moving bar stimuli potentiate some retinotectal synapses while depressing others (Engert et al., 2002; Zhou et al., 2003), but whether these modifications resulted from spike timing-dependent LTP/LTD of individual retinotectal synapses remains unclear. To address this issue, we focused our study on the synaptic modification underlying moving bar-induced direction selectivity of tectal cell responses (Engert et al., 2002). In particular, we examined whether changes in the tectal cell responses induced by the moving bar conditioning can be accounted for by STDP of retinotectal synapses.

In vivo perforated whole-cell recordings were made from tectal neurons in stage 41–45 *Xenopus* tadpoles. The retina was exposed to moving bars (20 μm wide; speed, 0.2 μm/ms) in four orthogonal directions alternately (frequency, 0.008 Hz; Figure 1A), and the tectal cell was voltage clamped (v.c.) at the reversal potential of Cl^- currents ($V_c \sim -45$ mV) to monitor excitatory light-evoked compound synaptic currents (CSCs). For conditioning, the retina was exposed to 60 sweeps of moving bar (speed, 0.2 μm/ms; frequency, 0.2 Hz) in one randomly chosen direction (indicated as 0°, the other three directions were assigned clockwise as 90°, 180°, and 270°, respectively) while the cell was current clamped (c.c.) at −50 to −60 mV. During this stimulation, the tectal cell exhibited a train of spikes, elicited either by the moving bar itself or by periodic current injection in case that the moving bar did not evoke spiking. Consistent with the previous finding (Engert et al., 2002), excitatory CSCs in response to the moving bar in the

conditioned direction were significantly enhanced compared to those for all other three directions (Figure 1A).

A key feature of STDP is the temporal specificity in synaptic modification: inputs that arrive early with respect to the postsynaptic spiking become potentiated, whereas those that arrive later than the spiking become depressed. Moving bar-evoked CSCs consist of many retinal inputs arriving at different times. If spike timing-dependent LTP/LTD underlies moving bar-induced synaptic modification, these inputs will be modified in different directions, depending on their timing relative to the tectal neuron spiking. Based on the STDP rule, we predicted that early synaptic inputs that contribute to the early part of CSCs are more likely to fall into the *pre-post* window for potentiation with respect to the spike train triggered by the moving bar, whereas those contributing to the later part of the response are more likely to fall into the *post-pre* window for depression. Therefore, to test this hypothesis, we further analyzed the dependence of the changes in synaptic currents on the timing of the input by dividing the evoked CSCs into two halves of equal duration: the early and late CSCs. As shown by the superimposed traces recorded before (gray) and after (black) the conditioning (Figures 1B and 1C), moving bar conditioning resulted in a marked enhancement of early CSCs but a slight reduction of late CSCs. In comparison with the marked enhancement of early CSCs, the reduction of late CSCs was relatively small (Figure 1C). As discussed later, the reduction of late CSCs may be masked by the increase in early CSCs due to the partial overlap of potentiated and depressed synaptic currents. A direct consequence of this asymmetric modification is a "shift" of the receptive field (RF) toward the potentiated direction, as observed previously (Engert et al., 2002).

Bidirectional Synaptic Modification Induced by Visual Stimuli

To directly test our hypothesis that STDP is involved in visual stimuli-induced synaptic modification, we examined light-induced changes in the efficacy of retinotectal synapses by controlling the timing of postsynaptic spiking relative to the light bar stimulus located within the RF of the tectal neuron. As shown in Figure 2A, each light bar stimulus (duration 1.5 s) triggered both *on* and *off* responses, as reflected by CSCs recorded in the tectal neuron ($V_c \sim -70$ mV). Since *off* responses were usually larger and more consistent than *on* responses, we have thus used the *off* response to represent light-evoked response in the tectal cell. To determine the plausibility of the STDP mechanism, we first assessed the temporal precision of visual stimuli-induced synaptic responses. The onset latency of excitatory postsynaptic potentials (EPSPs) evoked by the decrement of light stimulus showed a relatively small variation over 100 trials (Figure 2B, left panel). Although the onset latency was long (>100 ms), its standard deviation (SD) was relatively small (5.0 ± 1.9 ms, SEM; n = 74 cells; Figure 2B, right panel). Thus for each visual stimulus, the timing of the presynaptic spiking could be predicted with a precision of about 5 ms, well within the critical window for STDP.

To monitor the strength of retinotectal synapses, we estimated the light-elicited synaptic response by taking the total integrated charge of CSCs associated with the *off* response within a time window of 50 ms following the onset of CSCs, in order to exclude contribution from inhibitory synaptic currents (Tao and Poo, 2005). In most recordings, a bar stimulus evoked subthreshold depolarization of the tectal neuron (c.c., $V_m = -45 \sim -50$ mV). Repetitive subthreshold light stimulation alone at 0.33 Hz had no significant effect on the synaptic efficacy, as shown by the absence of any change in the CSC charge (Figure 2C). However, when the subthreshold visual stimuli were repetitively paired with postsynaptic current injections that initiated spiking of the tectal neuron, we observed either potentiation or depression of CSCs, depending on the time interval between the tectal cell spiking and the onset of EPSPs (Figures 2D–2F). When the interval was varied from −500 to +500 ms, we found synaptic potentiation when the onset of EPSPs was within about 20 ms before the tectal cell spiking, but synaptic depression when spiking occurred within 20 ms before the EPSPs (Figures 2E–2H). This dependence on the time interval of pre/post activation was similar to that found for LTP/LTD of individual retinal inputs induced by direct electrical stimulation of RGCs (Zhang et al., 1998). Thus, natural visual stimulation can indeed trigger spike timing-dependent LTP/LTD at retinotectal synapses.

Synaptic Modification Induced by Sequential Visual Stimuli

To further examine whether STDP of retinotectal synapses can account for the moving bar-induced changes in tectal responses (Figure 1), we performed experiments using three light bars of the same orientation applied sequentially to mimic the moving bar stimulation. Baseline tectal responses to each bar stimulus were stable when tested at a frequency of 0.017 Hz. In the first set of experiments, three light bars (designated I, II, and III) were repetitively applied (0.33 Hz, 5 min) for conditioning in the sequence I → II → III with an interval of 16.7 ms between adjacent bars (Figure 3A). This interval corresponds to a speed (1.2 μm/ms) of the moving bar faster than that found to be required for inducing direction-selective tectal responses (Engert et al., 2002; see also Figure 1). For each sequence of stimulation, the luminance decrement of bar II was paired with one postsynaptic spike, which was initiated by current injection into the tectal neuron 10 ms after the average onset of EPSPs measured for each cell (see Figure 2B). At this timing, the postsynaptic spiking largely followed and preceded the EPSPs elicited by bar I and III, respectively. Figure 3B depicts *off* CSCs evoked by bar stimuli before and after the conditioning in a typical experiment. We found persistent enhancement of CSCs evoked by bar I and II, but persistent reduction for bar III-evoked CSCs (Figure 3C). Data from seven experiments are summarized in Figure 3D. When the bar interval was increased from 16.7 to 500 ms, which corresponds to a speed (0.04 μm/ms) of moving bar slower than that shown to be insufficient for inducing direction-selective tectal responses (Engert et al., 2002), we found that CSCs evoked by bar II were potentiated, but those elicited by bar I and III remained unchanged (Figures 3E and 3F), again consistent with the STDP window.

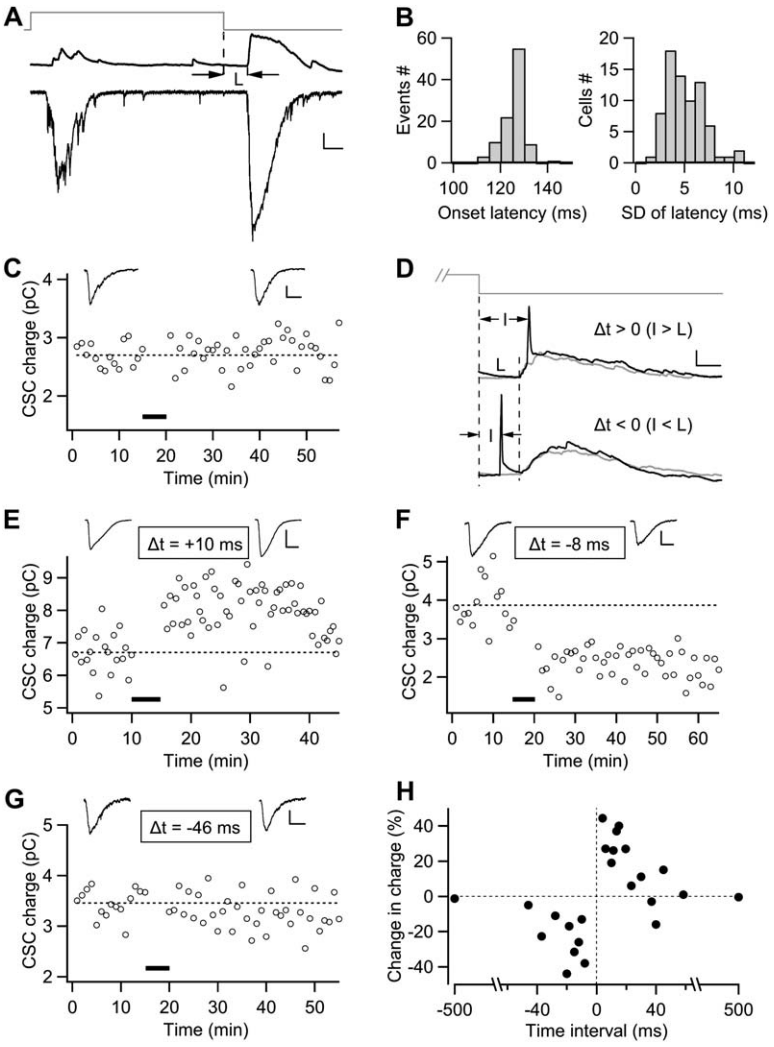

Figure 2. Spike Timing-Dependent LTP/LTD Induced by Repetitive Single-Bar Stimulation

(A) Sample traces of synaptic potentials (c.c., $V_m \sim -50$ mV) and CSCs (v.c., $V_c \sim -70$ mV; inward currents downward) recorded in a tectal neuron in response to a light bar stimulation (duration 1.5 s). The onset latency ("L") of the light response was defined as the time interval between the light decrement and the onset of synaptic responses. Scales: 12.5 pA, 150 ms.

(B) Temporal precision of light responses. (Left) The distribution of the onset latency for EPSPs recorded from a tectal neuron in response to a single light bar over 100 trials. (Right) The distribution of the standard deviation (SD) of the onset latency for 74 tectal cells.

(C) Repetitive conditioning with light stimuli that induced subthreshold tectal responses had no effect on CSCs. Data represent the total CSC charge within the first 50 ms of the CSCs evoked by a single light bar (1.5 s, 0.017 Hz) before and after conditioning (black bar, 1.5 s duration, 100 stimuli at 0.33 Hz, cell in c.c., $V_m = -45 \sim -50$ mV), normalized by the mean value (dotted line) before conditioning. Shown above are sample CSCs (average of 15; scales: 20 pA, 150 ms).

(D) Pairing of light stimuli with postsynaptic spikes. "I": interval between the light decrement and the onset of spiking induced by somatic current injection. The time interval between light bar-evoked EPSPs and spiking is indicated as Δt ($\Delta t = I - L$). Sample traces of synaptic potentials in the absence (gray) and presence (black) of the action potential (AP, evoked by current injection) were superimposed to depict the pattern of *pre-post* (left) and *post-pre* (right) pairing. Scales: 10 mV, 50 ms.

(E–G) Three examples of spike timing-dependent LTP/LTD. Data are presented as in (C). During conditioning, each light stimulus was paired with a postsynaptic AP, which was initiated by somatic current injection, at Δt = +10 ms (E), −8 ms (F), and +46 ms (G). Scales: 100 pA, 150 ms (E); 40 pA, 200 ms (F); 40 pA, 150 ms (G).

(H) Temporal window for spike timing-dependent enhancement and reduction of tectal responses. The conditioning protocol as described in (D) was applied, and the percentage of change in the CSC charge was plotted against Δt. Each point represents the result from one experiment.

Under physiological conditions, both RGCs and tectal neurons readily fire a train of spikes in response to natural visual stimuli (Zhang et al., 2000; Zhou et al., 2003; Du and Poo, 2004). Therefore, it is important to know whether pre- and postsynaptic spike trains can modify developing retinotectal synapses in vivo. To address this issue, we employed the same experimental design for conditioning stimuli as described above (Figure 3A) except that the bar II stimulus was adjusted (by increasing the width) to a level that reliably initiated a spike train in the tectal cell (Figure 3G), while inputs from bar I and III remained subthreshold in firing the tectal cell. We found that the sequential three-bar conditioning again induced a persistent enhancement of CSCs evoked by bar I and II, but depression of those evoked by bar III (Figure 3H). Therefore, visual stimuli that mimicked the moving bar conditioning resulted in simultaneous potentiation and depression of converging retinal inputs to the tectal neuron in a manner fully consistent with STDP, and the apparent effect of sequential three-bar conditioning on tectal light responses resembled moving bar-induced asymmetric modifications of early and late CSCs (Figure 1).

Dependence on Postsynaptic Spiking and NMDAR Activation

Consistent with the previous finding that the induction of spike timing-dependent LTP/LTD at retinotectal synapses

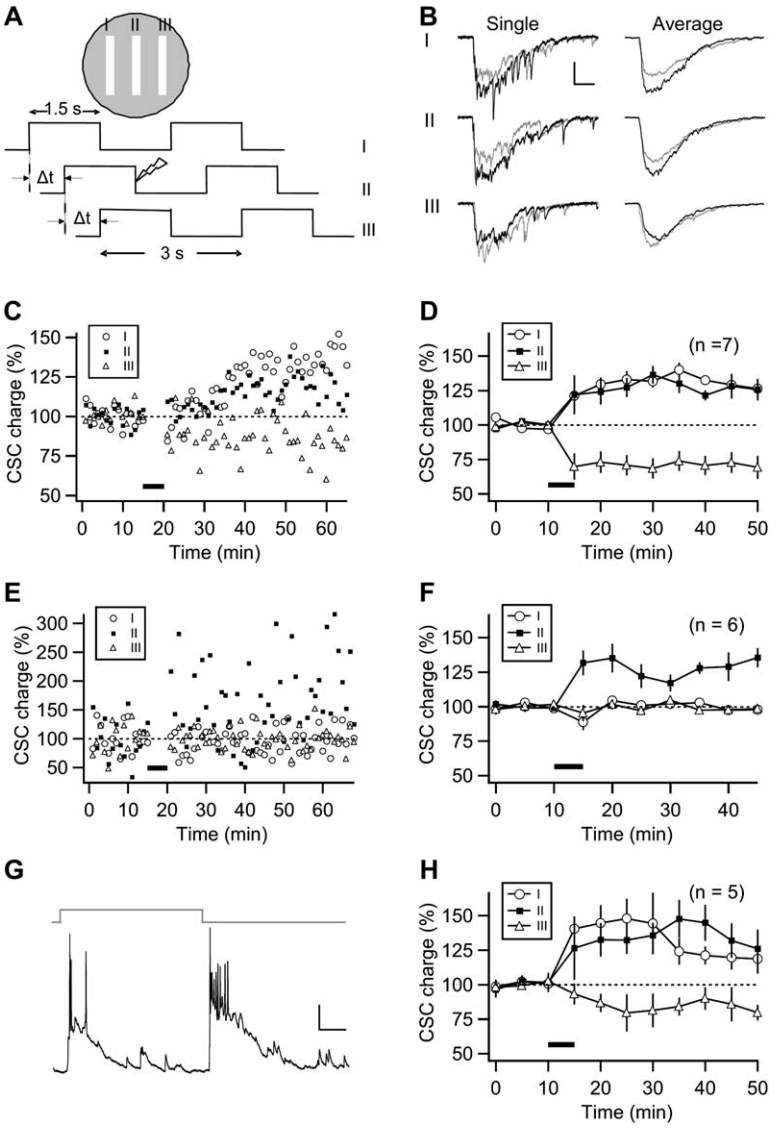

Figure 3. Bidirectional Changes of Tectal Cell Responses Induced by Three-Bar Conditioning

(A) Diagram of the experimental design. Sequential application of three light bars (I, II, and III) located within the RF of a tectal neuron, each of which elicited a subthreshold depolarization in the tectal neuron, was used to mimic the moving bar stimulus. Three bars were applied (1.5 s duration) sequentially at a low frequency (0.017 Hz) to monitor the baseline responses. During the conditioning session, bar I, II, and III were applied repeatedly (1.5 s duration, 100 times at 0.33 Hz) in the sequence of I → II → III with an interval of Δt (in ms). The decrement of bar II was paired with somatic current injection in the tectal neuron, which was set to be 10 ms after the mean delay of the onset of light responses.

(B) Sample traces of CSCs evoked by each light bar (I, II, or III) before and after three-bar conditioning with Δt = 16.7 ms. "Single": single test responses. "Average": averages of 15 consecutive responses. CSCs recorded before and after conditioning were shown as gray and black traces, respectively. Scales: 50 pA, 50 ms.

(C and D) Three-bar conditioning with Δt = 16.7 ms. Data in (C) represent the integrated CSC charge elicited by bar I (open circle), II (filled square), and III (open triangle) in an example tectal neuron, normalized by the mean CSC charge before conditioning. The summary of all data from experiments similar to (C) is shown in (D), with normalized CSC charge averaged over 5 min bins for each experiment. The changes in the mean CSC charge in response to bar I, II, and III at 5–40 min after conditioning were 30.4% ± 1.9%, 26.9% ± 1.7%, and −28.9% ± 0.7%, respectively (error = SEM).

(E and F) Three-bar conditioning with Δt = 500 ms. Data are presented in the same manner as in (C) and (D). After conditioning, the changes in the mean CSC charge in response to bar I, II, and III were −0.9% ± 2.0%, 28.4% ± 2.5%, and −1.1% ± 1.2%, respectively.

(G) Sample trace of the *on and off* responses elicited by suprathreshold light stimulation (c.c., $V_m \sim -50$ mV). Scales: 10 mV, 250 ms.

(H) Summary of all data obtained from cells conditioned by the standard three-bar conditioning (Δt = 16.7 ms; n = 5) similar to that in (D) except that bar II evoked suprathreshold tectal cell response, whereas bar I and III evoked subthreshold responses. The changes in the mean CSC charge in response to bar I, II, and III were 38.1% ± 12.3%, 35.1% ± 9.2%, and −15.8% ± 6.7%, respectively.

by electrical stimulation of RGCs requires postsynaptic spiking (Zhang et al., 1998), we found that when the postsynaptic tectal cell showed no spiking during the standard three-bar conditioning, there was no change in light-evoked CSCs following the conditioning (Figure 4A). Similarly, voltage clamping the tectal cell at −90 mV, which prevented spiking of the cell, also prevented three-bar conditioning-induced modification of CSCs (Figure 4B). Thus, postsynaptic tectal cell spiking is critical for light-induced modification of retinotectal synapses, consistent with STDP.

Both homo- and heterosynaptic LTP/LTD induced by neuronal activity at many synapses are known to require postsynaptic NMDAR activation (Malenka and Bear, 2004). We found that local perfusion of the tectum with NMDAR antagonist D-2-amino-5-phosphonopentanoic acid (D-APV) completely prevented the effect of three-bar conditioning on CSCs (Figure 4C). The dependence on NMDAR activation was also shown for the induction of direction selectivity of the tectal cell response by unidirectional moving bar (Engert et al., 2002), consistent with the notion that light-induced spike timing-dependent LTP/LTD of retinotectal synapses underlies moving bar-induced direction selectivity of the tectal neuron. Furthermore, our results are in agreement with the previous evidence showing that activity-dependent refinement of the topographic map in the optic tectum is NMDAR dependent (Debski et al., 1990).

Separate Signaling Pathways Mediate Spike Timing-Dependent LTP versus LTD

Neurotrophins (NTs) may play either a permissive or instructive role in activity-dependent synaptic modifications (Poo, 2001). Several lines of evidence suggest that

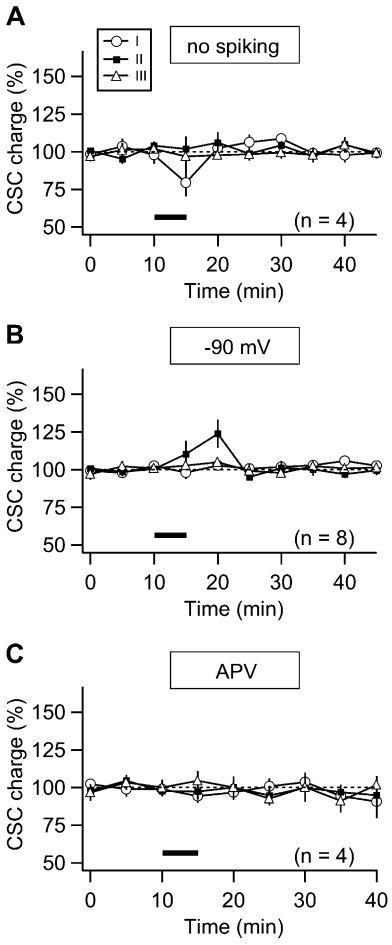

Figure 4. The Role of Postsynaptic Spiking and NMDARs

(A and B) Dependence on postsynaptic spiking. Experiments similar to that in Figure 3D, except that the conditioning with subthreshold three-bar stimuli was not paired with postsynaptic current injection (A) or that the tectal cell was voltage clamped at −90 mV during conditioning (B).

(C) Dependence on NMDARs. Experiments similar to that in Figure 3D, except that the tectum was perfused with APV (50 μM).

All of the error bars in this figure indicate SEM.

the secretion of NTs can be regulated by electrical activity (Blöchl and Thoenen, 1995; Balkowiec and Katz, 2000; Aicardi et al., 2004). Furthermore, persistent modification of developing *Xenopus* retinotectal synapses can be induced by local application of brain-derived neurotrophic factor (BDNF) to the tectum (Du and Poo, 2004). To examine the role of BDNF in STDP, we first used a low-frequency stimulation protocol (Zhang et al., 1998) to induce LTP or LTD. When spiking of the tectal neuron was initiated by injection of a depolarizing current pulse within 10 ms after EPSPs were repetitively elicited by extracellular stimulation of RGCs, LTP was induced (Figures 5A and 5B). However, in the presence of k252a, a tyrosine kinase inhibitor known to block BDNF-trkB signaling, the same stimulation protocol failed to induce significant LTP (Figure 5A). This dependence on BDNF is also consistent with the finding that downregulation of trkB expression in the retinotectal system by injection of morpholino antisense oligonucleotides abolished LTP of retinotectal synapses induced by the STDP protocol (Zhou et al., 2003). In contrast, when the spiking of the tectal neuron was initiated within 10 ms before the onset of EPSPs, LTD could be consistently induced either in the presence or in the absence of k252a (Figure 5A). Thus, the induction of spike timing-dependent LTP, but not LTD, requires BDNF-trkB signaling.

In addition to BDNF, nitric oxide (NO) has been recognized as a potential retrograde factor that mediates communication between pre- and postsynaptic neurons during topographic refinement of *Xenopus* retinotectal projections (Debski and Cline, 2002). Furthermore, NO synthesis is required for activity-induced LTD in cerebellar Purkinje cells (Linden and Connor, 1995). We thus examined whether NO signaling is involved in spike timing-dependent LTP/LTD, using the same STDP protocol described above. As shown in Figure 5B, we found that LTD was abolished in the presence of L-NAME, a potent NO synthase (NOS) inhibitor, whereas LTP induction was unaffected (Figure 5B).

We next examined whether visual stimuli-induced LTP/LTD also depends on similar BDNF/NO signaling as that found above for STDP induced by electrical stimulation of RGCs. When the standard three-bar conditioning stimuli (Figure 3A) were applied in the presence of k252a, we found that the enhancement of CSCs induced by *pre-post* conditioning was completely abolished, whereas the reduction of CSCs induced by *post-pre* conditioning remained largely intact (Figures 5C and 5D). Treatment with k252b, a membrane-impermeant analog of k252a, had no effect on either the enhancement or the reduction of CSCs (data not shown). Thus, light-induced LTP but not LTD of retinotectal synapses required BDNF-trkB signaling, fully consistent with the critical role of BDNF in spike timing-dependent LTP (Figure 5A). Similarly, when the standard three-bar conditioning stimuli were applied after bath perfusion of L-NAME, the induction of LTD but not LTP was completely prevented (Figures 5E and 5F), again consistent with the finding that NO signaling is required for LTD of retinotectal synapses induced by the STDP protocol (Figure 5B). Taken together, our results indicated that BDNF and NO signaling were required for spike-timing dependent LTP and LTD, respectively, induced by either electrical stimulation of the retina or by natural visual stimulation.

Blockade of LTP/LTD Affects Moving Bar-Induced Direction Selectivity

If direction selectivity and the asymmetric modification of tectal responses induced by repetitive unidirectional moving bar were indeed mediated by STDP, they should depend on the cellular signaling events similar to those underlying light bar-induced LTP/LTD. To test this prediction, conditioning with unidirectional moving bar, as described in Figure 1, was performed after bath application of either k252a or L-NAME, treatment that prevented light bar-induced LTP and LTD, respectively. We found that, in the presence of k252a, moving bar-induced enhancement of early CSCs was completely abolished, while the reduction of late CSCs became more significant as compared to that found under normal conditions (Figure 6A). This is fully consistent with the idea that the moving bar-induced enhancement of early CSCs is due to spike timing-dependent LTP and

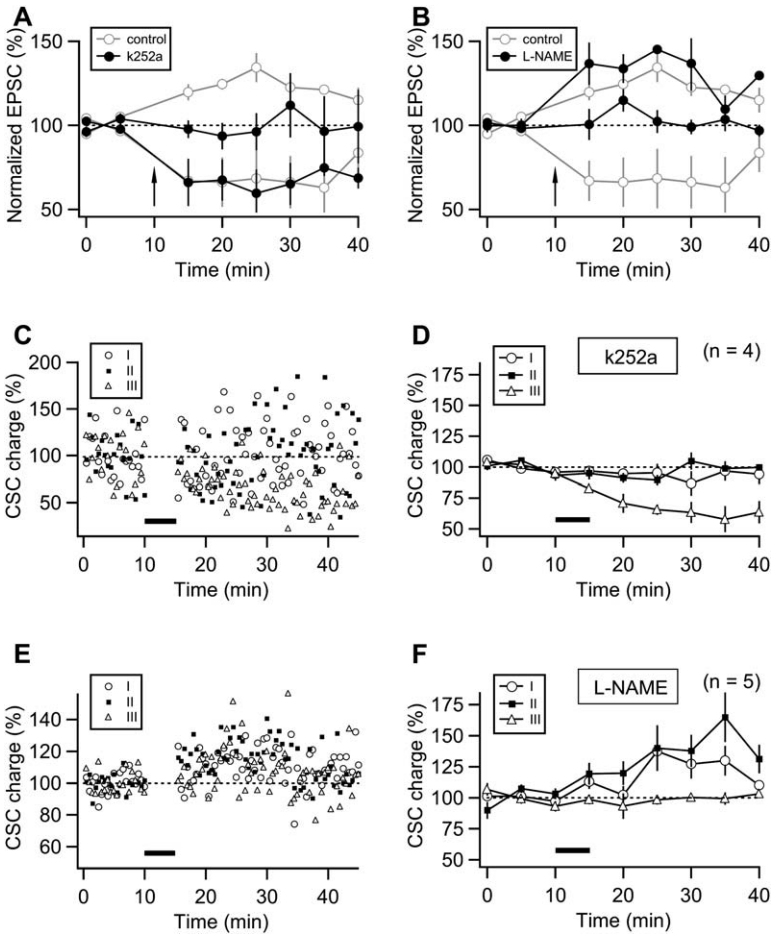

Figure 5. Dependence on BDNF-TrkB and NO Signaling

(A) Bath application of k252a impaired spike timing-dependent LTP but not LTD. LTP and LTD were induced by spike-timing protocol (arrow). Under normal conditions (gray, open symbols), the average amplitude of EPSCs was 127.2% ± 3.6% (n = 4) and 67.0% ± 0.8% (n = 6) of baseline values between 10 to 20 min after LTP and LTD induction, respectively. In the presence of k252a (black, filled symbols), the average amplitude of EPSCs was 100.6% ± 5.7% (n = 5) and 64.1% ± 2.3% (n = 4) of baseline values after LTP and LTD induction, respectively.

(B) Bath application of L-NAME abolished spike timing-dependent LTD but not LTP. The gray and open symbols represent the same data as in (A). In the presence of L-NAME (black, filled symbols), the average amplitude of EPSCs was 138.6% ± 3.4% (n = 4) and 105.4% ± 4.8% (n = 4) of baseline values after LTP and LTD induction, respectively.

(C and D) Dependence of light-induced LTP on BDNF-trkB signaling. Experiments similar to that in Figure 3D, except that the recordings were made in the presence of k252a (200 nM). The changes in the charge of CSCs in response to bar I, II, and III were 94.0% ± 6.0%, 96.5% ± 1.5%, and −32.7% ± 5.8%, respectively (n = 4).

(E and F) Dependence of light-induced LTD on NO synthesis. Experiments similar to that in Figure 3D, except that the recordings were performed under the bath perfusion of L-NAME (0.5–1 mM). The changes in the mean integrated charge of CSCs in response to bar I, II, and III were 23.9% ± 5.5%, 32.0% ± 12.6%, and 1.4% ± 3.1%, respectively.

The error bars in all of the panels indicate SEM.

that part of moving bar-induced depression of late CSCs was normally masked by the LTP of early CSCs. Furthermore, we found that blockade of BDNF-trkB signaling led to no enhancement, but a significant reduction, of moving bar-evoked total CSCs in the conditioned direction (Figure 6B). In contrast, when NO synthesis was blocked, both the enhancement of early CSCs and total CSCs induced by the moving bar in the conditioned direction remained unaffected (Figures 6C and 6D). However, late CSCs exhibited significant enhancement instead of reduction (Figure 6C), further supporting the notion that moving bar conditioning led to asymmetric synaptic modifications in the early- and late-phase CSCs via spike timing-dependent LTP/LTD.

Discussion

Spike Timing-Dependent LTP/LTD and Light-Induced RF Refinement

In the developing visual system of *Xenopus* tadpoles, LTP/LTD can be induced at retinotectal synapses by direct electrical stimulation of RGCs (Zhang et al., 1998), and natural visual stimuli can produce LTP-like modification of these synapses (Zhang et al., 2000; Engert et al., 2002). Our present results further demonstrate that natural visual inputs can induce both LTP and LTD at these retinotectal synapses by the STDP mechanism, leading to an experience-dependent refinement of receptive field (RF) properties. The case in point is the rapid development of persistent direction selectivity in light-evoked tectal cell responses following repetitive exposure to unidirectional moving bar (Engert et al., 2002; Figure 1A).

Four lines of evidence support the notion that spike timing-dependent LTP/LTD underlies moving bar-induced refinement of the retinotectal circuit. First, conditioning with the unidirectional moving bar caused a persistent enhancement of early CSCs but a reduction of late CSCs in the tectal neuron elicited by the moving bar in the conditioned direction (Figure 1), a temporal asymmetry consistent with the STDP rule. Second, repetitively pairing single light bar stimulation with the tectal cell spiking induced LTP- and LTD-like modifications of CSCs in a time interval-dependent manner, with a window similar to that found for STDP. Third, conditioning with three-bar stimuli that mimicked the moving bar stimulation led to LTP/LTD-like changes of CSCs, depending on the timing of light bar stimulation and tectal spiking (Figures 3D and 3H), and the overall effect mirrored closely the moving bar conditioning-induced

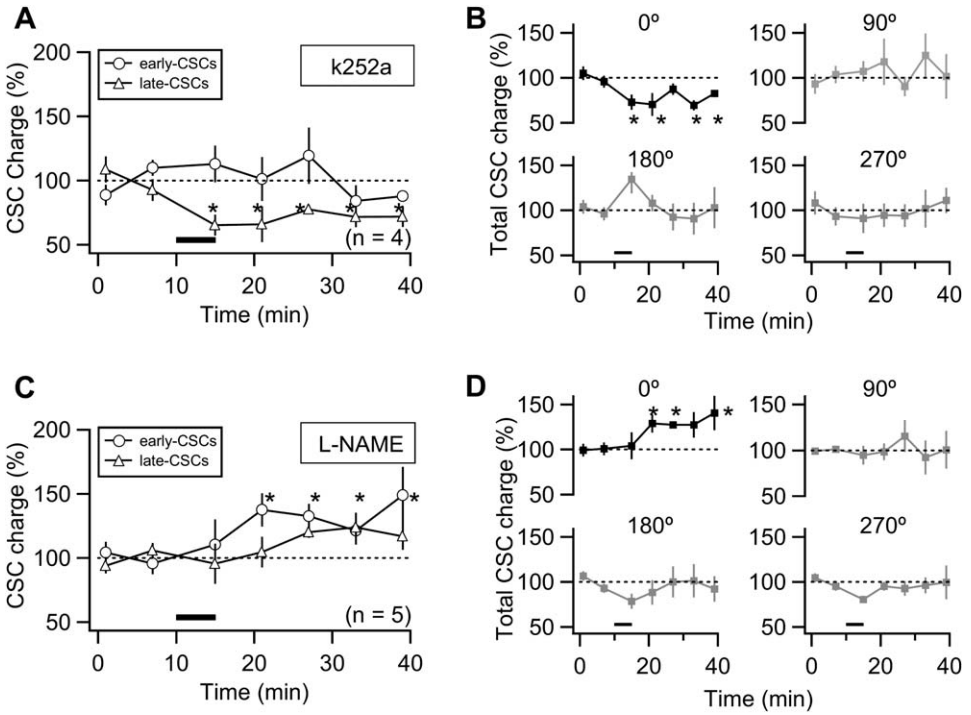

Figure 6. Moving Bar-Induced Synaptic Modification Depends on BDNF-TrkB and NO Signaling

(A) Enhancement of early CSCs was abolished by k252a. Experiments similar to that in Figure 1C except that k252a (200 nM) was present in the bath. Unlike that shown in Figure 1C, the integrated charge of early CSCs for the conditioned direction remained unchanged in comparison to the baseline value. In contrast, there was a significant reduction in the values for late CSCs (*$p < 0.05$, two-tailed t test).

(B) Data from the same set of experiments as in (A). In contrast to that shown in Figure 1A, the total CSC charge for the conditioned direction was significantly reduced (*$p < 0.05$, two-tailed t test).

(C and D) Reduction of late CSCs was abolished by blocking NO synthesis. Experiments similar to that in (A) and (B), except that L-NAME (0.5–1 mM) instead of k252a was present in the bath solution. The integrated charge of both early and late CSCs for the conditioned direction were significantly enhanced (C) in comparison to the baseline value (*$p < 0.04$, two-tailed t test). The total CSC charge for the conditioned direction was significantly enhanced (D), in comparison to its value before conditioning (*$p < 0.05$, two-tailed t test), whereas no significant change was found for all three other directions.

All of the error bars in this figure indicate SEM.

asymmetric modification of early and late CSCs. Finally, abolishing spike timing-dependent LTP and LTD of tectal light responses selectively by inhibiting BDNF-trkB and NO signaling, respectively, led to selective elimination of moving bar conditioning-induced enhancement/reduction of early CSCs/late CSCs. An immediate physiological consequence of this STDP-based synaptic modification is that both strengthening and weakening can be simultaneously induced among converging retinal inputs to the same tectal neuron, allowing selective synapse stabilization and elimination while keeping the overall synaptic drive to the neuron relatively constant. Importantly, this STDP also endows the retinotectal system with the capacity for imprinting sensory experience of specific spatiotemporal patterns into synaptic changes within the neural circuit.

STDP and the Development of Direction Selectivity

Frogs can respond to directional visual inputs by orienting themselves according to the direction, and this behavior may be evoked by electrical stimulation of the optic tectum (Mcilwain, 1972). Neurons sensitive to both the direction and speed of the movement of a visual object distribute uniformly in various layers of the frog optic tectum (Aleinikova and Gogoleva, 1981). A variety of mechanisms have been postulated to explain how visual neurons acquire direction selectivity. One class of models is based on feedforward excitation with a spatially asymmetric arrangement of the weight of excitatory afferents. Modeling studies showed that direction-selective responses can emerge as a result of an asymmetrically shaped RF generated by means of STDP (Mehta et al., 2000; Wenisch et al., 2005). Direction selectivity may also arise from short-term synaptic depression (Chance et al., 1998), and STDP can determine spatial distribution of depressing and nondepressing afferents within the RF, adjust the degree of depression, and thus help to develop direction selectivity (Buchs and Senn, 2002). The classic model for direction selectivity postulates the opposite timing of excitation and delayed inhibition within the RF for of a neuron (Barlow and Levick, 1965; Heeger, 1993). Recent studies showed that modification of excitatory synapses alone through STDP allows single cortical neurons to become direction selective via the Barlow-Levick model, in both simple feedforward-only and complex recurrent networks (Shon et al., 2004). Taken together, there is strong support for the notion that STDP may play a pivotal role in shaping cortical circuits responsible for direction-selective responses.

LTP/LTD and Refinement of Retinotectal Projections

During the refinement of retinotopic maps in the tectum, stabilization and elimination of synaptic connections are likely to be essential for shaping the initial rough pattern of connections into a more precise one (Tao and Poo, 2005). Real-time imaging of *Xenopus* RGC axons has revealed that retinotectal synapses are continuously formed and eliminated as the axons form topographic projections in the optic tectum, and this reorganization of synapses correlated directly to branching and remodeling of RGC axon arbors (Cohen-Cory, 2002). Furthermore, in vivo time-lapse imaging studies on *Xenopus* tadpoles have demonstrated structural changes in both axonal and dendritic arbors in response to visual inputs, mediated by NMDAR-dependent signaling events (Sin et al., 2002; Ruthazer et al., 2003). It is likely that LTP and LTD of retinotectal synapses represent functional prelude to structural changes—the stabilization and elimination of retinotectal connections, respectively. Studies of synapse elimination at developing neuromuscular junctions have suggested that synaptic weakening may precede structural elimination of synapses (Colman et al., 1997).

Specific Roles of BDNF and NO

Neurotrophins (NTs) are essential not only for the survival and differentiation of neurons, but also for the development and plasticity of synapses (Poo, 2001). Based on the findings that NTs can modulate synaptic efficacy and their expression can be upregulated by activity, it has been suggested that NTs may directly mediate activity-dependent plasticity. Our finding on the requirement of BDNF for LTP induction supports the notion that BDNF serves as an activity-regulated factor for the refinement of developing visual circuits (Katz and Shatz, 1996; Poo, 2001). Because exogenous application of BDNF can potentiate retinotectal synapses (Du and Poo, 2004), it is possible that light-induced BDNF secretion in the tectum directly mediates LTP at these synapses. However, we cannot rule out the alternative hypothesis that endogenously secreted BDNF serves merely a permissive role for LTP induction by facilitating use-dependent potentiation of these retinotectal synapses. The dependence of LTP but not LTD induction on BDNF is also consistent with the previous finding that BDNF promotes optic axonal arborization and formation of retinotectal synapses in *Xenopus* (Cohen-Cory, 1999; Alsina et al., 2001).

We found that light-induced LTD was selectively abolished when NO synthesis was inhibited (Figures 5E and 5F). Although moving bar conditioning-induced direction selectivity of the tectal cell response remained largely normal in the absence of NO synthesis (Figure 6D), the reduction of late CSCs evoked by moving bar in the conditioned direction was converted to enhancement, which may impede activity-dependent shrinkage of RFs. It is of interest to note that inhibition of NO synthesis reduces the loss of ipsilateral retinotectal connections and hinders the development of the chick visual system (Wu et al., 1994). The refinement of retinocollicular connections, a process accompanied by LTP/LTD (Mize and Lo, 2000), is also significantly delayed in the NOS gene knockout mice (Mize et al., 1998). Our observation that light-induced LTD requires NMDAR activation (Figure 4C) is consistent with the previous finding that blocking NMDARs reduces NOS activity and obstructs developmental elimination of inappropriate retinotectal projections (Ernst et al., 1998). However, in the doubly innervated optic tectum of three-eyed *Xenopus* tadpoles, NMDAR activation is required for the development of eye-specific segregation of RGC axons (Cline et al., 1987), but NOS inhibition does not abolish the eye-specific segregation (Renteria and Constantine-Paton, 1999), despite the fact that NO can cause collapse of RGC growth cones of retinal explants (Renteria and Constantine-Paton, 1996). These results suggest that the modulation of RGC growth cones by NO represents only a part of the cellular events downstream of NMDAR activation during synaptic competition. Our studies showed that light-induced LTD depends on both NMDAR and NO. Although NMDAR activation elevates NO synthesis, other effectors besides NO may be necessary for the induction of LTD and synapse elimination.

Functional Implications of STDP

Visual stimuli of specific pattern could drive immediate changes in intracortical connections and shifts in RFs in a manner consistent with STDP (Yao and Dan, 2001; Fu et al., 2002). Multi-whisker stimulation and principal-whisker deprivation caused rapid changes in spike timing of layer 4 and layers 2/3 neurons in the somatosensory cortex, suggesting a potential role for STDP in shaping the sensory cortical map (Celikel et al., 2004). Our present results directly demonstrate that visual stimulation can modify retinotectal synaptic efficacy in a spike timing-dependent manner and this type of plasticity can account for moving bar-induced functional changes. Through STDP, temporal sequence of sensory experience may be encoded in the neural circuit in a spatially distributed manner that depends on specific timing of pre/post spiking at individual synapses (Bi and Poo, 1999). Furthermore, the same spiking activity in the postsynaptic neuron may cause simultaneous strengthening and weakening among converging inputs on the same neuron, depending on the timing of pre/post spiking, thereby allowing coordinated refinement and remodeling of neural circuits.

Experimental Procedures

Tadpole Preparation and Electrophysiology

Xenopus laevis staged 41–45 by the criteria of Nieuwkoop and Faber (1967) were anesthetized with HEPES-buffered saline containing 0.02% MS222 (Sigma). The saline was composed of (in mM): NaCl, 135; KCl, 3; HEPES, 10; $CaCl_2$, 3; $MgCl_2$, 1.5; glucose, 10 (pH 7.4). For recording from tectal neurons, the tadpole was secured to a sylgard-coated dish by insect pins. The skin on top of the head was removed, and the brain was split open along the midline to expose the inner surface of the tectum on one side. To prevent occasional muscle contraction, we applied a low dose of α-bungarotoxin (1 mg/ml), which does not affect the retinotectal response. The tadpole was constantly perfused with fresh external solution, and all experiments were performed at the room temperature.

Whole-cell perforated-patch recording was performed by methods described previously (Zhang et al., 1998). The micropipettes were made from borosilicate glass capillaries (Kimax), with a resistance in the range of 5–6 MΩ. The pipette was tip-filled with internal solution and then back-filled with internal solution containing amphotericin B (200 μg/ml). The internal solution contained (in mM): K-gluconate, 128; KCl, 17.5; NaCl, 9; $MgCl_2$, 1; EGTA, 0.2;

HEPES, 10 (pH 7.3). The same patch pipette was used for extracellular stimulation of retinal ganglion cells (RGCs), except that the tip opening was increased to 3 μm, and the filling solution was the bath solution. Recordings were made using patch-clamp amplifiers (Axopatch 1D and Axopatch 200B; Axon Instruments). Signals were filtered at 2 kHz and sampled at 5 kHz. Input resistance (0.5–1 GΩ) and series resistance (20–40 MΩ) were monitored continuously during recordings. Data were accepted for analysis only if the series resistance and input resistance remained relatively constant (<10% change) throughout the experiment. Cells were held at a constant potential of −70 mV unless otherwise indicated. In local perfusion experiments, a glass pipette (opening 30–40 μm) filled with the extracellular solution containing either 50 μM D-2-amino-5-phosphonovaleric acid (D-APV) or 100 μM picrotoxin plus 60 μM strychnine hydrochloride, was used. In the latter case, the spontaneous Cl^- currents (with a characteristic slow time course) were completely abolished and light-evoked CSCs were reversed at 1.9 ± 1.6 mV (n = 10), consistent with the blockade of inhibitory transmission. All drugs were purchased from Sigma. For experiments using k252a (200 nM) and L-NAME hydrochloride (0.5–1 mM), the drugs were purchased from Tocris and applied at least 30 min before the recordings were made.

Visual Stimulation and Measurements of Evoked Responses

For light stimulation, the lens of the eye contralateral to the recorded tectum was removed. The retina was then flattened and stabilized with a glass coverslip. A small LCD screen (from a virtual reality goggle, Sony, PLM-A35) was mounted on the camera port of the microscope, which allowed projection of computer-generated images onto the retina of the tadpole. The images for visual stimulation were generated using custom software. For both single- and triple-bar experiments, white bars (duration 1.5 s) located within the RF of the tectal neuron were applied at the frequency of 0.017 or 0.033 Hz for testing tectal responses and at 0.33 Hz for conditioning. A total of 100 sets of light bar stimuli, each paired with current pulse injection (100–200 pA) in the tectal cell, were applied for conditioning while the cell was current clamped at −45 to −50 mV. The total integrated charge associated with the CSCs was analyzed by custom-made Matlab programs, which read each single current trace, determined the onset of CSCs evoked by light stimulation, and calculated the total charge within the first 50 ms time window of the CSCs. In moving bar experiments, white bars (20 μm in width) swept across the screen at a speed of 0.2 μm/ms. To assay baseline tectal responses, we made the recordings in voltage-clamp mode ($V_c \sim -45$ mV). Each test session includes bars moving in four orthogonal directions (in the sequence of right, down, left, and up) with 30 s intervals. The test sessions (with 2 min interval) were repeated seven to ten times during the control period. The conditioning session consisted of 60 sweeps of moving bar in one randomly chosen direction (at 0.2 Hz), during which the recordings were switched to current-clamp mode ($V_m = -50 \sim -60$ mV). In case that the cell did not fire action potentials reliably during the conditioning, each sweep of moving bar was paired with a spike train elicited by somatic current injection (one train included five pulses at 30 Hz). To test the prediction based on STDP rules that the early and late part of moving bar-evoked CSCs reflects LTP and LTD, respectively, the CSCs was divided in half and the total charge associated the two halves were integrated respectively.

Acknowledgments

We thank Y. Fu for the software; and Y. Dan and J. Du for helpful discussion. This work was supported by grants from USNIH (NS 36999 and EY014979).

Received: October 11, 2005
Revised: February 2, 2006
Accepted: March 1, 2006
Published: April 5, 2006

References

Abbott, L.F., and Nelson, S.B. (2000). Synaptic plasticity: taming the beast. Nat. Neurosci. *3* Suppl., 1178–1183.

Aicardi, G., Argilli, E., Cappello, S., Santi, S., Riccio, M., Thoenen, H., and Canossa, M. (2004). Induction of long-term potentiation and depression is reflected by corresponding changes in secretion of endogenous brain-derived neurotrophic factor. Proc. Natl. Acad. Sci. USA *101*, 15788–15792.

Aleinikova, T.V., and Gogoleva, L.M. (1981). Distribution of neurons related to movement direction velocity in the frog tectum opticum. Fiziol. Zh. SSSR Im. I M Sechenova *67*, 542–546.

Alsina, B., Vu, T., and Cohen-Cory, S. (2001). Visualizing synapse formation in arborizing optic axons in vivo: dynamics and modulation by BDNF. Nat. Neurosci. *4*, 1093–1101.

Balkowiec, A., and Katz, D.M. (2000). Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA in situ. J. Neurosci. *20*, 7417–7423.

Barlow, H.B., and Levick, W.R. (1965). The mechanism of directionally selective units in rabbit's retina. J. Physiol. *178*, 477–504.

Bell, C.C., Han, V.Z., Sugawara, Y., and Grant, K. (1997). Synaptic plasticity in a cerebellum-like structure depends on temporal order. Nature *387*, 278–281.

Bi, G., and Poo, M. (1998). Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. J. Neurosci. *18*, 10464–10472.

Bi, G., and Poo, M. (1999). Distributed synaptic modification in neural networks induced by patterned stimulation. Nature *401*, 792–796.

Blöchl, A., and Thoenen, H. (1995). Characterization of nerve growth factor (NGF) release from hippocampal neurons: evidence for a constitutive and an unconventional sodium-dependent regulated pathway. Eur. J. Neurosci. *7*, 1220–1228.

Buchs, N.J., and Senn, W. (2002). Spike-based synaptic plasticity and the emergence of direction selective simple cells: simulation results. J. Comput. Neurosci. *13*, 167–186.

Celikel, T., Szostak, V.A., and Feldman, D.E. (2004). Modulation of spike timing by sensory deprivation during induction of cortical map plasticity. Nat. Neurosci. *7*, 534–541.

Chance, F.S., Nelson, S.B., and Abbott, L.F. (1998). Synaptic depression and the temporal response characteristics of V1 cells. J. Neurosci. *18*, 4785–4799.

Cline, H.T., Debski, E.A., and Constantine-Paton, M. (1987). N-methyl-D-aspartate receptor antagonist desegregates eye-specific stripes. Proc. Natl. Acad. Sci. USA *84*, 4342–4345.

Cohen-Cory, S. (1999). BDNF modulates, but does not mediate, activity-dependent branching and remodeling of optic axon arbors in vivo. J. Neurosci. *19*, 9996–10003.

Cohen-Cory, S. (2002). The developing synapse: construction and modulation of synaptic structures and circuits. Science *298*, 770–776.

Colman, H., Nabekura, J., and Lichtman, J.W. (1997). Alterations in synaptic strength preceding axon withdrawal. Science *275*, 356–361.

Constantine-Paton, M., Cline, H.T., and Debski, E. (1990). Patterned activity, synaptic convergence, and the NMDA receptor in developing visual pathways. Annu. Rev. Neurosci. *13*, 129–154.

Dan, Y., and Poo, M. (2004). Spike timing-dependent plasticity of neural circuits. Neuron *44*, 23–30.

Debanne, D., Gahwiler, B.H., and Thompson, S.M. (1998). Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. J. Physiol. *507*, 237–247.

Debski, E.A., Cline, H.T., and Constantine-Paton, M. (1990). Activity-dependent tuning and the NMDA receptor. J. Neurobiol. *21*, 18–32.

Debski, E.A., and Cline, H.T. (2002). Activity-dependent mapping in the retinotectal projection. Curr. Opin. Neurobiol. *12*, 93–99.

Du, J., and Poo, M. (2004). Rapid BDNF-induced retrograde synaptic modification in a developing retinotectal system. Nature *429*, 878–883.

Egger, V., Feldmeyer, D., and Sakmann, B. (1999). Coincidence detection and changes of synaptic efficacy in spiny stellate neurons in rat barrel cortex. Nat. Neurosci. *2*, 1098–1105.

Engert, F., Tao, H.W., Zhang, L.I., and Poo, M. (2002). Moving visual stimuli rapidly induce direction sensitivity of developing tectal neurons. Nature *419*, 470–475.

Ernst, A.F., Jurney, W.M., and McLoon, S.C. (1998). Mechanisms involved in development of retinotectal connections: roles of Eph receptor tyrosine kinases, NMDA receptors and nitric oxide. Prog. Brain Res. *118*, 115–131.

Feldman, D.E. (2000). Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. Neuron *27*, 45–56.

Fregnac, Y., Burke, J.P., Smith, D., and Friedlander, M.J. (1994). Temporal covariance of pre and postsynaptic activity regulates functional connectivity in the visual cortex. J. Neurophysiol. *71*, 1403–1421.

Froemke, R.C., and Dan, Y. (2002). Spike-timing-dependent synaptic modification induced by natural spike trains. Nature *416*, 433–438.

Fu, Y.X., Djupsund, K., Gao, H., Hayden, B., Shen, K., and Dan, Y. (2002). Temporal specificity in the cortical plasticity of visual space representation. Science *296*, 1999–2003.

Goodhill, G.J. (1993). Topography and ocular dominance: a model exploring positive correlations. Biol. Cybern. *69*, 109–118.

Goodman, C.S., and Shatz, C.J. (1993). Developmental mechanisms that generate precise patterns of neuronal connectivity. Cell *72* Suppl., 77–98.

Heeger, D.J. (1993). Modeling simple-cell direction selectivity with normalized, half-squared, linear operators. J. Neurophysiol. *70*, 1885–1898.

Katz, L.C., and Shatz, C.J. (1996). Synaptic activity and the construction of cortical circuits. Science *274*, 1133–1138.

Linden, D.J., and Connor, J.A. (1995). Long-term synaptic depression. Annu. Rev. Neurosci. *18*, 319–357.

Malenka, R.C., and Bear, M.F. (2004). LTP and LTD: an embarrassment of riches. Neuron *44*, 5–21.

Markram, H., Lubke, J., Frotscher, M., and Sakmann, B. (1997). Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science *275*, 213–215.

Mcilwain, J.T. (1972). Central vision: visual cortex and superior colliculus. Annu. Rev. Physiol. *34*, 291–314.

Mehta, M.R., Quirk, M.C., and Wilson, M.A. (2000). Experience-dependent asymmetric shape of hippocampal receptive fields. Neuron *25*, 707–715.

Miller, K.D., Keller, J.B., and Stryker, M.P. (1989). Ocular dominance column development: analysis and simulation. Science *245*, 605–615.

Mize, R.R., and Lo, F. (2000). Nitric oxide, impulse activity, and neurotrophins in visual system development (1). Brain Res. *886*, 15–32.

Mize, R.R., Wu, H.H., Cork, R.J., and Scheiner, C.A. (1998). The role of nitric oxide in development of the patch-cluster system and retinocollicular pathways in the rodent superior colliculus. Prog. Brain Res. *118*, 133–152.

Nieuwkoop, P.D., and Faber, J. (1967). A Normal Table of Xenopus laevis (Amsterdam: North Holland).

Nishiyama, M., Hong, K., Mikoshiba, K., Poo, M.M., and Kato, K. (2000). Calcium stores regulate the polarity and input specificity of synaptic modification. Nature *408*, 584–588.

Poo, M.M. (2001). Neurotrophins as synaptic modulators. Nat. Rev. Neurosci. *2*, 24–32.

Rao, R.P., and Sejnowski, T.J. (2001). Predictive learning of temporal sequences in recurrent neocortical circuits. Novartis Found. Symp. *239*, 208–229.

Renteria, R.C., and Constantine-Paton, M. (1996). Exogenous nitric oxide causes collapse of retinal ganglion cell axonal growth cones in vitro. J. Neurobiol. *29*, 415–428.

Renteria, R.C., and Constantine-Paton, M. (1999). Nitric oxide in the retinotectal system: a signal but not a retrograde messenger during map refinement and segregation. J. Neurosci. *19*, 7066–7076.

Ruthazer, E.S., Akerman, C.J., and Cline, H.T. (2003). Control of axon branch dynamics by correlated activity in vivo. Science *301*, 66–70.

Schuett, S., Bonhoeffer, T., and Hubener, M. (2001). Pairing-induced changes of orientation maps in cat visual cortex. Neuron *32*, 325–337.

Shon, A.P., Rao, R.P., and Sejnowski, T.J. (2004). Motion detection and prediction through spike-timing dependent plasticity. Network *15*, 179–198.

Sin, W.C., Haas, K., Ruthazer, E.S., and Cline, H.T. (2002). Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases. Nature *419*, 475–480.

Song, S., Miller, K.D., and Abbott, L.F. (2000). Competitive Hebbian learning through spike-timing-dependent synaptic plasticity. Nat. Neurosci. *3*, 919–926.

Stent, G.S. (1973). A physiological mechanism for Hebb's postulate of learning. Proc. Natl. Acad. Sci. USA *70*, 997–1001.

Tao, H.W., and Poo, M. (2005). Activity-dependent matching of excitatory and inhibitory inputs during refinement of visual receptive fields. Neuron *45*, 829–836.

van Rossum, M.C., Bi, G.Q., and Turrigiano, G.G. (2000). Stable Hebbian learning from spike timing-dependent plasticity. J. Neurosci. *20*, 8812–8821.

Wenisch, O.G., Noll, J., and Hemmen, J.L. (2005). Spontaneously emerging direction selectivity maps in visual cortex through STDP. Biol. Cybern. *93*, 239–247.

Wiesel, T.N., and Hubel, D.H. (1963). Single-cell responses in striate cortex of kittens deprived of vision in one eye. J. Neurophysiol. *26*, 1003–1017.

Willshaw, D.J., and von der Malsburg, C. (1976). How patterned neural connections can be set up by self-organization. Proc. R. Soc. Lond. B. Biol. Sci. *194*, 431–445.

Wu, H.H., Williams, C.V., and McLoon, S.C. (1994). Involvement of nitric oxide in the elimination of a transient retinotectal projection in development. Science *265*, 1593–1596.

Yao, H., and Dan, Y. (2001). Stimulus timing-dependent plasticity in cortical processing of orientation. Neuron *32*, 315–323.

Zhang, L.I., and Poo, M. (2001). Electrical activity and development of neural circuits. Nat. Neurosci. *4* Suppl., 1207–1214.

Zhang, L.I., Tao, H.W., Holt, C.E., Harris, W.A., and Poo, M. (1998). A critical window for cooperation and competition among developing retinotectal synapses. Nature *395*, 37–44.

Zhang, L.I., Tao, H.W., and Poo, M. (2000). Visual input induces long-term potentiation of developing retinotectal synapses. Nat. Neurosci. *3*, 708–715.

Zhou, Q., Tao, H.W., and Poo, M. (2003). Reversal and stabilization of synaptic modifications in a developing visual system. Science *300*, 1953–1957.