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Activity-Dependent Transcription of BDNF Enhances Visual Acuity during Development

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SUMMARY

In the developing *Xenopus* tadpole, conditioning with 20 min of visual stimulation leads to increased proBDNF protein levels in the tectum measured 4 hr later. Following conditioning, the ability to induce direction selectivity in tectal neurons, as well as both retinotectal long-term potentiation and depression, thought to underlie this phenomenon, was strongly facilitated. This facilitation was blocked by knockdown of BDNF expression in tectal neurons. Animals that had been exposed to visual conditioning and subsequently received normal visual input for 7–11 hr exhibited higher spatial frequency thresholds of tectal cell responses to counterphasing gratings than nonconditioned control animals. An improvement in visual acuity was confirmed by enhanced sensitivity to counterphasing gratings in a behavioral test. These results indicate that brief sensory stimulation, by initiating nuclear transcription and de novo protein synthesis of BDNF, can facilitate the refinement of response properties in the developing visual system.

INTRODUCTION

Developing neural circuits adapt to their environments through a process of activity-dependent refinement, in which sensory inputs contribute to the concurrent strengthening of appropriate synapses and weakening of inappropriate synapses (Fox et al., 2010; Maurer and Lewis, 2001). This developmental process of synapse selection is believed to utilize plasticity mechanisms akin to long-term potentiation (LTP) and depression (LTD) (Feldman and Knudsen, 1998; Katz and Shatz, 1996; Zhang and Poo, 2001). In addition to its continual participation in the process of developmental refinement, synaptic plasticity also occurs in response to strong or salient environmental stimuli (Engert et al., 2002; Feldman, 2009; Li et al., 2008; Malenka and Bear, 2004; Smith et al., 2009). Plasticity-inducing stimuli can further initiate the production of different neuromodulators, including neurotrophins. In turn, plasticity mechanisms are themselves subject to regulation by neurotrophins (Cohen and Greenberg, 2008; Lu et al., 2008; Poo, 2001). Thus, gene products synthesized in response to a strong or salient, brief stimulus can play a dual role by directly inducing changes related to that stimulus, and by modulating the ongoing process of circuit refinement.

The neurotrophin brain derived neurotrophic factor (BDNF) can be synthesized in an activity-dependent manner primarily through regulation of the BDNF exon IV promoter (Greenberg et al., 2009). Its immature form proBDNF has been shown to play a role in LTD, through activation of the p75 neurotrophin receptor (Rosch et al., 2005; Woo et al., 2005). It is believed that proBDNF can either be cleaved intracellularly to form the mature protein mBDNF, or it can be cleaved in response to LTP inducing stimuli extracellularly through tissue plasminogen activator (tPA) mediated activation of plasmin. Upon cleavage, mBDNF plays a role in LTP through activation of the TrkB receptor (An et al., 2008; Barker, 2009; Lessmann and Brigadski, 2009; Nagappan et al., 2009; Pang et al., 2004). Thus, proBDNF and mBDNF are both regulated by activity, but are thought to regulate LTD and LTP, respectively. Therefore, as circuit refinement is a process of concurrently strengthening appropriate synapses and weakening inappropriate synapses, BDNF synthesis is positioned to regulate both arms of this process and improve the functional characteristics of the circuit.

To test if upregulation of BDNF synthesis in response to an acute visual stimulus facilitates ongoing synaptic plasticity and functional refinement during development, we used the developing visual system of the *Xenopus* tadpole. Previously, we observed in this system that visual conditioning upregulated the activity of the transcriptional regulator Nuclear Factor of Activated T cells (NFAT), and that inhibition of NFAT led to a decrease in the levels of BDNF transcript (Schwartz et al., 2009). In this study we observed that visual conditioning upregulated levels of proBDNF protein. As a consequence of BDNF upregulation by visual stimulation, retinotectal LTP, LTD, and plasticity of stimulus direction selectivity were all facilitated. We further examined whether ongoing functional refinement was affected, by visually conditioning animals to induce the upregulation in BDNF levels, and then returning them to their rearing environment to continue to receive normal sensory input. Interestingly, we found that visual acuity was improved in conditioned animals compared to controls. As acuity is a measure of visual system function (Maurer et al., 1999; Sale et al., 2009), these results imply that elevated neurotrophin levels induced by earlier visual conditioning facilitated subsequent functional circuit refinement.

RESULTS

Repeated Visual Stimulation Upregulates Activity of the BDNF Exon IV Promoter and Expression of proBDNF Protein in the Optic Tectum

BDNF is transcribed in response to neuronal activity primarily through regulation of the BDNF exon IV promoter (Greenberg

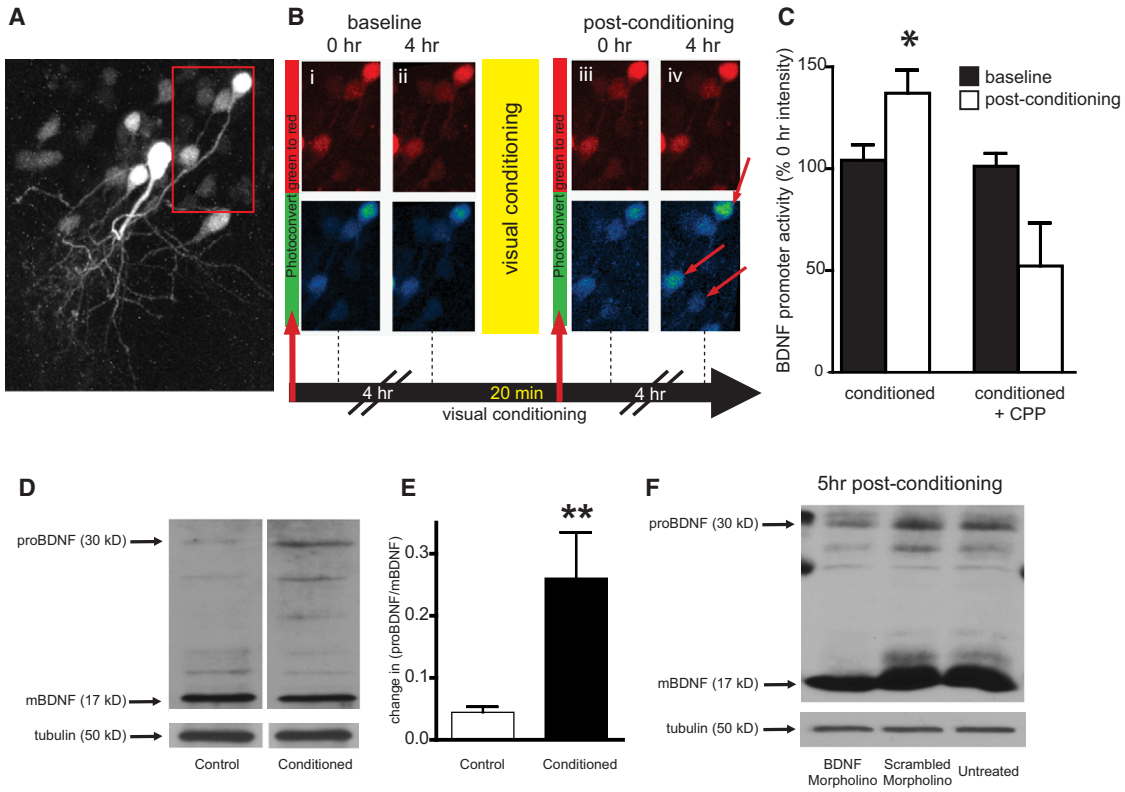

Figure 1. ProBDNF Levels Are Upregulated 4 hr after a Conditioning Visual Stimulus

(A–C) Visual stimulation activates the BDNF exon IV promoter.

(A) Maximum intensity two-photon projection of tectal neurons in vivo, electroporated with a plasmid containing 1500 bp of the BDNF exon IV promoter driving Kaede. Box indicates region of interest in (B).

(B) Timeline with example of average intensity projections of Kaede fluorescence through 10 μm depth. Images 4 hr after the first photoconversion reflect baseline Kaede synthesis. These were compared to images collected 4 hr after visual conditioning and a second photoconversion. Arrows show cells with higher promoter activity after conditioning. Top: red (converted Kaede); bottom: green (newly synthesized and residual unconverted Kaede).

(C) Kaede produced 4 hr after conditioning compared with the 4 hr baseline period. Visual conditioning upregulates the activity of the BDNF exon IV promoter, which is blocked in the NMDAr antagonist CPP (*$p < 0.5$).

(D and E) The ratio of proBDNF to mBDNF protein is increased 4 hr after conditioning (**$p < 0.01$).

(F) MO knockdown of BDNF blocks the increase in proBDNF protein produced in response to conditioning.

et al., 2009). Thus, to determine if a brief period of intensive visual stimulation could regulate the activity of this promoter, neurons in the optic tectum, the principal visual nucleus in the *Xenopus* brain, were electroporated with a pGL3 basic plasmid in which a 1500 bp fragment of the BDNF exon IV promoter was inserted to drive expression of the green-red photoconvertible fluorescent protein Kaede. In nonconditioned animals, basal levels of promoter activity produced sufficient Kaede protein to allow visualization of the tectal cell somata by two-photon microscopy (Figure 1A). To determine the effect of visual conditioning on promoter activity, the amount of Kaede produced in the 4 hr after exposing animals to a low-frequency simulated motion sequence was compared to the amount produced in the 4 hr before conditioning (Figures 1B and 1C). A similar visual stimulation paradigm has been shown to activate the transcriptional regulator NFAT through the activation of N-methyl D-aspartate type glutamate receptors (NMDAr), as well as to induce NMDAr-mediated changes in dendritic growth (Schwartz et al., 2009; Sin et al., 2002). De novo protein synthesis was assessed by photoconverting the Kaede to red at the beginning of each 4 hr period and then quantifying the change in green fluorescence produced by newly synthesized Kaede by the end of the period. Average projections of a two-photon z-series through a fixed volume of tissue were used for quantification as described previously (Schwartz et al., 2009). In the 4 hr following 20 min of visual conditioning, fluorescence from new Kaede protein increased (137% ± 11.7%, n = 14) to a greater degree than during the 4 hr baseline period (104.8% ± 8.0%) preceding conditioning (Figure 1C; $p < 0.05$). Incubating animals in the blood-brain barrier permeant NMDAr antagonist 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP, 20 μM) starting 25–30 min before conditioning, prevented the increase in Kaede produced during the 4 hr period after conditioning (52.2% ± 21% n = 6) despite baseline levels (101.2% ± 6.3%) similar to the untreated animals. These results demonstrate that 20 min of visual conditioning is sufficient to increase transcription under control of the BDNF exon IV promoter in an NMDAr-dependent manner in tectal neurons in the intact animal (Figures 1B and 1C).

Next, we tested whether this enhanced BDNF exon IV promoter activity led to a change in BDNF protein levels in the tectum. At 5 hr after visual conditioning, midbrains including the optic tectum, were surgically isolated and homogenized for western blotting. Blots were probed with an antibody that recognizes both the immature and mature forms of BDNF. Visual conditioning led to an increase in the ratio of proBDNF to mBDNF (control: 0.04 ± 0.01, conditioned: 0.26 ± 0.04; Figures 1D and 1E, n = 3 repeats, 5 animals per condition for each experiment). Because the antibody gave several bands, we confirmed the identity of the BDNF bands by introducing a BDNF antisense Morpholino (BDNF MO) oligonucleotide, fluorescently tagged with lissamine rhodamine. At 5 hr postconditioning, brains that had been previously electroporated with the BDNF MO showed reduced expression of proBDNF compared with brains electroporated with a scrambled MO or conditioned animals without MO treatment (Figure 1F, n = 2 experiments, 4-5 animals per experiment). As a retrograde spread of plasticity from the tectum to the eye has been reported (Du et al., 2009), we also assayed proBDNF levels in the eyes of conditioned animals. However, conditioning did not induce a detectable change in proBDNF levels in the eye (Figure S1 available online). Thus, the activation of the BDNF exon IV promoter by visual conditioning resulted in increased proBDNF protein levels in the tectum.

Enhanced Levels of proBDNF Facilitate Visual System Plasticity

The activity-dependent regulation of BDNF levels is significant, as BDNF has been reported to modulate the susceptibility of synapses to undergo plasticity. In the hippocampus, proBDNF has been shown to facilitate LTD and in *Xenopus* mBDNF is thought to be required for retinotectal LTP (Du et al., 2009; Mu and Poo, 2006; Woo et al., 2005). To determine if the proBDNF synthesized in response to visual conditioning affected retinotectal plasticity, we first examined the effects of visual conditioning in a plasticity protocol designed to enhance stimulus direction sensitivity of tectal neurons, believed to engage both LTP and LTD at tectal cell synapses (Engert et al., 2002; Mu and Poo, 2006; Zhou et al., 2003).

To increase proBDNF levels, animals were visually conditioned and then returned to their normal visual environments. At 4–6 hr postconditioning, animals received three bouts of training with a moving bar projected onto the retina. The training bouts were delivered at 4 min intervals. This spaced training protocol is designed to induce direction selectivity in tectal neurons as previously described (Engert et al., 2002; Zhou et al., 2003). (n.b., throughout this article, the term "conditioning" refers to the visual stimulation used to upregulate BDNF expression whereas the term "training" refers to the visual stimulation protocol used to rapidly shift receptive field properties.) Thirty minutes to two hours after training, the compound synaptic current (CSC) elicited by a bar moving in each of the four cardinal directions was measured in whole cell voltage clamp recordings of tectal neurons. The response to each direction was normalized to the average response across all four directions. A schematic of the experimental timeline is shown in Figure 2A. Cells from conditioned animals (n = 14) developed a significant preference for the bar moving in the trained direction (untrained: 91.6% ± 7.4% versus trained: 143% ± 18.74%). On the other hand, cells from the group that had not been conditioned (untrained: 100.7% ± 6.6% versus trained: 109% ± 12.9%, n = 12), or conditioned cells with BDNF MO knockdown (untrained: 100.5% ± 8.2% versus trained: 95.9% ± 10.7%, n = 11) did not exhibit significant direction training for the entire population of neurons studied (Figures 2B and 2C). The slight increase in sensitivity to the trained direction observed in the nonconditioned group is comparable to that previously reported by Zhou et al. (2003). In that study, an approximate 25% change was observed in 12 out of 25 cells. There was no significant difference between cells from animals that had not been electroporated (n = 8) and those that had been electroporated with the scrambled MO (n = 4). These groups were therefore combined. These results suggest that the upregulation of proBDNF induced by prior visual conditioning facilitated a change in direction sensitivity in tectal neurons. As plasticity of direction sensitivity in these neurons is thought to involve the induction of LTD and LTP (Mu and Poo, 2006), we next examined how conditioning may have impacted retinotectal synaptic plasticity.

Although spike-timing-dependent LTP and LTD have been proposed as possible mechanisms underlying the induction of direction selectivity at the retinotectal synapse (Engert et al., 2002; Mu and Poo, 2006; Vislay-Meltzer et al., 2006), we instead used a synaptic pairing protocol (holding −35 mV, 300 pulses at 1 Hz) to induce LTD in this study. This protocol was selected because the sensitivity of the retinotectal synapse to spike-timing protocols has been shown to be greatly reduced by the stage of development used in this study (Tsui et al., 2010). In nonconditioned animals, pairing depolarization of the tectal neuron with repeated electrical stimulation at the optic chiasm induced a transient depression of retinotectal α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type glutamatergic excitatory postsynaptic current (EPSC) amplitudes that recovered (100.6% ± 5.8% of baseline before induction) around 20 min after stimulation (Figures 3A and 3B). In striking contrast, this protocol induced a robust LTD in animals that had earlier undergone visual conditioning (44% ± 10.6%, $p < 0.05$ versus nonconditioned) (Figure 3A–3Bi). The induced depression was stable for as long as recordings were made, up to 1 hr after induction. In animals that had been bathed in the transcription inhibitor actinomycin D (50 μM) for 90 min, starting 30 min before conditioning, the facilitation of LTD did not occur (105% ± 10.5%, $p < 0.01$ versus conditioned), suggesting that gene transcription initiated by the conditioning stimulus mediated this facilitation of LTD (Figure 3Bii).

Activation of the p75-neurotrophin receptor by proBDNF has been reported to facilitate synaptic LTD in area CA1 of mouse hippocampus (Woo et al., 2005). Several lines of evidence suggested that transcription leading to proBDNF synthesis following visual conditioning might also underlie the facilitation of retinotectal LTD that we observed. Conditioning failed to facilitate LTD in cells in which proBDNF expression had been knocked down by BDNF MO electroporation (85% ± 10.2%, $p < 0.05$ versus conditioned; Figures 3A and 3Biii). Furthermore, we found that inhibition of the p75-neurotrophin receptor by applying the

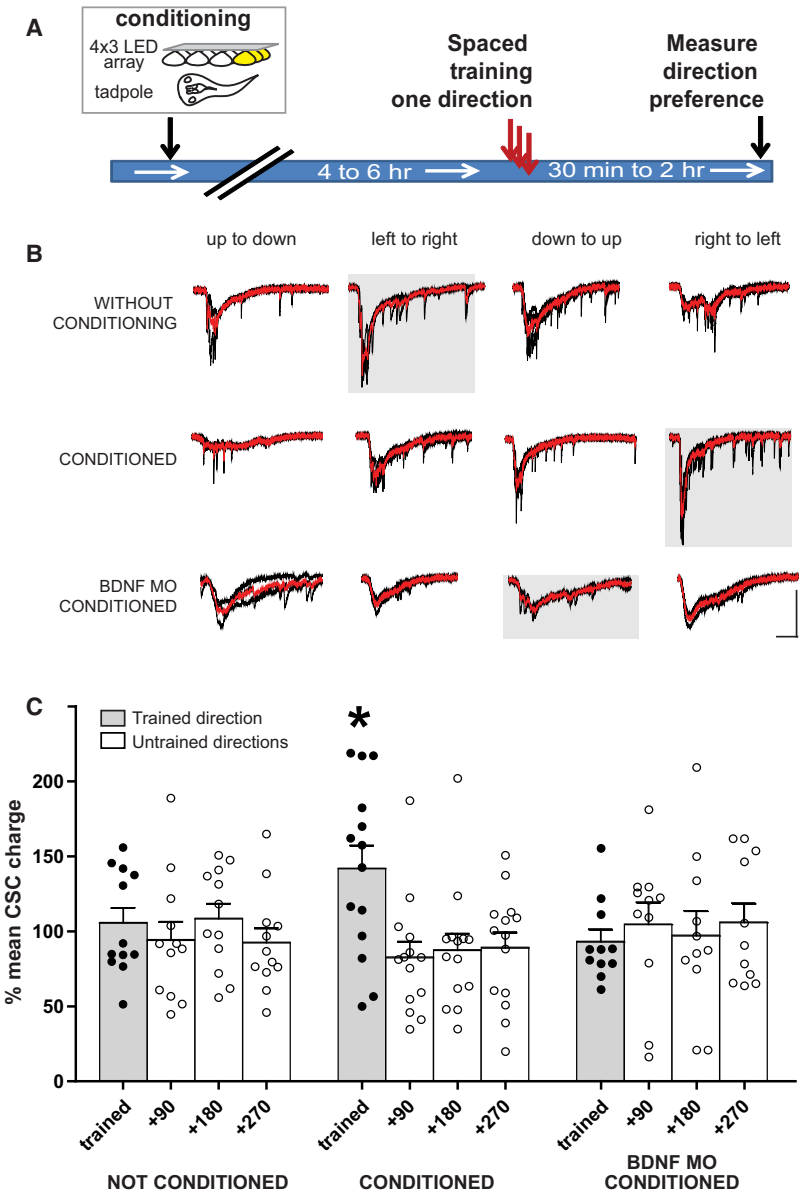

Figure 2. Visual Conditioning Makes Tectal Neurons More Susceptible to Direction Training

(A) Timeline of experiment: Animals were conditioned and returned to their rearing bowls. After 4–6 hr, tadpoles were trained using a spaced protocol by repeatedly moving a bar across the retina in the same direction. Thirty minutes to two hours after training, the compound synaptic current (CSC) elicited by bars moving in each of the four cardinal directions was measured.

(B) Gray traces are representative examples of CSCs elicited by a bar moving in each of the four listed directions. Red traces are the average of the underlying gray traces. Gray boxes indicate the trained direction in each example (scale 75 pA, 100 ms).

(C) Cells from animals trained 4–6 hr after conditioning exhibit a larger response to the trained direction. This effect was blocked by MO knockdown of BDNF (*$p < 0.05$).

has a well-established role in the modulation of synaptic transmission and plasticity in many systems, including the retinotectal synapse in *Xenopus* (Du and Poo, 2004; Mu and Poo, 2006). We therefore tested the effects of visual conditioning on retinotectal LTP.

We examined a number of different pairing protocols to induce a weak LTP at retinotectal synapses that might be sensitive to modulation by BDNF. We found that while three bursts of 40 pulses at 10 Hz, holding the cell at −12 mV, induced only a transient synaptic facilitation (Figure S3), two spaced repetitions of this protocol resulted in a modest, but stable increase of the EPSC amplitude to 128% ± 6.5% of baseline in animals that had not undergone visual conditioning. This spaced pairing protocol was therefore used for subsequent LTP experiments. In animals that had been conditioned, this small potentiation was facilitated, resulting in a stable increase to 204% ± 8.5% baseline amplitude ($p < 0.05$ versus nonconditioned) (Figure 4A–4Bi). Next we blocked tPA activity to determine if extracellular cleavage of proBDNF to mBDNF was required for the visually induced facilitation. Bath application of the inhibitor tPA-stop blocked LTP induction (80% ± 7.1%, $p < 0.01$ versus conditioned) (Figure 4Bii). As tPA can be involved in cascades other than the cleavage of proBDNF, we tested LTP induction in tectal cells in which BDNF expression had been knocked down by BDNF MO electroporation. Knockdown of BDNF prevented the facilitation induced by conditioning (125% ± 5.3%, $p < 0.05$ versus conditioned; Figure 4Biii). In contrast, electroporation of a control scrambled MO ($n = 3$) did not interfere with facilitation of LTP by visual conditioning, resulting in a potentiation that was indistinguishable from that observed in untreated, conditioned animals ($n = 6$). These groups were therefore combined. These findings imply that proBDNF synthesized in response to visual conditioning may be cleaved

REX function-blocking antibody (Mischel et al., 2001) also prevented facilitation of LTD (92% ± 19.8%, $p < 0.05$ versus conditioned Figure 3Biv). In contrast, conditioned animals treated with preimmune serum exhibited normal LTD (71.7% ± 9.8%) (Figure S2). Application of exogenous proBDNF (2 ng/ml), together with tPA-stop, an inhibitor of tissue plasminogen activator (tPA), to prevent its rapid breakdown to mBDNF, produced no detectable changes in baseline synaptic transmission (Figure 3Bv), but mimicked the effects of visual conditioning on LTD induction (56% ± 5%, $p < 0.05$ versus nonconditioned) (Figure 3Bvi). These results suggest that the increased levels of proBDNF protein that resulted from earlier visual conditioning facilitated induction of LTD at the retinotectal synapse.

ProBDNF can be cleaved to mBDNF intracellularly by various convertases or extracellularly by plasmin (Barker, 2009). mBDNF

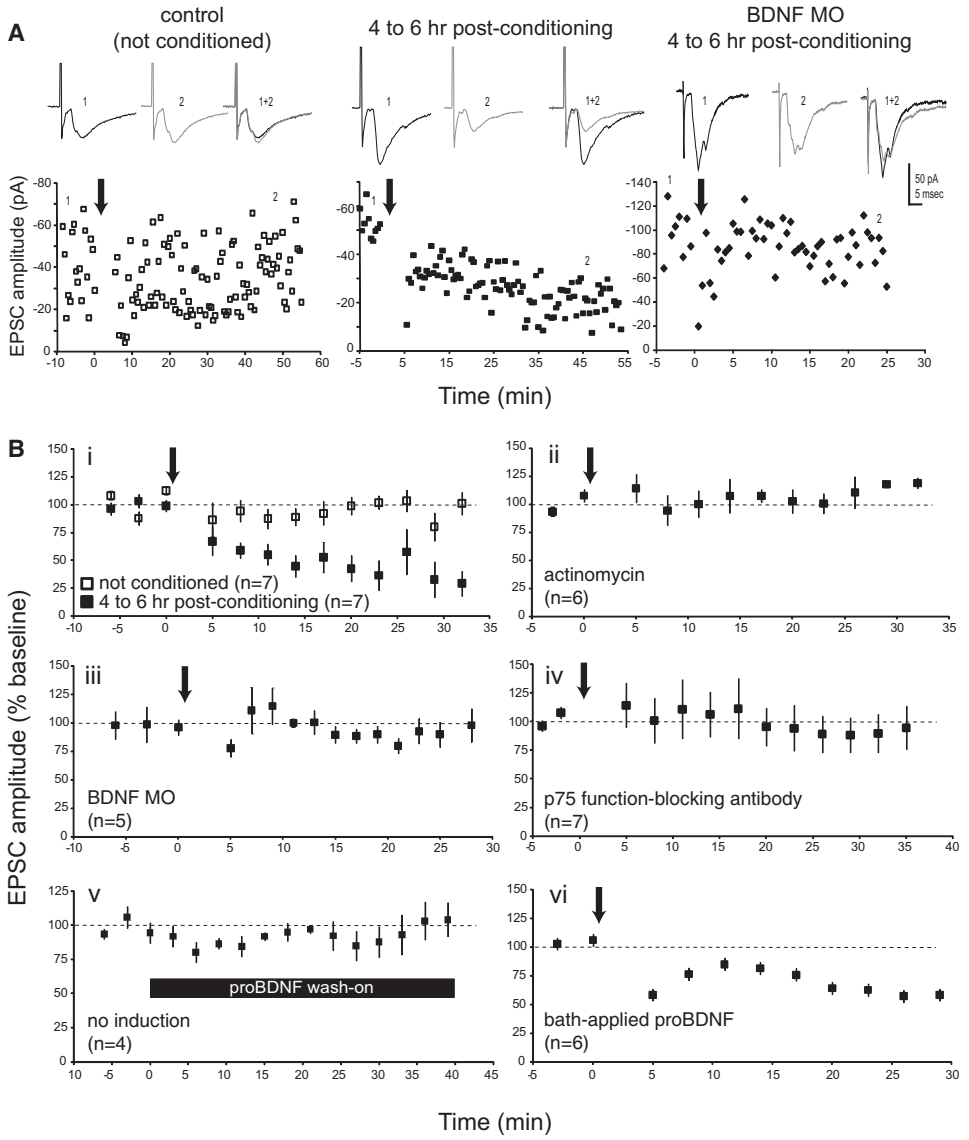

Figure 3. Visual Conditioning Facilitates Retinotectal LTD by Increasing proBDNF Synthesis

(A) Representative examples of LTD experiments in control, visually conditioned, and BDNF MO-electroporated conditioned tadpoles. EPSCs recorded at −70 mV in response to electrical stimulation at the optic chiasm (ten events averaged), before (1) and after (2) LTD induction. Only the conditioned animal shows LTD in response to a weak induction protocol.

(B) Averaged EPSC amplitudes as a percentage of baseline in all animals. (i) LTD induction is facilitated in animals 4–6 hr after visual conditioning. Facilitation of LTD by conditioning is blocked by (ii) treatment with the transcriptional inhibitor actinomycin D during and after conditioning, (iii) knockdown of BDNF expression by MO electroporation into tectal neurons prior to conditioning, and (iv) application of a p75-ntr function-blocking antibody during recording. (v) Evoked EPSC amplitude is not modulated by wash-on (black bar) of exogenous proBDNF, but (vi) preincubation of animals in exogenous proBDNF for 30 min before induction facilitates LTD. Arrows indicate onset of LTD induction protocol.

in a tPA-dependent manner in response to the LTP protocol, and that the resulting production of mBDNF facilitates LTP. Activation of the TrkB receptor tyrosine kinase is the main pathway by which mBDNF initiates downstream signaling. Inhibition of TrkB signaling with the receptor tyrosine kinase inhibitor K252a entirely blocked LTP induction in conditioned animals (97% ± 3.8%, $p < 0.05$ versus no drug; Figure 4iv) in agreement with previous reports (Du et al., 2009; Mu and Poo, 2006).

Together, our findings demonstrate that the BDNF synthesized in response to 20 min of robust visual conditioning, can facilitate bidirectional plasticity at the retinotectal synapse hours later. As developmental circuit refinement is thought to rely upon environmentally driven strengthening of appropriate, and weakening of inappropriate, synapses through mechanisms like LTP and LTD (Katz and Shatz, 1996; Zhang and Poo, 2001), we next tested whether visual conditioning might facilitate the ongoing process of circuit refinement.

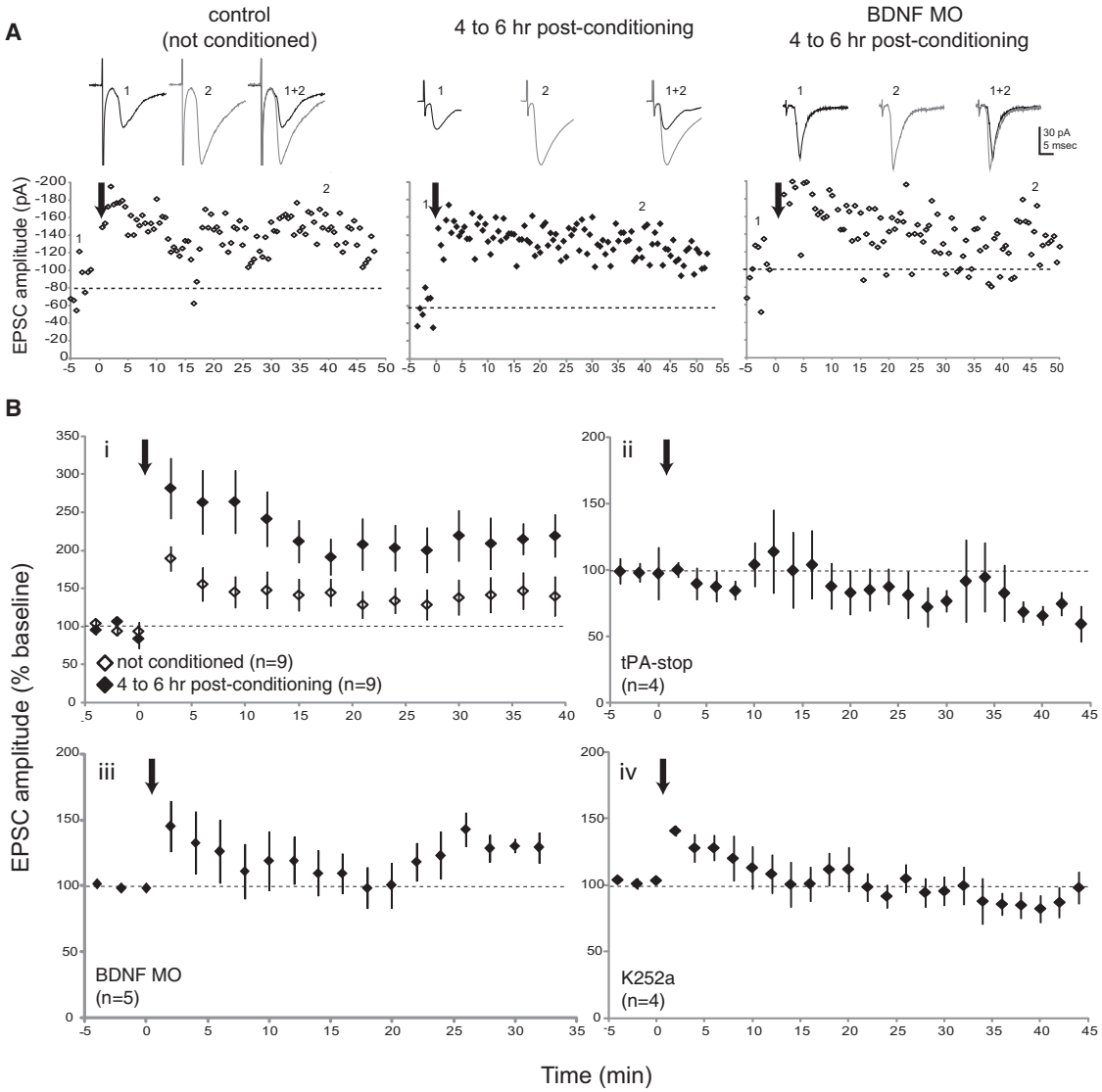

Figure 4. Visual Conditioning Facilitates Retinotectal LTP through a BDNF-Dependent Mechanism

(A) Representative examples of LTP experiments in control, visually conditioned, and BDNF MO-electroporated conditioned tadpoles. EPSCs recorded at −70 mV in response to electrical stimulation at the optic chiasm (ten events averaged), before (1) and after (2) LTP induction.

(B) Averaged EPSC amplitudes as a percentage of baseline in all animals. (i) LTP induced in animals 4–6 hr following visual conditioning was greater than that induced in nonconditioned controls. Facilitation of LTP in conditioned animals was blocked by (ii) inhibition of tPA, (iii) MO knockdown of BDNF production in tectal neurons, and (iii) inhibition of TrkB receptor tyrosine kinase activity by K252a. Arrows indicate onset of LTP induction protocol.

Animals Exhibit Improved Visual Acuity 7–11 hr after Conditioning

Visual acuity is a measure of the ability to resolve spatial details. One method for measuring acuity in humans is the Teller acuity test (Dobson and Teller, 1978), in which preverbal infants will preferentially look at a grating that they can resolve, compared to either a gray screen of comparable luminance or a higher spatial frequency grating that they cannot resolve. Furthermore, cortical responses to gratings of different sizes determined by measuring transcranial visually evoked potentials can be extrapolated to determine a subject's acuity thresholds, with comparable results to the behavioral tests (Campbell and Maffei, 1970; Good, 2001).

To determine if proBDNF produced by visual conditioning participates in the ongoing process of circuit refinement, we subjected tadpoles to visual conditioning and then returned them to their normal rearing environment for 7–11 hr. This period permitted at least 3–7 hr of visual experience during the time of enhanced LTD and LTP, as determined above. We measured CSC responses of tectal cells to full-field flash stimuli at holding potentials of −70 mV and +40 mV. Recordings at −70 mV predominantly show AMPAr currents and

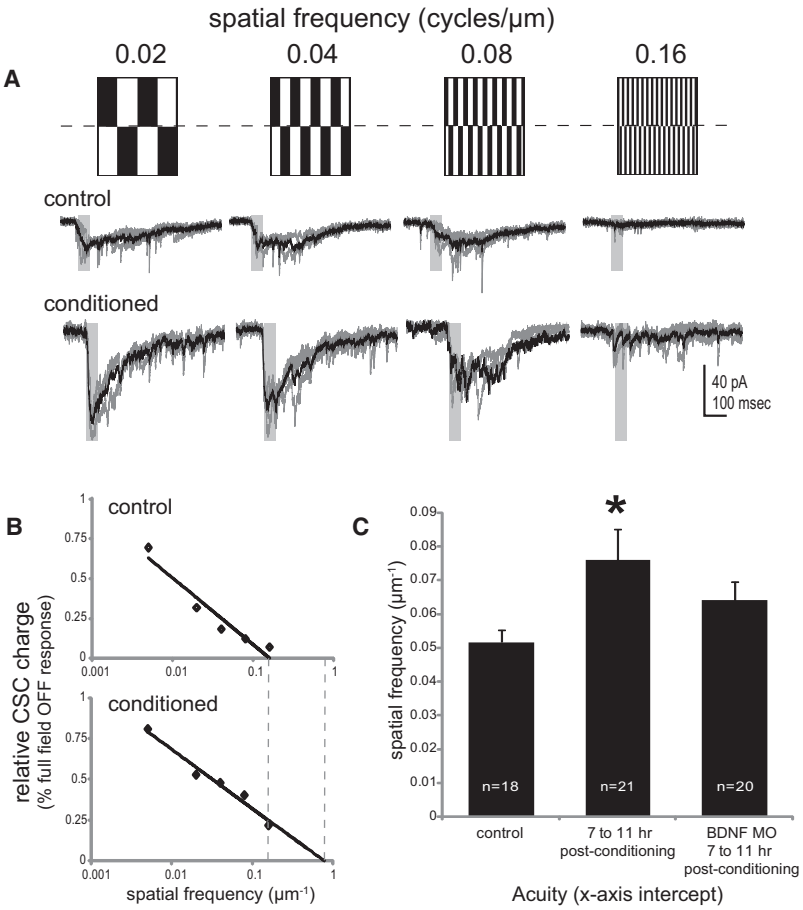

Figure 5. Tectal Neurons Exhibit a BDNF-Dependent Improvement in Visual Spatial Frequency Sensitivity 7–11 hr after Conditioning

(A) Examples of different spatial frequency gratings projected onto the retina (top). Under the dashed line is the corresponding counterphased grating. Below the gratings are representative examples of AMPAr mediated synaptic currents elicited by counterphasing the grating images. Black lines are the average of three to five individual traces (gray lines). Gray boxes indicate the period analyzed.

(B) Examples of linear regressions of total CSC charge evoked by presentation of counterphasing gratings over a range of different spatial frequencies, normalized to full-screen OFF response for each cell.

(C) Spatial frequency thresholds (x-intercepts) were extrapolated from linear regression of plots from multiple neurons from control, conditioned and BDNF MO treated conditioned animals. Visual acuity of tectal neurons from animals that had been visually conditioned 7–11 hr prior was enhanced (*$p < 0.05$, ANOVA with Dunnett post-test). This enhancement was prevented in neurons BDNF MO loaded neurons.

recordings at +40 mV are dominated by long-lasting NMDAr currents. The recording pipette included CsF in the internal solution to inhibit chloride flux through GABA-A receptors without inducing epileptiform activity, as can occur when GABA antagonists are applied in the bath (Marchionni and Maccaferri, 2009) (Figure S4A). The visually evoked responses consist of a mixure of early monosynaptic inputs from the retina and polysynaptic inputs from local tectal connections. A higher AMPA/NMDA ratio has been shown to correlate with synapse maturity and synaptic potentiation, as new AMPArs are trafficked to immature NMDAr-only silent synapses (Wu et al., 1996). Interestingly, the AMPA/NMDA ratio of responses to full-field OFF stimuli, but not ON stimuli, was greater in conditioned animals (0.85 ± 0.23) compared to nonconditioned controls (0.35 ± 0.23; $p < 0.05$). This increase in AMPA/NMDA ratio was prevented by MO knockdown of BDNF (0.48 ± 0.13) (Figures S4B and S4C). There was no significant difference in AMPA/NMDA ratios of cells from untreated animals and those electroporated with the scrambled MO. These respective groups were therefore combined. Tectal cells receive three classes of retinal input, namely ON, OFF, and ON/OFF (Edwards and Cline, 1999). Thus, the selective change in the OFF ratio, suggests that only specific inputs were affected. A possible reason for this selectivity is that OFF responses are generally larger in tectal cells, and therefore these synapses may have been more robustly activated (Figure S4B) (Zhang et al., 2000). These findings indicate that by 7–11 hr after conditioning, a BDNF-dependent change in glutamatergic transmission could be detected among tectal cells consistent with synaptic plasticity having occurred in the developing retinotectal system in response to ambient visual input.

To determine whether the synaptic changes might have contributed to an improvement in stimulus sensitivity by the visual system, we measured the responses of tectal cells to counterphasing square wave gratings of various spatial frequencies focused through the microscope objective directly onto the contralateral retina with its lens removed. Tectal cells predominantly responded in a graded fashion to gratings of increasing spatial frequency (Figure 5A), with a full-field OFF stimulus eliciting the largest CSC in 18 of 20 cells from controls, in 21 of 21 cells from the conditioned group, and in 20 of 21 cells from the BDNF MO group. Responses were analyzed only from these cells, which permitted us to normalize all other responses to the robust full-field OFF response for each cell. The sensitivity threshold for each cell was calculated as the x-intercept value from a linear extrapolation in log spatial frequency versus response plots (Figure 5B). Cells from animals that had been conditioned and returned to their normal rearing environment for 7–11 hr had higher spatial frequency thresholds than nonconditioned control, or conditioned cells with MO knockdown of BDNF (Figure 5C) ($p < 0.05$, conditioned: 0.076 ± 0.009 cycles μm^{-1}, nonconditioned: 0.052 ± 0.003 cycles μm^{-1}, conditioned BDNF MO: 0.064 ± 0.005 cycles μm^{-1}). Thus, normal visual experience during the time following conditioning, when plasticity was facilitated, led to a BDNF-dependent improvement in the spatial resolution thresholds of tectal responses to visual stimuli.

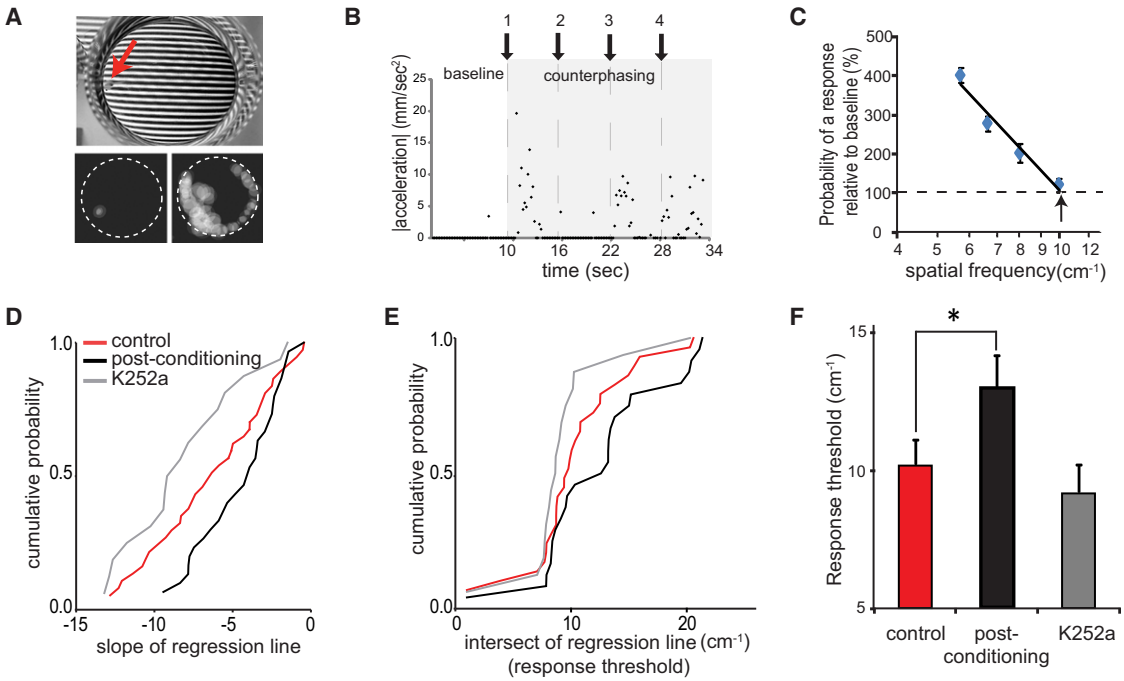

Figure 6. Conditioning Improves Response Thresholds in a Visual Task in Unrestrained Tadpoles

(A) Picture of a tadpole (arrow) in a single well of a six-well dish positioned over a display presenting a sine wave grating (top). In the bottom panels, white dots plot the positions of the tadpole during the stable 10 s baseline period before (left) and the 10 s after the onset of counterphasing (right).

(B) Example plot of absolute acceleration relative to the onset of counterphasing. Counterphasing of gratings increases the number of rapid changes in acceleration that tadpoles exhibit.

(C–E) The relative probabilities of eliciting a change in acceleration in response to four counterphases are plotted as a semilog function of the spatial frequencies of the gratings tested for a representative animal. Linear regression lines for the individual tadpoles were analyzed, and (D) the slopes of the regression lines and (E) response thresholds (extrapolated intercept with y = 100%, the mean probability of observing a response during baseline; e.g., arrow in C) were calculated.

(F) A shift to higher spatial frequency response thresholds was observed in animals 7–9 hr after visual conditioning. This improvement was prevented in animals treated with K252a during the period following conditioning when plasticity would have been facilitated.

Behavioral Consequences of Improved Visual Acuity

Previous behavioral experiments in *Xenopus* have shown that as tectal circuitry matures, animals develop improved visual avoidance behaviors (Dong et al., 2009). It has also been reported that the kinematics of *Xenopus* tadpoles has evolved such that they are better adapted to bursts of rapid maneuvering, rather than to sustained high-speed swimming (Wassersug, 1989). For example, increments in the intensity of thermal stimuli elicit more frequent but briefer bouts of swimming by tadpoles (Sillar and Robertson, 2009). Thus, it is anticipated that more salient stimuli will elicit more erratic swimming dominated by frequent changes in acceleration.

To test if the improved spatial sensitivity of tectal responses affected the behavioral response of the animals to visual stimuli, we measured the responses of freely swimming tadpoles to counterphasing gratings. One animal was placed into each well of a 6-well dish mounted above a video monitor. Swimming behavior in response to the onset of counterphasing of sine wave gratings of different spatial frequencies was then monitored by video and acceleration in swimming trajectories was calculated by measuring changes in tadpole position over time using ImageJ (Figure 6A). Tadpoles typically showed constant unidirectional motion or were stationary during the 10 s baseline period when gratings were present and stable, but counterphasing of the gratings every 6 s caused swimming patterns to become more erratic, reflected in higher rates of trajectory acceleration (Figures 6A and 6B).

A subset of the tadpoles responded to three or more of the spatial frequencies tested. In these animals, the probability of exhibiting rapid changes in trajectory in response to the counterphasing of a grating was inversely proportional to its spatial frequency. The probability of observing an acceleration shift was plotted against log spatial frequency to estimate the behavioral thresholds of nonconditioned tadpoles (n = 39 tadpoles of 64 tested) and tadpoles examined 7–9 hr after conditioning (n = 31 tadpoles of 59 tested) for this task as shown in Figure 6C. The behavioral response thresholds for each animal were calculated by extrapolating to the value where the log spatial frequency versus acceleration response line intersects with the baseline acceleration probability (100%). In addition the slopes of these linear regressions were also calculated as an indication of behavioral dynamic range (Figure 6D). Interestingly, animals that had been conditioned and returned to their normal rearing environment for 7–9 hr had thresholds at higher spatial frequencies (13.1 ± 1.14 cycles cm^{-1}) than nonconditioned controls (10.3 ± 0.8 cycles cm^{-1}, $p < 0.05$, Figures 6E and 6F).

To determine if the enhanced BDNF signaling resulting from visual conditioning played a role in this change, we injected K252a twice into the tectal ventricle at 3.5 and 4.5 hr after conditioning, corresponding to the period when we found facilitation of synaptic plasticity. Animals were then tested at 7–9 hr after conditioning. TrkB inhibition (n = 16), but not control vehicle injection (n = 12), prevented the improvement in spatial sensitivity produced by conditioning (K252a: 9.2 ± 1.0 cycles cm^{-1}; vehicle: 9.8 ± 1.11 cycles cm^{-1}) (Figure 6F).

The fact that only about half the tadpoles responded to three or more of the counterphasing gratings most likely reflects independent modulation of the behavioral output rather than low-order visual system differences between animals as the fall-off of visually evoked responses measured electrophysiologically in tectal neurons correlated well with spatial frequency in nearly all animals tested (Figure 5). Thus, the data show that the observed increase in tectal cell sensitivity to finer gratings can affect the visually-evoked behavior of the awake unrestrained animal in a BDNF-dependent manner. However, for the reasons mentioned above, this behavioral assay provides an estimate of the visual sensitivity of the animals rather than a measurement of absolute acuity.

To confirm that the observed change in swimming acceleration in response to visual stimuli involved retinotectal transmission, we thermally lesioned the optic tract just anterior to the optic tectum using the two-photon microscope with the infrared laser set at high intensity (~200 mW on the stage at 810 nm) (Figure S5). At 5 hr after lesioning, we performed the behavioral test. Although animals that had undergone optic tract lesions still exhibited normal startle responses to full-screen ON stimuli, their response to counterphasing gratings was dramatically impaired. This finding is in agreement with previous studies attributing the visual acuity of behavioral responses to sensory processing in the optic tectum (Yolen and Hodos, 1976). Taken together, our data demonstrate that BDNF signaling induced by visual conditioning is able to facilitate bidirectional retinotectal synaptic plasticity, resulting in a behaviorally significant improvement in the response thresholds of tectal neurons to visual stimuli.

DISCUSSION

We previously reported that a repeating visual stimulus was able to upregulate plasticity-related gene transcription in the *Xenopus* optic tectum (Schwartz et al., 2009). Here, we have extended this finding by showing that sensory stimulation-mediated activation of the BDNF exon IV promoter, and subsequent synthesis of proBDNF, can modulate synaptic plasticity in the developing visual system. These increased levels of proBDNF, as well as an accompanying enhancement of signaling downstream of mBDNF, did not appear to induce synaptic changes on their own, but rather facilitated ongoing plasticity mechanisms. Importantly, enhanced BDNF signaling contributed to a behaviorally detectable improvement in visual acuity. In summary, our findings reveal that the BDNF synthesized in response to 20 min of visual conditioning can facilitate bidirectional plasticity at the retinotectal synapse with direct behavioral consequences for the developing animal. A summary is presented in Figure 7.

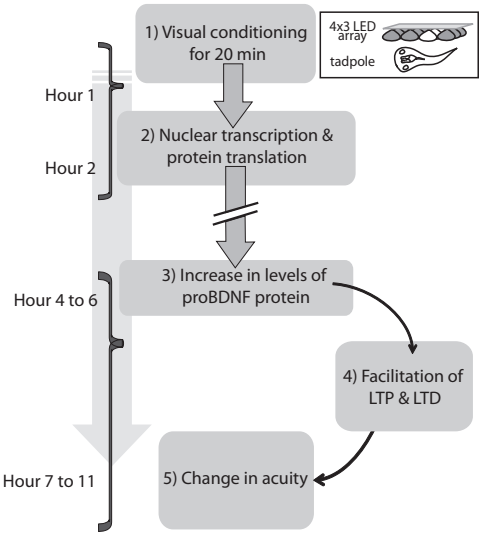

Figure 7. Summary of Experimental Design and Results
Animals were conditioned with a repeating visual stimulus (1). In response to conditioning, activity of the BDNF exon IV promoter is upregulated (2). Four to 6 hr later, proBDNF levels were increased in the tectum (3). In the presence of higher levels of proBDNF, bidirectional plasticity of tectal neurons was facilitated (4). Visual acuity was improved at 7–11 hr postconditioning in a BDNF-dependent manner (5).

BDNF Regulation of Synaptic Plasticity

Recent studies, carried out mainly in the CA1 area of mouse hippocampus, have revealed key roles for BDNF signaling and processing in synaptic LTP and LTD. Late-phase LTP (L-LTP) in CA1 is largely absent in transgenic mice lacking BDNF, and early-phase LTP is also substantially reduced in these animals (Korte et al., 1995; Patterson et al., 1996). Neurons are able to release both the precursor and mature forms of BDNF; however, the site of release may be a critical determinant of what form the released protein takes (Matsuda et al., 2009; Yang et al., 2009). As the protein synthesis machinery present in most dendrites lacks the Golgi-like organelles that process constitutively secreted proteins (Horton et al., 2005), it is likely that dendritically synthesized BDNF is secreted in its precursor form (An et al., 2008). Secreted proBDNF at synapses would then be cleaved to mBDNF by plasmin, activated from plasminogen by the activity of tPA, consistent with reports that tPA is also required for L-LTP (Pang et al., 2004). Our findings in the retinotectal system suggest a similar requirement for the synaptic release and cleavage of proBDNF, as acute inhibition of tPA activity reduced retinotectal LTP to the same degree as pharmacological inhibition of TrkB signaling. Furthermore, the knockdown of BDNF by MO antisense electroporation into tectal neurons reveals that BDNF from the postsynaptic cell is required for LTP.

On the other hand, the activation of the p75NTR by proBDNF has been reported to facilitate hippocampal LTD (Woo et al., 2005). Our retinotectal data confirmed the facilitation of LTD by recently synthesized proBDNF, and demonstrated that this could be mimicked by exogenous application of proBDNF if tPA activity is inhibited. In light of these findings, it is interesting to consider how the regulation of the rate of proBDNF cleavage

could regulate not only the efficacy but also the direction of synaptic plasticity (Nagappan et al., 2009). In contrast to these findings during development, inhibiting BDNF signaling in the mature visual cortex does not appear to affect plasticity, but rather reduces responsiveness to high-spatial frequency stimuli (Heimel et al., 2010). As the roles of BDNF appear to change during development, it will be important to determine if different mechanisms regulate the effects of BDNF on circuit development and circuit function.

Locus of Action of BDNF in the Retinotectal System

Application of exogenous BDNF to the optic tectum rapidly and profoundly impacts the retinotectal circuit. In vivo imaging of puncta of the synaptic vesicle protein GFP-synaptobrevin in *Xenopus* retinotectal axons has revealed a rapid increase in axonal branching and presynaptic punctum number within minutes to hours of BDNF application (Alsina et al., 2001; Hu et al., 2005). A BDNF-mediated increase in the number of PSD95-GFP positive postsynaptic specializations appears to occur subsequent to presynaptic changes, becoming evident only many hours after neurotrophin application (Sanchez et al., 2006). Functionally, a rapid increase in mEPSC frequency, but not amplitude, has been reported in response to application of BDNF to the tectum (Du and Poo, 2004). Our experimental protocol differed from these approaches in two important ways. First, the elevation of BDNF levels in our experiments relied on activity-dependent synthesis and release of endogenous protein rather than application of exogenous neurotrophin. Second, the BDNF-mediated changes that we described occurred only in response to specific LTP- and LTD-inducing electrical and visual stimulation protocols. Thus, the specific timing and location of neurotrophin delivery may determine its effects on the circuit. This is consistent with the report that the human BDNF val66met polymorphism which impairs dendritic trafficking and activity-dependent, but not constitutive secretion of BDNF results in abnormal hippocampal function (Egan et al., 2003).

While our own experiments do not distinguish between pre- and postsynaptic sites of action of the BDNF synthesized in response to visual conditioning stimuli, the efficacy with which MO knockdown of tectal BDNF synthesis fully prevented facilitation of both LTP and LTD clearly points to the postsynaptic cell as the source of newly synthesized BDNF. Retinotectal LTP experiments by Du and colleagues (2009) using MO antisense against TrkB targeted to presynaptic retinal or postsynaptic tectal neurons suggested that BDNF signaling onto both synaptic partners contributed to BDNF-dependent LTP expression. Quite remarkably, this same study also observed a retrograde change in synaptic transmission back in the retina within minutes of BDNF applied exclusively to the tectum. It is clear from our optic chiasm stimulation experiments that endogenous BDNF directly facilitated plasticity at the retinotectal synapse. While we cannot exclude the additional possibility that the newly synthesized BDNF may also have had retrograde effects in the retina that could have contributed to the refinement of visually evoked and behavioral responses that we observed, we did not detect changes in proBDNF levels in the retinae of tadpoles that had undergone visual conditioning (Figure S1).

Other Signaling Pathways Activated by Visual Conditioning

Visual stimulation broadly drives glutamatergic and GABAergic synaptic transmission in the tectum. Our experiments demonstrated a requirement for NMDAr activation for upregulation of BDNF synthesis in tectal neurons, but does not exclude additional roles for other neurotransmitter receptor types. Because postsynaptic depolarization helps relieve the Mg block of NMDArs, AMPAr activation might also indirectly contribute to enhancing BDNF levels. A direct effect, for example through Ca-permeable AMPARs is also possible, but difficult to test as blocking AMPArs would necessarily also reduce NMDAr currents. Activation of GABA-A receptors could also contribute to this process as the equilibrium potential for Cl may still be depolarizing in some neurons at this developmental stage (Akerman and Cline, 2006).

It should be noted that modulation of glutamatergic synaptic transmission by de novo BDNF synthesis, is only one of many elements that contribute to the changes induced by visual conditioning. Diverse protocols using visual stimulation of *Xenopus* tadpoles have been shown to regulate the expression of Homer 1a, the synthesis of polyamines which modulates ion channel properties, and the activity of small GTPases which regulate cytoskeletal growth (Aizenman et al., 2002; Sin et al., 2002; Van Keuren-Jensen and Cline, 2006). However, the unique feature of BDNF we report here is its ability to bidirectionally facilitate plasticity in its cleaved and uncleaved forms (Woo et al., 2005). Because of this bidirectional facilitation, experiments that disrupt BDNF signaling are likely to have a more profound effect on refinement compared to manipulations that modulate plasticity in only one direction.

Experience-Dependent and Experience-Expectant Developmental Plasticity

Early sensory activity can influence circuit development both permissively and instructively. Greenough et al. (1987) provided an insightful framework for considering these influences by categorizing developmental plasticity as either "experience-expectant" or "experience-dependent." The former represents those processes that have evolved to be part of normal development through generations of interactions between the developing brain and a predictable sensory landscape, whereas the latter constitutes a mechanism for adaptation to the different forms of sensory information each unique organism receives. A classic example of experience-dependent plasticity would be the ocular dominance shift observed in response to monocular occlusion. Recent experiments have revealed that while TrkB signaling appears to be dispensable for the deprivation-induced loss of responsiveness to the deprived eye, it is required to mediate the recovery of binocular responses following reopening of the deprived eye (Kaneko et al., 2008).

There are also many experience-expectant aspects of response selectivity development, including receptive field refinement in the superior colliculus, and the emergence of orientation selectivity in visual cortex, which occur to a remarkable extent even in naive animals deprived of visual experience (Carrasco et al., 2005; Crair et al., 1998). Despite this initial developmental progress, long-term dark-rearing is not benign and eventually leads to the decline of these response properties.

Remarkably, BDNF overexpression is able to prevent these detrimental effects of dark-rearing (Gianfranceschi et al., 2003). The ability of BDNF overexpression to substitute for normal sensory experience has been proposed to reflect the acceleration of GABAergic circuit maturation downstream of BDNF signaling (Hanover et al., 1999; Huang et al., 1999).

Our experiments offer an alternative role for BDNF in the control of circuit development. We found that robust sensory stimulation led to the delayed upregulation of BDNF protein, resulting in a facilitation of both synaptic LTP and LTD. Under this condition of bidirectionally elevated synaptic plasticity, experience-dependent direction selectivity training, as well as experience-expectant visual acuity refinement, was readily enhanced. Interestingly, we observed a preferential effect on OFF stimuli. This result implies that BDNF preferentially modulated a specific subset of functional synaptic inputs in this case, and argues against it having exerted its action via nonspecific, homeostatic mechanisms or a general enhancement of GABAergic transmission.

Our use of CsF in the intracellular recording solution to block GABAergic currents in recordings of visually evoked responses, as well as our having restricted analysis of LTP and LTD to the short-latency responses evoked by direct optic chiasm stimulation, allow us to conclude that the improved visual acuity observed in conditioned animals was likely attributable to a BDNF-mediated facilitation of plasticity at retinotectal synapses. Nonetheless, when making measurements in a complex functional circuit it is difficult to fully exclude possible contributions of local interneurons to the changes in visual processing, especially in the case where the improvements in visual acuity took place during a period of natural visual input. Recent evidence in *Xenopus* demonstrates that the instructive contribution of plasticity mechanisms to visual field refinement depends on GABAergic inputs (Richards et al., 2010), which themselves undergo concurrent refinement during development (Tao and Poo, 2005). In addition, spike-timing-dependent plasticity of recurrent excitatory inputs also may play an important role in altering how neurons change their responses to visual stimuli over time (Pratt et al., 2008). All of these components could potentially be influenced by changes in tectal levels of BDNF in response to recent visual experience.

What might be the benefit of the several hour delay between the conditioning stimulus and the elevation in BDNF expression levels? Given that BDNF expression bidirectionally facilitates ongoing experience-expectant developmental plasticity, it may serve as a kind of "gain control," setting the kinetics of baseline circuit refinement. Immediately after an intensive sensory stimulus that might have rapidly driven a disproportionate plastic change in the circuit (Tsui et al., 2010), such a mechanism would help to normalize the response properties of the cell by temporarily making it more sensitive to ambient sensory inputs, thus efficiently resetting its basal synaptic input strengths in accord with the sensory environment.

EXPERIMENTAL PROCEDURES

Animals

Stage 47-48 albino *X. laevis* tadpoles were bred by human chorionic gonadotropin-induced mating of adults from our in-house breeding colony. Embryos were reared in standard Modified Barth's Saline-H. All experiments were approved by the MNI Animal Care Committee in accordance with Canadian Council on Animal Care guidelines.

Electroporation

Cells in the tectum were bulk electroporated as described previously (Ruthazer et al., 2005). Fluorescently labeled neurons were used roughly 48 hr after electroporation. Sequences for the BDNF MO and scrambled MO have been previously published (Yang et al., 2009).

Reporter Assays

Kaede was cloned in the pGL3 basic plasmid downstream of a 1500 bp fragment of the BDNF exon IV promoter (Tao et al., 1998) as described in the Supplemental Experimental Procedures. Kaede-expressing tectal neurons were photoconverted to red by 15 s exposure to excitation light using the DAPI filter of an Olympus BX41 fluorescence microscope. Four hours later, the increase in green fluorescence from newly synthesized Kaede was imaged on the two-photon microscope. Following visual conditioning, cells were photoconverted again and the change in green fluorescence assessed 4 hr later. Details provided in the Supplemental Experimental Procedures.

Western Blots

Protein extracted from five brains per experiment was run on polyacrylamide gels for western blotting with rabbit anti-BDNF (sc-546, 1:1000, Santa Cruz) and with rabbit anti β-tubulin (sc-9104, 1:20000, Santa Cruz) as a loading control. Additional controls described in Supplemental Experimental Procedures.

Electrophysiology

Tectal whole-cell patch clamp recordings were made through a dorsal midline incision in intact animals. Electrical stimuli were generated with an ISO-flex stimulus isolation unit (AMPI, Israel), delivered to the optic chiasm through a custom bent 25 μm cluster electrode (FHC, Maine). Events and responses were selected for analysis based upon published criteria: Series resistance was monitored throughout all experiments and cells with changes > 20% were not included (Schwartz et al., 2009). Comparisons of plasticity between groups were based on mean response amplitudes at 24–30 min after plasticity induction normalized to baseline amplitudes. Details provided in the Supplemental Experimental Procedures.

Visually Evoked Responses

Visual stimuli were generated using custom ImageJ macros. After patching onto a tectal neuron, full field stimuli, moving bars or gratings were displayed on a 600 × 800 pixel, 9 × 12 mm SVGA OLED-XL display (eMagin,100304-01) projected from the eyepiece through the microscope objective directly onto the retina after removing the lens (Engert et al., 2002). For direction selectivity and spatial frequency response experiments, the integrated current for the first 50 ms of the response was used (Mu and Poo, 2006). Analysis was performed using MATLAB. Details are provided in the Supplemental Experimental Procedures.

Behavior

One animal was placed in each well of a six-well dish on a flat screen monitor (1280 × 1024, LG Flatron model #L17NT-A). ImageJ was used to generate full screen sine wave gratings of 50% contrast at spatial frequencies 5.7, 6.67, 8 and 10 cycles/cm. Stimuli were delivered in pseudorandom order. Initially the screen was black for 4 min, the first grating appeared for 90 s before counterphasing 4 times at 6 s intervals. Then the screen was held black for 90 s and the next size grating was presented in a similar manner. Images of tadpoles were captured at 15 frames/s and resampled at five frames/s for analysis with ImageJ and MATLAB. To quantify behavior we calculated the average change in acceleration at a 5 Hz sampling rate. A response was scored as a change greater than the average change observed during the 10 s period immediately before the first counterphase.

Statistics

Two-tailed t tests were used to compare two groups. Multiple groups were compared using ANOVA with Bonferroni post-tests, unless otherwise indicated. Data are presented as mean ± SEM.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and five figures and can be found with this article online at doi:10.1016/j.neuron.2011.02.055.

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Neuron, Volume *70*
Supplemental Information

Activity-Dependent Transcription of BDNF Enhances Visual Acuity during Development

Neil Schwartz, Anne Schohl, and Edward S. Ruthazer

Supplemental Experimental Procedures:

ANIMALS: Stage 47-48 albino *X. laevis* tadpoles were bred by human chorionic gonadotropin-induced mating of adults from our in-house breeding colony. Embryos were reared in standard Modified Barth's Saline-H. All experiments were approved by the MNI Animal Care Committee in accordance with Canadian Council on Animal Care guidelines.

ELECTROPORATION: Cells in the tectum were bulk electroporated as described previously (Ruthazer et al., 2005). Fluorescently labeled neurons were used roughly 48 hr after electroporation. Sequences for the BDNF MO and scrambled MO have been previously published (Yang et al., 2009).

REPORTER EXPERIMENTS: For the reporter plasmid the luciferase region of the pGL3 basic plasmid (Promega) was replaced with kaede at HindIII and XbaI sites. These sites were introduced surrounding kaede by PCR using primers:

forward: CGCGAAGCTTATGAGTCTGATTAAACCAG, and

reverse: ATCGTCTAGATTACTTGACGTTGTCCGGCA.

After insertion of kaede into the reporter plasmid, a 1500 base pair fragment of the BDNF exon IV promoter cut with SacI and Hind III from the reporter plasmid used in Tao et al. (1998) was inserted upstream.

For reporter assays, animals expressing Kaede were illuminated 15 sec with UV illumination from the DAPI filter set on an Olympus BX41 microscope. This resulted in photoconversion of a large fraction of the green Kaede to red. Significant, though not complete, photoconversion occurred while minimizing the toxicity associated with longer conversions. 5 to 10 min after conversion a green fluoresence image was acquired (G1) (Schwartz et al 2009). A second image (G2) was acquired after 4 hr to determine baseline Kaede synthesis (G2-G1). Animals were then conditioned or kept on the bench without conditioning for 30 min. At the end of this period a second photoconversion was done, and a third image (G3) was collected. After another 4 hr period, the final image (G4) was acquired to assess Kaede synthesis during the second period (G4-G3). ImageJ software was used to calculate fluorescence changes due to new green Kaede produced during the 4 hr periods. Background was subtracted from measurements (Schwartz et al 2009).

WESTERN BLOTS: Brains extracted in a HEPES extraction buffer with protease inhibitor SetV (Calbiochem, San Diego) were run on acrylamide gel, transferred to PVDF membrane and stained alternatively with rabbit anti BDNF (sc-546, 1:1000, Santa Cruz) or a rabbit anti ß-tubulin (sc-9104, 1:20000, Santa Cruz) loading control. The anti-BDNF antibody has been used previously in Xenopus (Calle et al., 2006; Yang et al., 2009). Two specificity control experiments were done in addition to MO knockdown: First, we obtained the cDNA encoding full-length Xenopus BDNF from Open Biosystems, fused it with GFP and expressed it in intact animals. After confirming expression of BDNF-GFP,

brains were used for WB. Blots were stained with anti-GFP (Invitrogen, A6455) and then stripped and re-stained with rabbit anti-BDNF antibody. The rabbit anti-BDNF antibody recognized the GFP-tagged bands and endogenous protein at appropriate molecular weights (data not shown). Second, we probed blots with a different anti-BDNF antibody (Chemicon AB9042).

ELECTROPHYSIOLOGY: Tectal whole-cell patch clamp recordings were made through a dorsal midline incision in intact animals. Electrical stimuli were generated with an ISO-flex stimulus isolation unit (AMPI, Israel), delivered to the optic chiasm through a custom bent 25 μm cluster electrode (FHC, Maine). The external solution consisted (in mM) of 115 NaCl, 2 KCl, 1.5 CaCl2, 3 MgCl2, 5 HEPES and 10 glucose, - pH 7.4 and osmolarity 255 mOsm. For plasticity experiments 3 mM CaCl2 was used. Recordings were made from the same area within the tectum. For recording electrically evoked responses, pipettes (5 to 9 MΩ) were filled with (in mM): 120 K gluconate, 5 NaCl, 1.5 MgCl2, 1 EGTA, 20 Hepes, 2 NaATP and 0.3 Na2GTP. LTD was induced with a pairing protocol: depolarization to -35 mV, 300 pulses at 1 Hz (Daw et al., 2002). LTP was induced with a pairing protocol that consisted of depolarization to -12 mV, then 3 x (40 pulses at 10Hz) with 20 sec rest between each set. For most experiments we applied this protocol twice at a 3 min interval. During the interval cells were held at -40 mV. To isolate AMPA events evoked by visual stimuli, cells were held at -70 mV, and pipettes were filled with (in mM): 114 CsF, 1.5 MgCl2, 20 TEA-Cl, 10 EGTA, 20 HEPES, 2 NaATP and 0.3 Na2GTP. The CsF effectively reduced chloride flux in these experiments (Supplemental Figure S4) (Marchionni and Maccaferri, 2009). In some experiments, K252a (200 nM, Calbiochem), tPA-stop (4.5 μM + 0.1% BSA; American Diagnostics) or

proBDNF (2 ng/ml + 0.1% BSA, Alomone), were applied 20 to 30 min before induction unless otherwise stated. CPP (20 μM) was obtained from Tocris. Events and responses were selected for analysis based upon published criteria: Series resistance was monitored throughout all experiments and cells with changes >20% were not included (Schwartz et al., 2009). Comparisons of plasticity between groups were based on mean response amplitudes at 24 to 30 min after plasticity induction normalized to baseline amplitudes.

VISUALLY EVOKED RESPONSES: Visual stimuli were generated using custom ImageJ macros. After patching onto a tectal neuron, full field stimuli, moving bars or gratings (0.005, 0.02, 0.04, 0.08, or 0.16 cycles/μm on the retina) were displayed on a 600x800 pixel, 9 mm x 12 mm SVGA OLED-XL display (eMagin,100304-01) projected from the eyepiece through the microscope objective directly onto the retina after removing the lens (Engert et al., 2002). For direction selectivity and spatial frequency response experiments, the integrated current for the first 50 ms of the response was used (Mu and Poo, 2006). Visual stimulus presentation and spaced training used the protocol of Zhou et al. (2003). Full screen and grating stimuli were presented in pseudorandom order. A minimum interval of 20 sec between full screen stimuli and 8 sec between grating stimuli was used to avoid habituation. Gratings were presented in groups. At the start of each group, a full screen stimulus was presented, 20 sec later this was switched to the first grating and 10 sec later this grating was counterphased. The same grating was then counterphased 2-3 times at 8-12 sec intervals. The next sized grating was then presented for 10 sec until its counterphase. This sequence repeated and then ended with a full-field stimulus. There was 20 to 30 sec rest between groups. For quantification, the average of a minimum of 4 responses was used. To determine AMPA/NMDA ratios, the

first 100 ms and 200 ms of the respective responses were quantified. AMPA currents were recorded at -70 mV. To isolate visually evoked NMDAr-mediated currents, a holding potential of +40 mV was used and 100 μm GYKI52466 (Tocris) was puffed onto the tectum with a Picospritzer.

BEHAVIOR: One animal was placed in each well of a six-well dish on a flat screen monitor (1280x1024, LG Flatron model #L17NT-A). ImageJ was used to generate full screen sine wave gratings of 50% contrast at spatial frequencies 5.7, 6.67, 8 and 10 cycles/cm. Stimuli were delivered in pseudorandom order. Initially the screen was black for 4 min, the first grating appeared for 90 sec before counterphasing 4 times at 6 sec intervals. Then the screen was held black for 90 sec and the next size grating was presented in a similar manner. Images of tadpoles were captured at 15 frames/sec and re-sampled at 5 frames/sec for analysis with ImageJ and MATLAB. To quantify behavior we calculated the average change in acceleration at a 5Hz sampling rate. A response was scored as a change greater than the average change observed during the 10 sec period immediately before the first counterphase.

STATISTICS: Two-tailed t-tests were used to compare two groups. Multiple groups were compared using ANOVA with Bonferroni post-tests, unless otherwise indicated. Data are presented as mean ± SEM.

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

Supplemental Figures
Schwartz, Schohl & Ruthazer

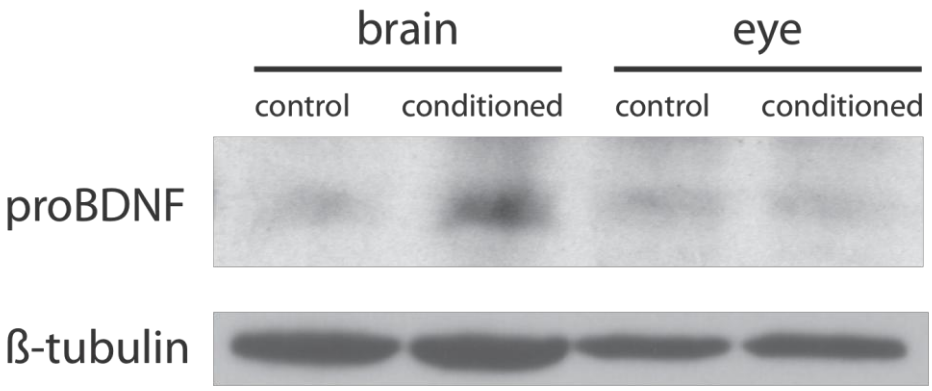

Figure S1, related to Fig. 1 Western blot shows increased proBDNF levels in the brain after visual conditioning, but no change in the eyes.

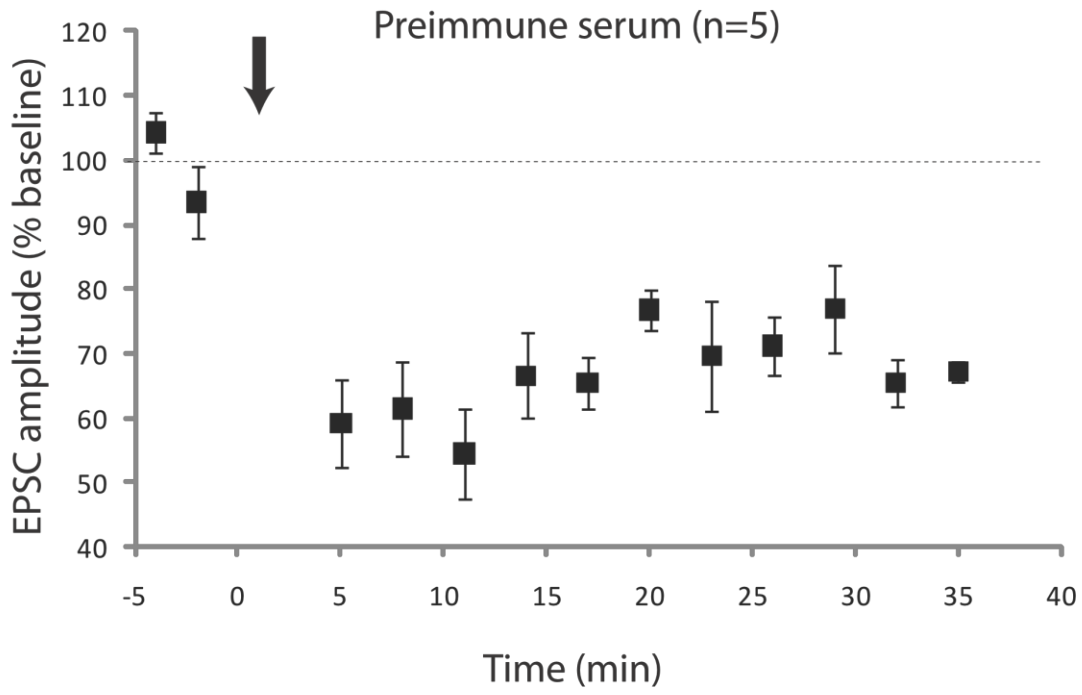

Figure S2, related to Fig. 3 Incubation of animals in pre-immune serum does not prevent the facilitation of retinotectal LTD in visually conditioned tadpoles.

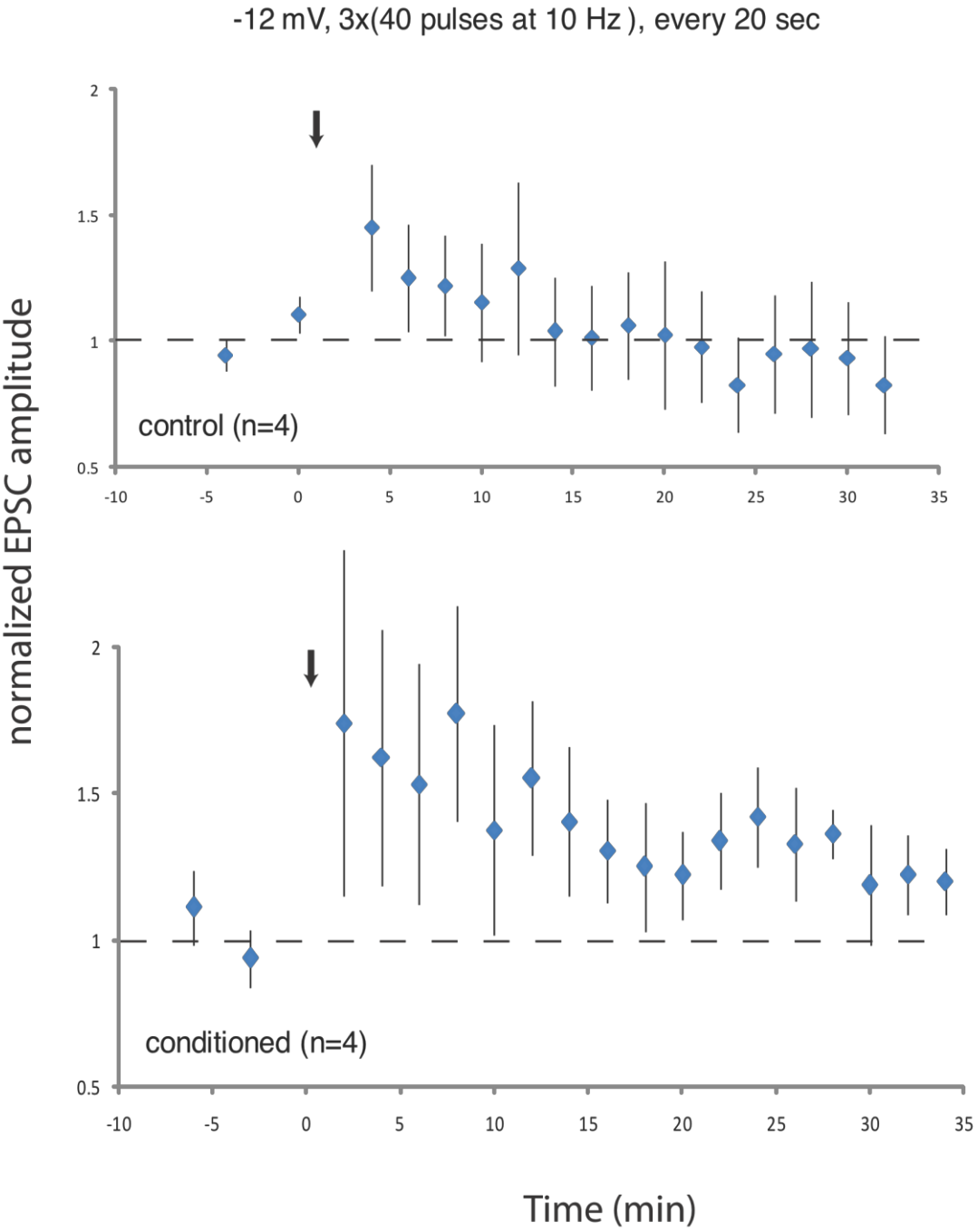

Figure S3, related to Fig. 4. Results using a weak LTP induction protocol. A retinotectal LTP induction protocol of depolarization to -12 mV with 3 x (40 pulses at 10 Hz) with 20 sec rest between each set, induced only a transient potentiation in control tadpoles, but a small, stable LTP in visually conditioned animals (control 93±23%, conditioned 118±23%).

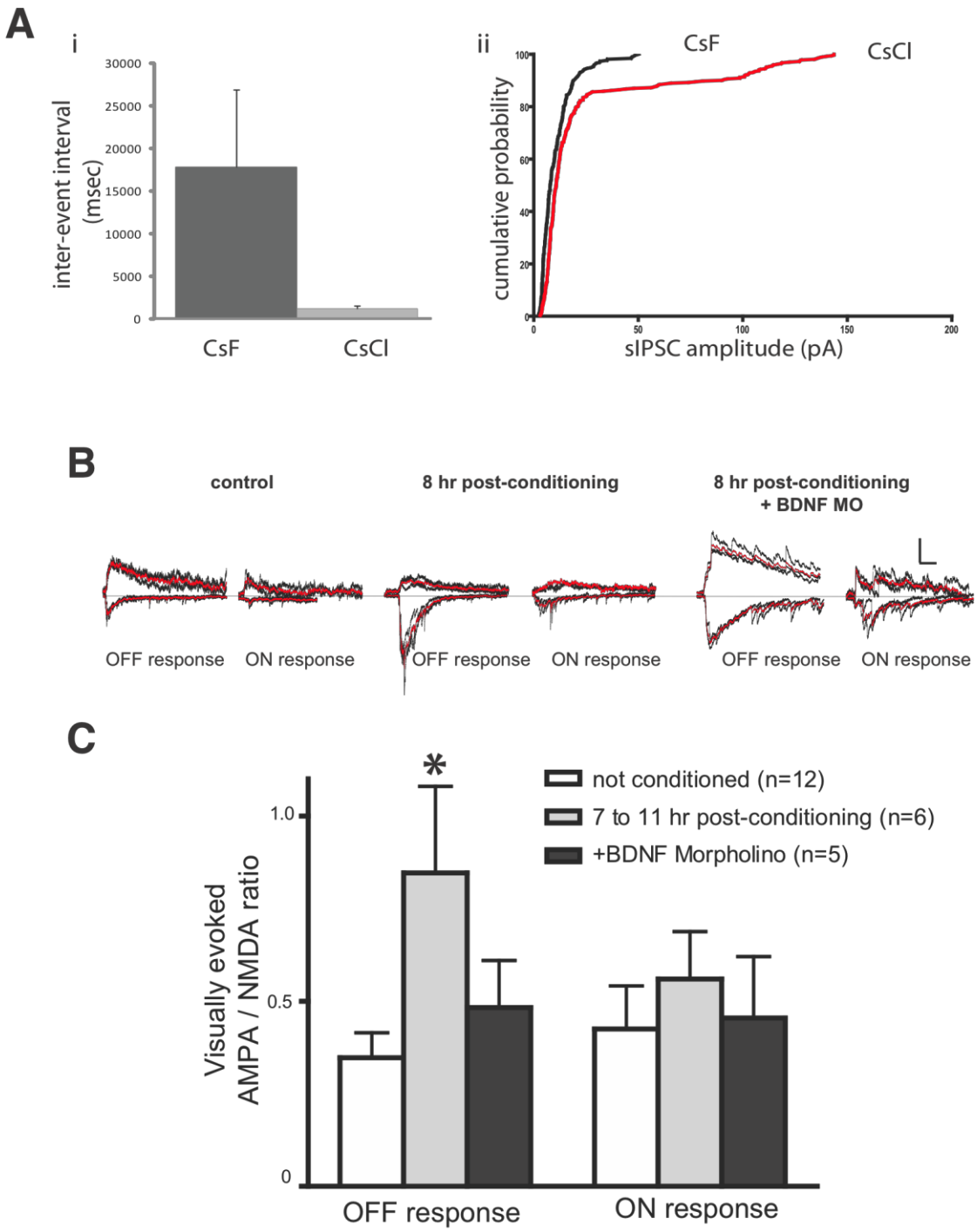

Figure S4, related to Fig. 5. Measurements of visually-evoked AMPA/NMDA ratios in tectal neurons. A) CsF internal reduces chloride flux in tectal neurons. Neighboring tectal cells were patched with pipettes containing 114 mM CsF or 114 mM CsCl. (i) The mean inter-event interval of spontaneous IPSCs was dramatically increased in tectal cells

patched with CsF-containing pipettes. (ii) Cumulative probability plot of IPSC amplitudes shows a shift to the left and the loss of larger spontaneous IPSC events. B) The AMPA/MNDA ratios of responses elicited by a full screen off visual stimulus, are higher in conditioned animals that are returned to their normal rearing environment for 7 to 11 hours. Gray traces are 3-4 representative examples of synaptic currents elicited by full-screen OFF and ON stimuli. Red traces are the average of the underlying gray traces. *Top* pharmacologically isolated NMDAr currents at +40 mV. *Bottom* AMPAr mediated current at -70 mV. From left to right the vertical scale bar corresponds to 40 pA, 45 pA and 50 pA. The horizontal scale bar corresponds to 100 msec for all traces. C) Note that only the AMPA/NMDA ratio of OFF responses was modulated in animals that had undergone conditioning. (*$p<0.05$ ANOVA with Dunnetts post-test)

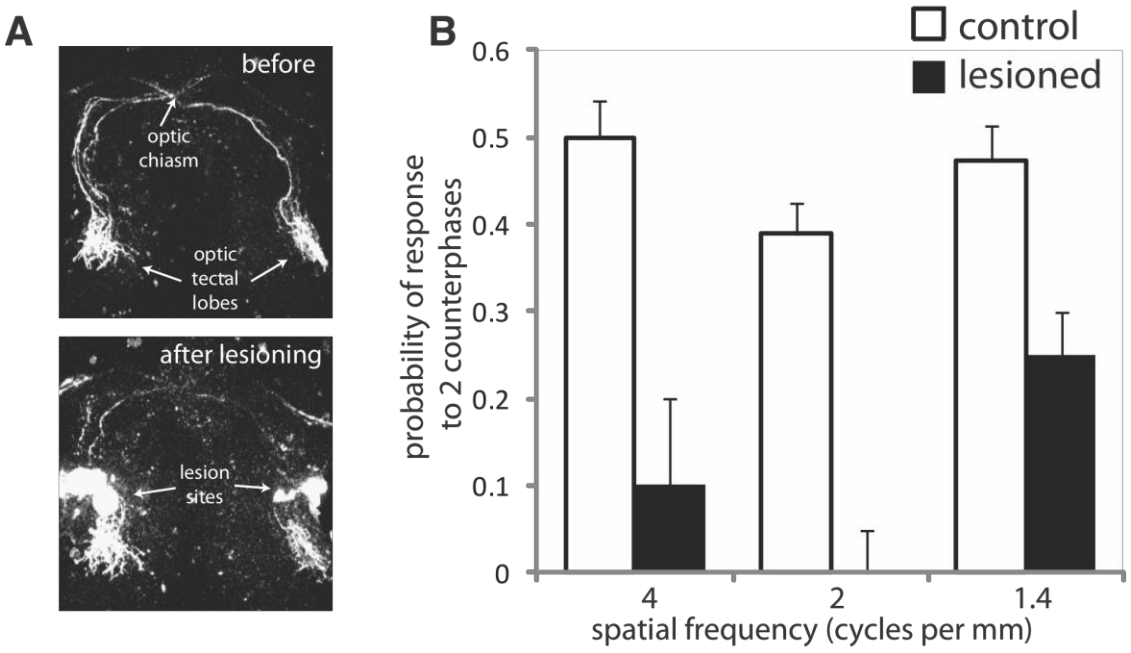

Figure S5, related to Fig. 6. Counterphasing gratings do not affect the swimming behavior in animals with lesions to the optic tract. A) Maximum intensity projection of GFP-transfected retinal ganglion cell axons and their projections into both tecta, before (top) and after (bottom) lesioning the optic tract with the infrared laser on a 2-photon microscope. B) 5 hours after the lesion the response to counterphasing gratings was greatly reduced.