

# Visual input induces long-term potentiation of developing retinotectal synapses

Li I. Zhang, Hui-zhong W. Tao and Mu-ming Poo

Department of Biology, University of California at San Diego, La Jolla, California 92093-0357, USA

The first two authors contributed equally to this work.

Correspondence should be addressed to M.-m.P. ([mpoo@ucsd.edu](mailto:mpoo@ucsd.edu))

Early visual experience is essential in the refinement of developing neural connections. *In vivo* whole-cell recording from the tectum of *Xenopus* tadpoles showed that repetitive dimming-light stimulation applied to the contralateral eye resulted in persistent enhancement of glutamatergic inputs, but not GABAergic or glycinergic inputs, on tectal neurons. This enhancement can be attributed to potentiation of retinotectal synapses. It required spiking of postsynaptic tectal cells as well as activation of NMDA receptors, and effectively occluded long-term potentiation (LTP) of retinotectal synapses induced by direct electrical stimulation of retinal ganglion cells. Thus, LTP-like synaptic modification can be induced by natural visual inputs and may be part of the underlying mechanism for the activity-dependent refinement of developing connections.

Although some aspects of visual system development do not require visual inputs<sup>1–3</sup>, there is ample evidence that neuronal activity induced by visual inputs can influence the development of ocular dominance and orientation columns in the visual cortex<sup>4–10</sup> and the refinement of retinotopic maps in the optic tectum<sup>11–14</sup>. Several lines of evidence suggest that LTP similar to that in the hippocampus<sup>15,16</sup> may serve as an underlying mechanism for activity-dependent development of the visual system. LTP can be induced at many developing synapses by repetitive electrical stimulation through an NMDA-receptor-dependent mechanism<sup>17–22</sup>. Moreover, in many parts of the developing nervous system, refinement of nerve connections requires a functional NMDA subtype of glutamate receptor<sup>23–31</sup>. Finally, there is a temporal correlation between the susceptibility of developing synapses to LTP and the critical period for the refinement of neural circuits by early visual experience<sup>21,32,33</sup>.

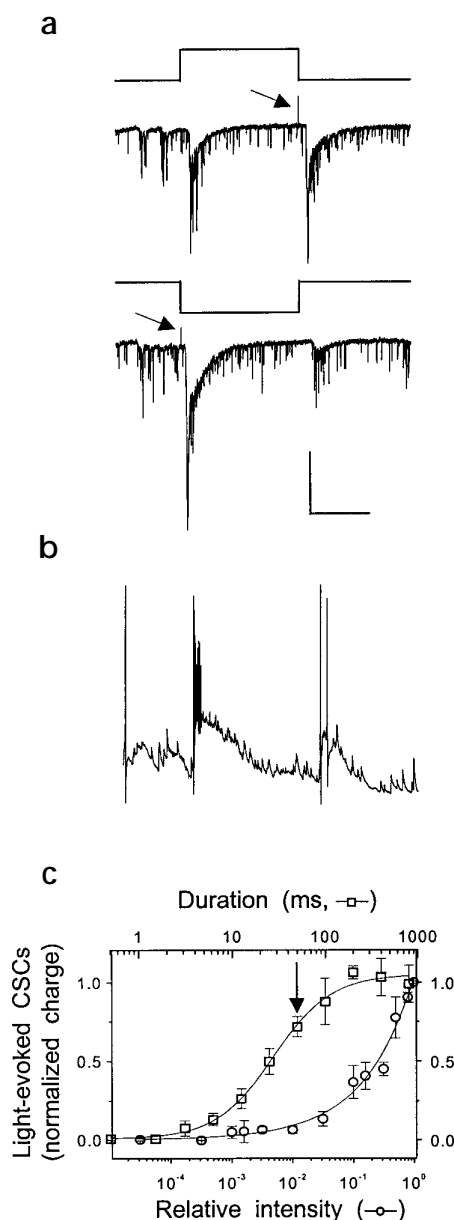
In the adult rat brain, sensory stimuli associated with fear conditioning induce LTP of glutamatergic connections in the amygdala<sup>34</sup>. Auditory inputs also can induce LTP of inhibitory afferents on goldfish Mauthner neurons<sup>35</sup>. Saturating induction of LTP in the rat hippocampus by electrical stimulation impairs spatial learning<sup>36</sup>, and motor-skill learning results in strengthening of horizontal cortical connections, accompanied by a reduction in the extent of electrically induced LTP<sup>37</sup>. Moreover, following exposure to a new environment, previously induced LTP in the rat hippocampus is reversed<sup>38</sup>. These results suggest that natural sensory stimuli may induce long-term synaptic modification in adult nervous systems in the form of LTP. To understand the causal link between synaptic plasticity and activity-dependent refinement of the developing nervous system, it is important to determine whether LTP-like potentiation can be induced by sensory inputs. Previous studies on the function of activity in developing connections have rarely addressed the direct consequence of activity at the level of synaptic function.

Furthermore, as activity evoked by electrical stimulation commonly used for the induction of LTP does not resemble that evoked by sensory inputs, it would be useful to determine whether synaptic potentiation induced by sensory inputs, if it occurs at all, shares common cellular mechanisms with electrically induced LTP. Using *in vivo* whole-cell recording, we examined the properties of visually evoked responses in the developing optic tectum of living *Xenopus* tadpoles and the effects of repetitive visual inputs on synaptic efficacy in the retinotectal system.

## RESULTS

### Tectal responses to step changes in light intensity

*In vivo* perforated whole-cell recording<sup>39,40</sup> from tectal cells of stage 40–41 *Xenopus* tadpoles<sup>41</sup> was used to monitor compound synaptic currents (CSCs) evoked by light stimuli applied to the intact contralateral eye. *Xenopus* tectal cells before stage 47 have large receptive fields and crude retinotopic organization and respond only to intense visual stimuli such as sharp changes in illumination or movement of a large black object against a light-colored background<sup>22,42–44</sup>. Thus, we used a step change in the intensity of illumination across the entire retina as a standard form of visual input. Light stimuli consisting of either a step increment or a step decrement in light intensity evoked CSCs of complex profiles (clamp voltage,  $V_c = -70$  mV; Fig. 1a). These CSCs could be attributed to monosynaptic retinotectal inputs and other polysynaptic pathways within the brain activated by visual stimuli. When recorded in current-clamp mode, light stimuli evoked compound synaptic potentials, which often triggered spiking of the tectal cell (Fig. 1b). The response to an initial step decrement in light intensity ('dimming stimulus') was usually larger and more consistent than that induced by a light increment. When the tectal cell was voltage clamped at  $-70$  mV, CSCs were all inward



**Fig. 1.** CSCs recorded in tectal neurons in response to visual stimuli in developing *Xenopus* tadpoles. (a) Membrane currents recorded in a tectal cell ( $V_c = -70$  mV; inward currents downward) in response to a 2-s step of light increment (top) and decrement (bottom). Arrows, artifact associated with shutter switching. Scales represent 100 pA and 1 s. (b) The response to a 2-s step decrement in light intensity (dimming) of the same cell as in (a), recorded under current clamp. Scales represent 25 mV and 1 s. (c) Dependence of the tectal cell's response on the duration and background light intensity of the dimming stimuli. Recordings from the same tectal neuron to a series of dimming stimuli of progressively higher background intensities (circles) and with progressively longer duration at the maximal background illumination (squares). Arrow, duration chosen for the standard stimulus used. The response of the tectal cell was quantified by measuring the total integrated charge associated with each CSC. Data represent mean  $\pm$  s.d. obtained during repeated tests ( $n = 4$ ) of the same cell.

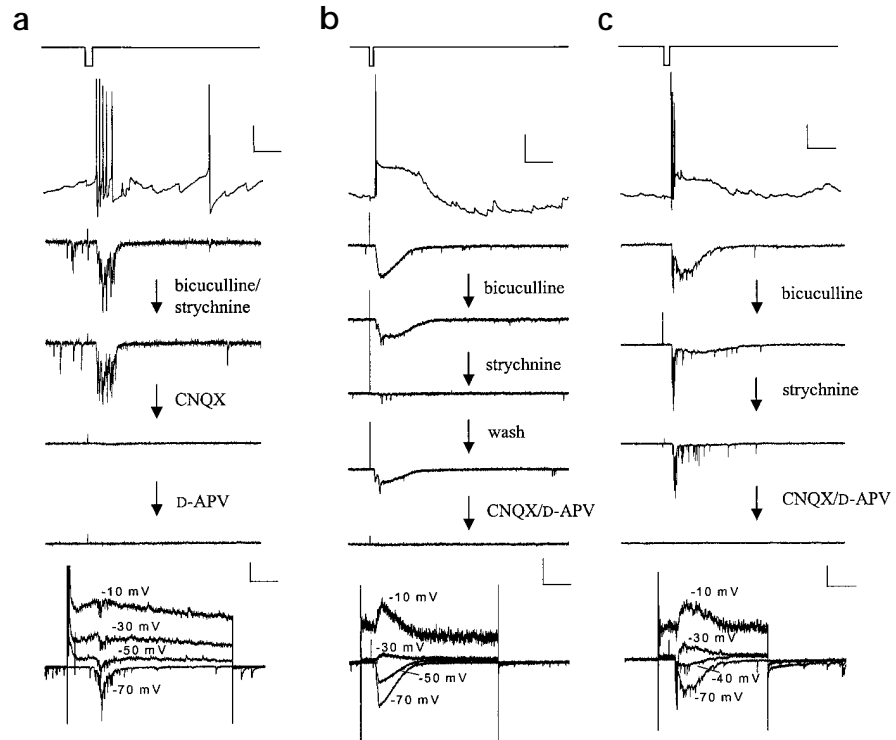
The responses evoked by the standard dimming stimulus in single tectal cells were classified into three major types according to the effects of pharmacological agents and the reversal potentials of the synaptic currents. In cells exhibiting type A responses (41 of 205 cells; Fig. 2a), dimming-stimulus-evoked CSCs consisted mainly of a cluster of fast excitatory postsynaptic currents (EPSCs) with a time course of normally less than 100 ms. These currents had a reversal potential close to 0 mV and were completely abolished by sequential perfusion of the tectum with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and ( $\pm$ )-2-amino-5-phosphonopentanoic acid (D-APV) (antagonists for AMPA and NMDA subtypes of glutamate receptors, respectively) but were not significantly affected by perfusion with bicuculline and strychnine (antagonists of GABA<sub>A</sub> and glycine receptors, respectively). In type B responses (58 of 205 cells; Fig. 2b), CSCs were dominated by inward currents with a slow time course of a few hundred milliseconds. The slow currents had a reversal potential in the range of  $-30$  to  $-40$  mV and were completely eliminated by sequential perfusion with bicuculline and strychnine, suggesting that they were largely due to chloride currents associated with inhibitory inputs within the tectum. However, when recorded under current clamp (resting membrane potential of tectal cells ranging from  $-45$  to  $-65$  mV), the slow inward currents could depolarize the cell to a plateau level of  $-30$  to  $-40$  mV for 300–500 ms. This is reminiscent of the depolarizing action of GABAergic currents in many developing nervous systems<sup>45</sup>. The slow inward currents also depended on the activation of more 'upstream' glutamatergic synapses within the tectum, as they were abolished completely by glutamate receptor antagonists CNQX and D-APV. In type C responses (79 of 205 cells; Fig. 2c), CSCs seemed to be composed of an initial phase of fast glutamatergic currents followed by a slow phase of GABAergic and glycinergic currents. The reversal potential of the latter was the same as that of the slow currents in type B responses. In classifying the three types of visual responses, pharmacological agents were used in initial experiments, whereas reversal potential measurements were made for all experiments. In addition to these three major types of responses, two other minor types of responses were also observed (Methods).

#### Enhancement of tectal responses following dimming stimuli

To test the effect of repetitive exposure to dimming stimuli, we first obtained a baseline recording of evoked CSCs in the tectal cell ( $V_c = -70$  mV) in response to the dimming stimulus at a low frequency (0.033 Hz). Eighty dimming stimuli were then applied at a higher frequency (0.25–0.3 Hz), during which the recording

currents. The total integrated charge associated with CSCs was used as a measure of evoked response size. After examining tectal responses evoked by dimming stimuli of various durations and amplitudes, we chose a dimming step of 50 ms in duration and a 300-fold decrement from the maximal light intensity as a standard stimulus. This yielded a response 70% of the maximal response in this experimental condition (Fig. 1c). The onset latency of the evoked response (due to delay in phototransduction and transmission along the visual pathway) was  $79 \pm 14$  ms (mean  $\pm$  s.d.;  $n = 142$ ) following the step decrement in light intensity. Finally, in the presence of constant, low background illumination without step changes in intensity, tectal neurons (under current clamp) exhibited spontaneous random firing of single spikes at a low average frequency (0.01–2 Hz;  $n = 11$ ) and occasionally bursting activity. The latter resembled the response evoked by step changes in light intensity, suggesting that spontaneous activity in the tectum may also be involved during development of this visual system.

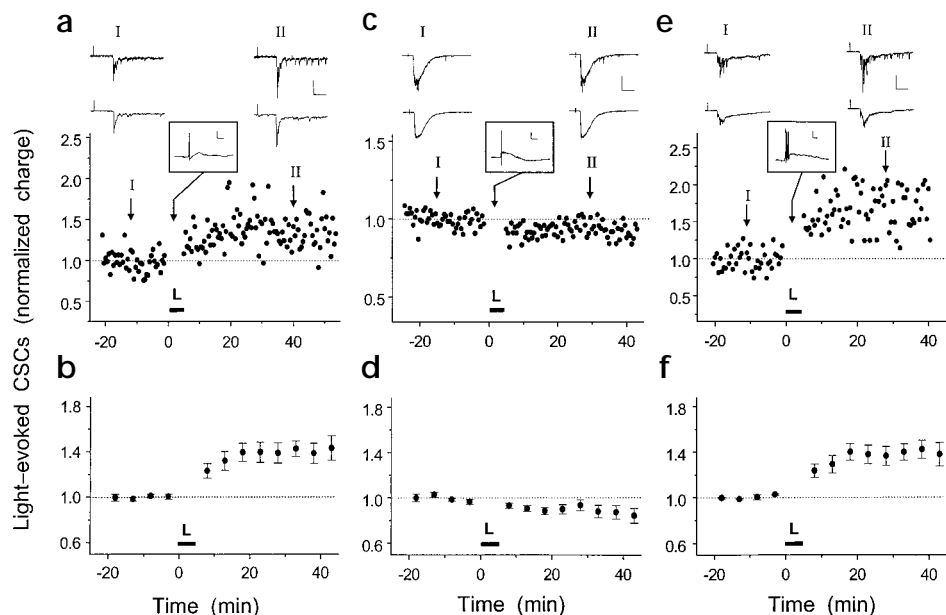
**Fig. 2.** Three major types of tectal responses triggered by the dimming stimulus. **(a)** Type A responses. Membrane potential (second trace; scales represent 15 mV and 250 ms) and membrane currents (lower traces) evoked by a dimming stimulus of 50 ms duration (top trace). The current traces depict CSCs before and after sequential local perfusion of bicuculline and strychnine, CNQX and D-APV ( $V_c = -70$  mV; scales represent 30 pA and 250 ms). The superimposed traces below showed CSCs recorded at four different holding potentials. Scales represent 80 pA and 250 ms. **(b)** Type B responses. Data presented as in **(a)**. Scales represent 80 pA and 500 ms for currents; 15 mV and 500 ms for potentials. **(c)** Type C responses. Scales represent 60 pA and 250 ms for currents and 15 mV and 250 ms for potentials.



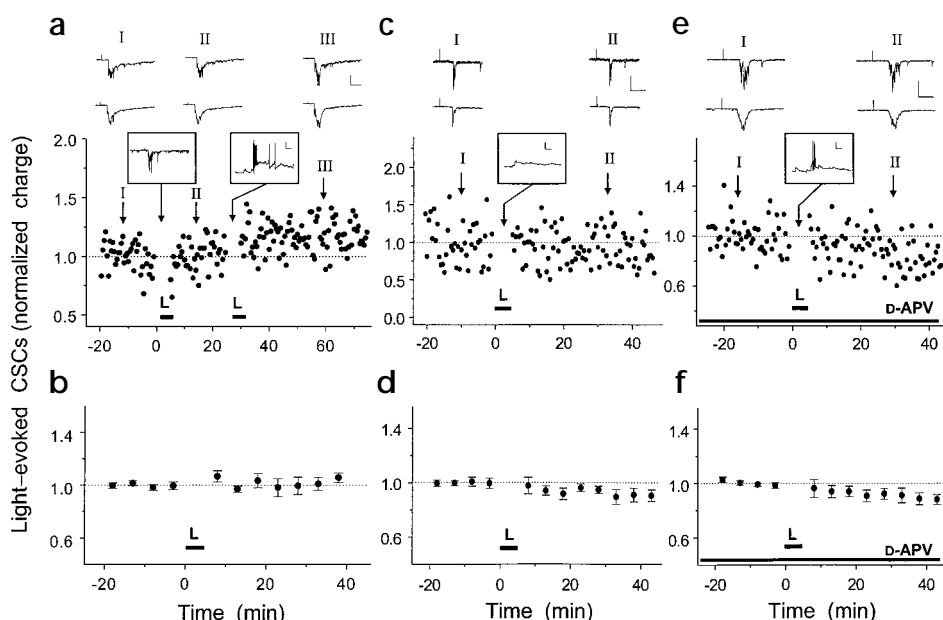
was switched to current clamp to allow firing of the tectal cell. Test recording of CSCs (at 0.033 Hz) following repetitive dimming stimuli showed that CSCs of type A responses were markedly enhanced, as indicated by an increase in the total integrated charge (Methods) associated with CSCs (Fig. 3a and b). The enhancement persisted for as long as stable recording could be made (up to 1.5 h). In cells exhibiting type B responses, we observed no increase in the total charge associated with CSCs after repetitive dimming stimuli (Fig. 3c and d). The gradual reduction in the response was attributed to slow 'rundown' of chloride currents during the course of recording, which was also

observed in the absence of repetitive stimulation. Finally, in cells exhibiting type C responses, repetitive dimming stimuli resulted in an enhancement of the fast phase of CSCs, as indicated by the total charge of CSCs within the first 50-ms window following the onset of CSCs (Fig. 3e and f). This enhancement was associated mostly with excitatory inputs, as found for type A responses. In contrast, there was no increase in the total charge over a 100- to 350-ms window following the onset of CSCs (data not

**Fig. 3.** Enhancement of light-evoked responses by repetitive dimming stimuli. **(a)** Recording from a tectal neuron exhibiting type A responses. Data represent the total integrated charge of CSCs evoked by a 50-ms dimming stimulus (Methods). L, repetitive dimming stimuli (50-ms duration, 0.3 Hz for 80 pulses); bar (below L), time of application. All values were normalized against the mean value of those recorded before 'L'. Above, traces of single CSCs (upper) and average of 10 CSCs (lower) at various times (I and II). Scales above represent 40 pA and 50 ms. The recording was switched to current-clamp mode during 'L'. Inset, membrane potential induced by the dimming stimulus during 'L'. Scales represent 20 mV and 200 ms (same for those in the insets of c and e). **(b)** Summary of all data on type A responses. Normalized total charges associated with the CSCs within the first 100-ms window following the onset of responses were averaged over 5-min bins before and after 'L' for each experiment. Data represent mean  $\pm$  s.e.m. **(c, d)** All data on type B responses, presented as in **(a)** and **(b)**. Scales above represent 150 pA and 150 ms. **(e, f)** All data on type C responses. Integrated charge of the first fast component of CSCs was measured before and after 'L'. Scales above represent 75 pA and 100 ms. The changes in the mean integrated charge of CSCs 15–40 min after 'L' were  $40.8 \pm 8.8\%$  (s.e.m.;  $n = 8$ ) in **(b)**,  $-9.6 \pm 4.0\%$  ( $n = 9$ ) in **(d)** and  $39.1 \pm 8.2\%$  ( $n = 9$ ) in **(f)**.



**Fig. 4.** Involvement of postsynaptic spiking and NMDA receptors in synaptic modification by dimming stimuli. All data were obtained from tectal cells exhibiting type A or C responses. (a, b) The effect of preventing postsynaptic spiking. (a) A tectal cell exhibiting type C responses was stimulated with two episodes of repetitive dimming stimuli (L). The first 'L' was applied while the tectal cell was voltage-clamped at  $-90$  mV, and the second 'L' was applied while the tectal cell was held in current clamp. Data are presented as in Fig. 3. Scales above represent 200 pA and 100 ms. Scales in the box represent 20 mV and 100 ms (same in c and e). (b) Summary of results from all experiments under the condition of voltage clamp at  $-90$  mV. (c, d) Modification was absent for non-spiking type A responses. Data are plotted as in (a) and (b). Scales above represent 30 pA and 100 ms. (e, f) The effect of blocking NMDA receptors. Tadpole brain was perfused with  $50$   $\mu$ M D-APV throughout the experiment. Data were obtained from tectal cells that exhibited type A responses and spiking during 'L'. Scales above represent 80 pA and 100 ms. The changes in the mean integrated charge of CSCs 15–40 min after 'L' were  $1.6 \pm 4.9\%$  (s.e.m.;  $n = 7$ ) in (b),  $-6.8 \pm 4.2\%$  ( $n = 6$ ) in (d) and  $-8.8 \pm 4.3\%$  ( $n = 7$ ) in (f).



shown). The latter window corresponded mainly to the time course of the slow inward component (Fig. 2c). These results suggest that repetitive visual inputs selectively potentiated glutamatergic responses on the tectal cell, leaving GABAergic/glycinergic responses relatively unchanged.

Several possible changes along the visual pathway could have contributed to the observed enhancement of excitatory components of CSCs evoked by visual stimuli. Spiking activity of retinal ganglion cells (RGCs) could have been elevated, resulting in an increased glutamate release by retinal axons in the tectum. Furthermore, the excitatory retinotectal connection could have been potentiated through mechanisms that depend on the postsynaptic tectal cell. Finally, polysynaptic excitatory inputs on the tectal cell recorded could have been elevated through either an activity-dependent potentiation of the inputs themselves or an increased excitability of 'upstream' excitatory cells. Several lines of evidence suggested that the enhancement of CSCs reflected a strengthening of excitatory synapses on the recorded tectal neuron. First, the enhancement of type A and type C CSCs was abolished in all cases in which the tectal cell was voltage clamped at a hyperpolarized potential ( $-90$  mV) during the repetitive dimming stimuli (Fig. 4a and b). In contrast, the same episode of repetitive dimming stimuli induced an enhancement of CSCs when the cell was held in current clamp (Fig. 4a). Second, in the cases for which responses to dimming stimuli were too weak to elicit spiking of the tectal cell, the stimuli had no effects on CSCs (Fig. 4c and d). Third, there was no change in type A CSCs when  $50$   $\mu$ M D-APV was present in the recording medium (Fig. 4e and f). Although APV may also affect synapses in the earlier stages of visual pathway, the requirements for both postsynaptic spiking and activation of NMDA receptors suggests that the enhancement of CSCs resulted mainly from activity-induced strengthening of excitatory synapses on the tectal cell.

#### Retinotectal synapses potentiated by dimming stimuli

To further determine whether potentiation can be induced at individual monosynaptic retinotectal synapses by repetitive dimming stimuli, we monitored monosynaptic EPSCs in the tectal cell evoked by direct electrical stimulation of RGCs in the contralateral eye (after removal of the lens)<sup>22</sup>. Eighty repetitive dimming stimuli (duration, 50 ms; 0.3 Hz) were applied to the retina, with the tectal cell held under current clamp. In 9 of 12 cases (summarized in Fig. 5d) that exhibited type A or C responses (Fig. 5a), repetitive dimming inputs resulted in a persistent increase in the amplitude of EPSCs (over 20% above control level) when the dimming stimulus had triggered spiking of tectal cells. The lack of potentiation found in 3 of 12 cases could be attributed to the possibility that these electrically stimulated retinotectal connections may not have been activated by the dimming stimulus. In contrast, no change in the EPSC amplitude was observed in nine of nine cases in which the dimming stimuli failed to trigger postsynaptic spiking (Fig. 5b and d). Furthermore, in the presence of  $50$   $\mu$ M D-APV, repetitive dimming stimulation failed to potentiate any retinotectal connection monitored (in seven of seven cases), although each dimming stimulus did reliably trigger spiking of the tectal cell (Fig. 5c). Thus, the enhanced responses of excitatory inputs could be attributed, at least in part, to the potentiation of retinotectal synapses.

The requirements for postsynaptic spiking and NMDA receptor activation suggested that visual input-induced potentiation of retinotectal synapses shares the same cellular mechanisms with LTP of these synapses induced by direct electrical stimulation of the RGCs<sup>22</sup>. Moreover, potentiation of retinotectal synapses induced by sequential episodes of repetitive dimming stimulation (0.3 Hz; 80 pulses) was additive (Fig. 6a). Potentiation by repetitive dimming stimulation was also additive to that induced by repetitive electrical stimulation of the ganglion cells (at 1 Hz; 80 pulses; Fig. 6b). However, following prolonged exposure of

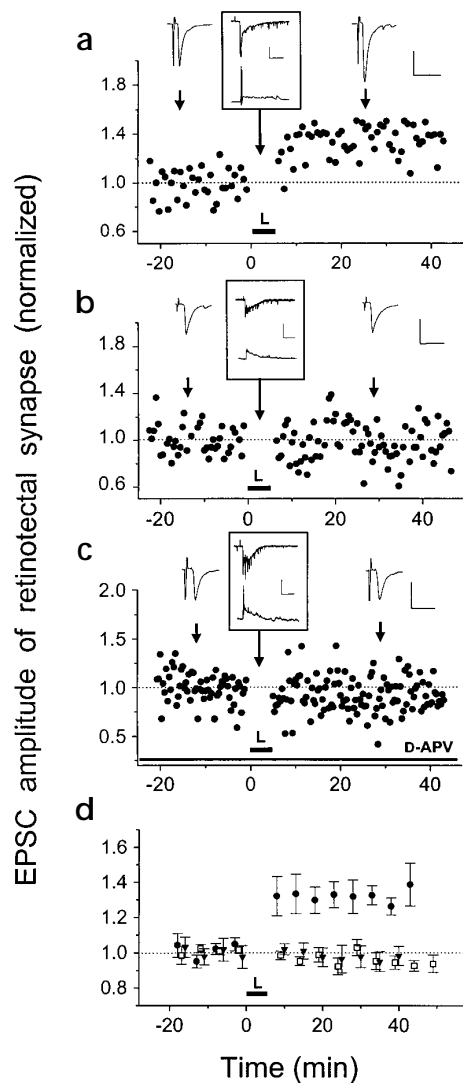


**Fig. 5.** Induction of LTP at retinotectal synapses by repetitive dimming stimuli. (a) 'L'-potentiated retinotectal synapses. Data points depict the amplitudes of monosynaptic EPSCs evoked by minimal electrical stimulation of RGCs and normalized against the mean value obtained before 'L'. Sample traces above represent averages of 10 consecutive EPSCs at the time marked by the arrow. Scales represent 20 ms and 40 pA. In this experiment, each dimming stimulus triggered spiking of the tectal neuron during 'L' (inset, bottom). Inset, top, dimming-stimulus-evoked response when the cell was clamped at  $-70$  mV. Scales represent 100 pA and 30 mV (inset) and 200 ms for current and potential. (b) The dimming stimulus failed to trigger spiking of the tectal cell during 'L'. Scales represent 50 pA and 20 ms for EPSCs; 30 mV and 100 pA and 500 ms for potential and current of dimming-stimulus-evoked responses (inset). (c) Experiment in the presence of  $50 \mu\text{M}$  D-APV. Here dimming stimuli triggered spiking of the tectal cell during 'L' (inset). Scales are the same as (b). (d) Summary of the effects of repetitive dimming stimuli on the retinotectal synapses. Normalized EPSC amplitudes from each experiment were averaged over 5-min bins before and after 'L'. Circles, squares and triangles, experiments similar to those described in (a) (b) and (c), respectively. Some symbols are laterally displaced for clarity. Error bars represent s.e.m. The changes in the mean amplitude of EPSCs 15–40 min after 'L' were  $38.4 \pm 7.8\%$  (s.e.m.;  $n = 9$ ),  $-4.1 \pm 3.9\%$  ( $n = 9$ ) and  $-2.3 \pm 5.7\%$  ( $n = 7$ ) for circles, squares and triangles, respectively.

the retina to the dimming stimuli (0.3 Hz for 30 min), neither repetitive dimming stimulation nor electrical stimulation was effective in modifying retinotectal synapses (Fig. 6c and d), although each stimulus had triggered spiking of the tectal cell. In contrast, in two other cases (data not shown) in which dimming stimuli did not trigger spiking in the tectal cell, repetitive electrical stimulation potentiated the retinotectal synapses after prolonged dimming stimuli. These results suggested that potentiation of retinotectal synapses by electrical stimulation could be occluded by previous saturating potentiation of these synapses following prolonged dimming stimulation. To further examine the possibility that prolonged exposure of dimming light had produced a nonspecific desensitization of the cellular processes required for LTP rather than a saturated induction of LTP, we applied  $50 \mu\text{M}$  D-APV to the tectum during the prolonged dimming stimulation. When the drug was washed out before repetitive electrical stimulation of RGCs, robust LTP was induced (Fig. 6e). Thus, potentiation of retinotectal synapses induced by repetitive dimming stimulation was due to cellular processes similar to those induced by the electrical stimulation.

#### DISCUSSION

Previous studies of the adult frog visual system have described four different types of RGCs that are particularly sensitive to visual stimuli with contrast, convexity and moving edges, as well as to dimming of light<sup>46</sup>. In the developing tadpoles used here, most recorded tectal cells responded to both an increment and a decrement in light intensity, with different characteristic patterns. Although each tectal cell receives innervation from many RGCs, and there is no clear functional retinotopic organization<sup>22,42</sup>, the existence of several types of evoked responses suggests that distinct tectal circuits may have already emerged. Tectal cells exhibiting a type A response seemed to receive direct projections from RGCs, whereas those with a type B response received inputs mainly from GABAergic/glycinergic neurons located in the tectum or other brain regions and activated by the light stimulus. Cells with a type C response were encountered most frequently and were likely to receive both direct RGC projections and indirect GABAergic/glycinergic inputs of vary-



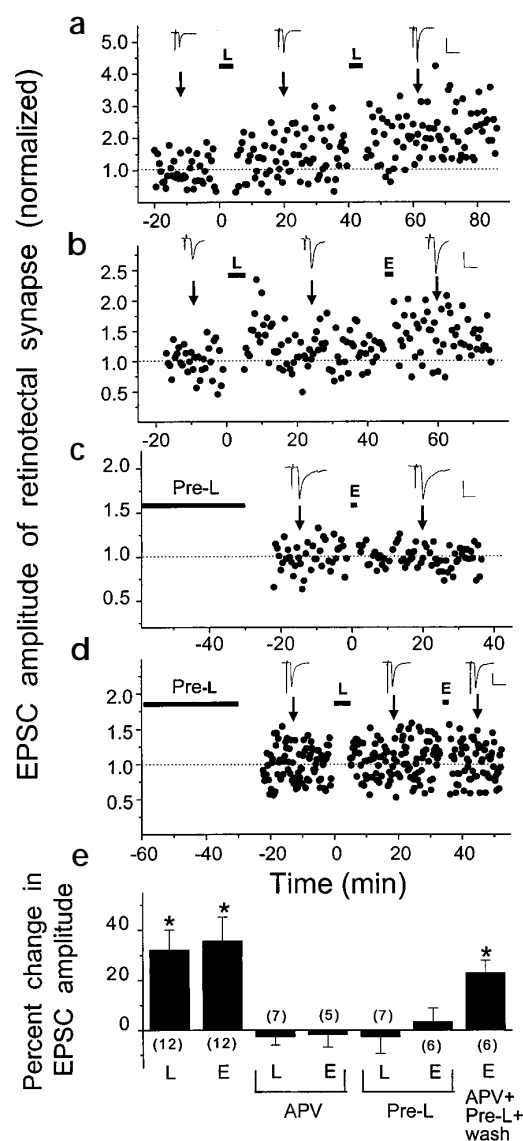
ing proportions. Direct recording of tectal responses elicited by minimal stimulation of RGCs has also revealed the co-existence of polysynaptic GABAergic inputs and direct retinotectal projections on the same tectal cell<sup>22</sup>.

The potentiation of the dimming response induced by repetitive visual stimuli (Fig. 3) was attributed mainly to changes in synaptic efficacy at monosynaptic retinotectal connections, although we cannot exclude the possibility that there was activity-induced plasticity in the retina or in the polysynaptic pathways leading to the recorded tectal cell. As the potentiation effect was largely abolished by voltage-clamping the postsynaptic tectal neuron at  $-90$  mV, the plasticity was likely to be associated with synapses made onto the tectal cell. If retinal plasticity had resulted in changes in the firing pattern of RGC axons evoked by the dimming stimulus, such changes were not revealed by CSCs recorded in the tectal cells. The relative timing in spiking of RGCs and postsynaptic tectal cells can determine the direction of synaptic modification (LTP or long-term depression, LTD) at retinotectal connections. Here postsynaptic spiking was also required for the induction of synaptic changes by dimming stimuli. Although it is not possible to precisely determine the timing of RGC spikes, the response evoked by the dimming stimulus in many tectal cells (types A and C) apparently involved many RGC

**Fig. 6.** Occlusion of electrically induced LTP by dimming stimuli. In these experiments, dimming stimuli were capable of triggering spiking of the recorded tectal cell. (a) Additive potentiation of the retinotectal synapses by two sequential episodes of 'L' with an interval of about 30 min. Data points depict the amplitudes of monosynaptic EPSCs that were evoked by minimal electrical stimulation of RGCs and normalized against the mean value obtained before 'L'. Scales represent 40 pA and 20 ms. (b) Additive potentiation of retinotectal synapses induced by sequential 'L' and repetitive electrical stimulation of RGCs (1 ms, 5–10 V at 1 Hz for 80 s) coupled with current injection (1 ms and 1 nA) to the recorded tectal neuron to trigger spike of the cell within 10 ms following the onset of EPSP when the cell was held in current clamp<sup>22</sup> (E). Scales represent 40 pA and 10 ms. (c, d) Experiments like those in (a) and (b), except that the retina was pre-exposed to dimming stimuli for a prolonged period (0.3 Hz for 30 min; Pre-L) before the onset of the experiment, in which the effects of 'E' (c) or sequential application of 'L' and 'E' (d) were examined. Scales represent 50 pA and 10 ms for (c) and 20 ms for (d). (e) Summary of the percentage change of mean EPSC amplitude ( $\pm$  s.e.m.) of retinotectal connections 15–40 min after repetitive stimulation, and changes in EPSCs induced by 'L' and 'E' in the presence of 50  $\mu$ M D-APV (APV) or prolonged prior exposure to dimming stimuli (Pre-L), as well as that induced by 'E' following extensive washing away of the 50  $\mu$ M D-APV present during 'Pre-L' (wash). Numbers in parentheses, number of experiments. \* $p < 0.01$ , significant potentiation (Student's *t*-test).

inputs that arrived within tens of milliseconds following the onset of response (Figs. 1 and 2). Most excitatory inputs contributing to the fast CSCs that showed enhancement by dimming stimuli had arrived before or coinciding with the time of postsynaptic spiking (Fig. 2a), consistent with the expectation of synaptic potentiation induced by positively correlated spiking<sup>22,47</sup>. The GABAergic/glycinergic inputs to the tectal cell, on the other hand, arrived mostly after postsynaptic spiking. The lack of any significant changes at the latter inputs is also consistent with the property of synaptic modification of GABAergic connections by correlated spiking in cultures of hippocampal neurons (K. Ganguly and M.-m.P., unpublished data).

Developing visual systems exhibit LTP and LTD in response to electrical stimulation, and there is a temporal correlation between the critical period for activity-dependent refinement of developing connections in the visual system and its susceptibility to the induction of LTP/LTD. To establish a causal link between synaptic modifications and activity-dependent refinement in the visual system, however, it is important to determine whether natural visual inputs can induce acute synaptic modification in an intact developing visual system by mechanisms similar to LTP/LTD. At defined monosynaptic retinotectal synapses, LTP/LTD can be induced by direct electrical stimulation of RGCs<sup>22</sup>. However, it is not clear whether and where LTP/LTD can occur following repetitive visual inputs, as visually evoked responses of a single neuron *in vivo* involve activation of a large network of neurons with complex patterns of activity. Our results showed that despite the complexity in the activity pattern, visual inputs were effective in producing LTP-like modification of retinotectal synapses. Although the causal relationship between the functional synaptic modification and structural refinement of developing connections remains to be determined, the effectiveness of natural sensory inputs in triggering long-term changes in synaptic efficacy underscores the sensitivity of developing connections to early visual experience. As suggested by the structural changes associated with long-term functional plasticity<sup>48,49</sup>, the persistent synaptic modification observed here is likely to be an integral part of the cellular processes responsible for structural refinement of developing connections in the nervous system.



## METHODS

**Tectal recording.** *Xenopus* tadpoles were staged by published criteria<sup>41</sup>. Tadpoles at stage 40–41 were anesthetized with saline containing 0.02% MS222 (Sigma, St. Louis, Missouri), secured by insect pins to a sylgard (Dow Corning Co., Midland, Michigan)-coated dish and incubated in HEPES-buffered saline containing 115 mM NaCl, 2 mM KCl, 10 mM HEPES, 2.5 mM CaCl<sub>2</sub>, 10 mM glucose and 1.5 mM MgCl<sub>2</sub>, pH 7.3. For recording, 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. A low dose (2  $\mu$ g per ml) of  $\alpha$ -bungarotoxin (RBI, Natick, Massachusetts) was applied to the bath to prevent occasional contraction of muscle fibers during the recording. As previously shown<sup>22</sup>, this toxin treatment did not affect the retinotectal responses recorded. The method of perforated-patch, whole-cell recording<sup>39,40</sup> was used for recording from tectal cells. The micropipets were made from borosilicate glass capillaries and had resistances in the range of 4–6 M $\Omega$ . The pipets were coated with a layer of sylgard except near the tip, tip-filled with internal solution and then back-filled with internal solution containing 200  $\mu$ g per ml amphotericin B (Sigma). The internal solution contained 110 mM potassium gluconate, 10 mM KCl, 5 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 20 mM HEPES and 0.5 mM EGTA, pH 7.3. The bath was constantly perfused with fresh recording medium at a slow rate (0.5 ml per min) throughout the recording, and all experi-

ments were performed at room temperature (20–22°C). Recordings were made with a patch-clamp amplifier (Axopatch 1-D; Axon Instruments, Foster City, California). Signals were filtered at 5 kHz using amplifier circuitry, and data were sampled at 5 kHz and analyzed using Axoscope (Axon Instruments) and Origin (Microcal, Northampton, Massachusetts) software. The whole-cell capacitance was fully compensated, and the series resistance (20–50 M $\Omega$ ) was compensated at 80% (lag, 60  $\mu$ s). Data accepted for analysis were only those in which the series resistance did not change more than 10%, and input resistance (0.5–1.5 G $\Omega$ ) remained relatively constant throughout the experiment. For direct retinal stimulation, a micropipet with an opening of about 3  $\mu$ m was filled with extracellular solution and used under loose-seal conditions for 'minimal stimulation' as described earlier<sup>22</sup>.

**Light stimulation and measurements of evoked responses.** To apply light stimuli, the eye ipsilateral to the tectum under recording was removed, and light illumination from a light source (Fiber-lite, Moburn, Massachusetts) was delivered to the contralateral eye through an optic fiber. Neutral-density filters were used to adjust the intensity of this illumination. The timing and duration of light stimuli were controlled by an electronic shutter (UniBlitz, Rochester, New York) coupled to a stimulator (NeuroData, Delaware Water Gap, New Jersey). Step changes in the light intensity from the background illumination were used as visual stimuli. The dimming stimulus consists of an 'off' step and an 'on' step; each could evoke responses in the tectal neurons when they were separated by more than 300 ms (Fig. 1a and b). However, for standard 50-ms dimming stimulus, the response to the 'on' step was absent. The light-evoked synaptic currents on tectal cells were characterized pharmacologically by applying 10  $\mu$ M CNQX (RBI) or the following antagonists: 50  $\mu$ M D-APV (RBI), 15  $\mu$ M bicuculline methiodide (RBI) or 60  $\mu$ M strychnine hydrochloride (Sigma). Those antagonists were either applied directly to the tectum through local perfusion pipets or perfused with fresh media across the tectum. To quantify the CSCs evoked by dimming stimuli, in cases of type A or B responses, we measured the total integrated charge associated with CSCs within the first 100-ms window following the onset of CSCs for A and that within the first 500-ms window for B. For type C responses, the integrated charges associated with the initial (glutamatergic) and late (GABAergic/glycinergic) phases of CSCs were separately measured within the first 50-ms and 100- to 350-ms windows following the response, respectively. This separation was justified by the observation that, near the reversal potential of glutamatergic currents ( $V_c = -10$  mV), the onset latency of the outward (GABAergic/glycinergic) components' CSCs was about 50 ms longer than that of CSCs under normal recording conditions ( $V_c = -70$  mV; Fig. 2c). Besides the three major types of evoked responses described in the text, two minor types were also observed. In 20 of 205 cells, the responses consisted mostly of small compound GABAergic currents with onset latency significantly longer than those of types A–C. In 7 of 205 cells, recordings were apparently made from glial cells, which showed a low input impedance (100 M $\Omega$ ) and a high whole-cell capacitance (20 pF) compared with those of tectal neurons (0.5–1.5 G $\Omega$  and 7–12 pF, respectively). The dimming-stimulus-evoked responses in the latter cells were insensitive to CNQX and D-APV, but were partially blocked by 300  $\mu$ M L-trans-2,4-pyrrolidinedione-2,4-dicarboxylate (RBI), an antagonist of glutamate transporters, suggesting they were due to glial cell transporter activity in response to dimming-stimulus-induced glutamate release in the tectum.

#### ACKNOWLEDGEMENTS

We thank G. Bi, Y. Dan, W. Harris, C. Holt and F. Engert for suggestions. This work was supported by a grant from the National Science Foundation.

RECEIVED 22 FEBRUARY; ACCEPTED 12 MAY 2000

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

- Penn, A. A., Riquelme, P. A., Feller, M. B. & Shatz, C. J. Competition in retinogeniculate patterning driven by spontaneous activity. *Science* 279, 2108–2112 (1998).
- Crowley, J. C. & Katz, L. C. Development of ocular dominance columns in the absence of retinal input. *Nat. Neurosci.* 2, 1125–1130 (1999).
- Wiesel, T. N. & Hubel, D. H. Single cell responses in striate cortex of kittens deprived of vision in one eye. *J. Neurophysiol.* 26, 1003–1017 (1963).
- LeVay, S., Stryker, M. P. & Shatz, C. J. Ocular dominance columns and their development in layer IV of the cat's visual cortex: a quantitative study. *J. Comp. Neurol.* 179, 223–244 (1978).
- LeVay, S., Wiesel, T. N. & Hubel, D. H. The development of ocular dominance columns in normal and visually deprived monkeys. *J. Comp. Neurol.* 191, 1–51 (1980).
- Stryker, M. P. & Harris, W. A. Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex. *J. Neurosci.* 6, 2117–2133 (1986).
- Weliky, M. & Katz, L. C. Disruption of orientation tuning in visual cortex by artificially correlated neuronal activity. *Nature* 386, 680–685 (1997).
- Crair, M. C., Gillespie, D. C. & Stryker, M. P. The role of visual experience in the development of columns in cat visual cortex. *Science* 279, 566–570 (1998).
- Rittenhouse, C. D., Shouval, H. Z., Paradiso, M. A. & Bear, M. F. Monocular deprivation induces homosynaptic long-term depression in visual cortex. *Nature* 397, 347–350 (1999).
- Fawcett, J. W. & Willshaw, D. J. Compound eyes project stripes on the optic tectum in *Xenopus*. *Nature* 296, 350–351 (1982).
- Udin, S. B. Abnormal visual input leads to development of abnormal axon trajectories in frogs. *Nature* 301, 336–338 (1983).
- Reh, T. A. & Constantine-Paton, M. Eye-specific segregation requires neural activity in three-eyed *Rana pipiens*. *J. Neurosci.* 5, 1132–1143 (1985).
- Schmidt, J. T. & Buzzard, M. Activity-driven sharpening of the retinotectal projection in goldfish: development under stroboscopic illumination prevents sharpening. *J. Neurobiol.* 24, 384–99 (1993).
- Bliss, T. V. & Lømo, T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol. (Lond.)* 232, 331–356 (1973).
- Malenka, R. C. & Nicoll, R. A. Long-term potentiation—a decade of progress? *Science* 285, 1870–1874 (1999).
- Perkins, A. T. & Teyler, T. J. A critical period for long term potentiation in the developing rat visual cortex. *Brain Res.* 439, 222–229 (1988).
- Komatsu, Y., Fujii, K., Maeda, J., Sakaguchi, H. & Toyama, K. Long-term potentiation of synaptic transmission in kitten visual cortex. *J. Neurophysiol.* 59, 124–141 (1988).
- Artola, A., Bröcher, S. & Singer, W. Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex. *Nature* 347, 69–72 (1990).
- Mooney, R., Madison, D. V. & Shatz, C. J. Enhancement of transmission at the developing retinogeniculate synapse. *Neuron* 10, 815–825 (1993).
- Crair, M. C. & Malenka, R. C. A critical period for long-term potentiation at thalamo-cortical synapses. *Nature* 375, 325–328 (1995).
- Zhang, L. I., Tao, H. W., Holt, C. E., Harris, W. A. & Poo, M. A critical window for cooperation and competition among developing retinotectal synapses. *Nature* 395, 37–44 (1998).
- Cline, H. T. & Constantine-Paton, M. NMDA receptor antagonists disrupt the retinotectal topographic map. *Neuron* 3, 413–426 (1989).
- Scherer, W. J. & Udin, S. B. N-methyl-D-aspartate antagonists prevent interaction of binocular maps in *Xenopus* tectum. *J. Neurosci.* 9, 3837–3843 (1989).
- Bear, M. F., Kleinschmidt, A., Gu, Q. A. & Singer, W. Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. *J. Neurosci.* 10, 909–925 (1990).
- Schmidt, J. T. Long-term potentiation and activity-dependent retinotopic sharpening in the regenerating retinotectal projection of goldfish: common sensitive period and sensitivity to NMDA blockers. *J. Neurosci.* 10, 233–246 (1990).
- Hahm, J. O., Langdon, R. B. & Sur, M. Disruption of retinogeniculate afferent segregation by antagonists to NMDA receptors. *Nature* 351, 568–570 (1991).
- Simon, D. K., Prusky, G. T., O'Leary, D. D. & Constantine-Paton, M. N-methyl-D-aspartate receptor antagonists disrupt the formation of a mammalian neural map. *Proc. Natl. Acad. Sci. USA* 89, 10593–10597 (1992).
- Schlaggar, B. L., Fox, K. & O'Leary, D. D. Postsynaptic control of plasticity in developing somatosensory cortex. *Nature* 364, 623–626 (1993).
- Li, Y., Erzurumlu, R. S., Chen, C., Jhaveri, S. & Tonegawa, S. Whisker-related neuronal patterns fail to develop in the trigeminal brainstem nuclei of NMDAR1 knockout mice. *Cell* 76, 427–437 (1994).
- Aamodt, S. M. & Constantine-Paton, M. The role of neural activity in synaptic development and its implications for adult brain function. *Adv. Neurol.* 79, 133–144 (1999).
- Kirkwood, A., Rioult, M. C. & Bear, M. F. Experience-dependent modification of synaptic plasticity in visual cortex. *Nature* 381, 526–528 (1996).
- Kirkwood, A., Lee, H. K. & Bear, M. F. Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. *Nature* 375, 328–331 (1995).
- Rogan, M. T., Staubli, U. V. & LeDoux, J. E. Fear conditioning induces associative long-term potentiation in the amygdala. *Nature* 390, 604–607 (1997).

35. Oda, Y., Kawasaki, K., Morita, M., Korn, H. & Matsui, H. Inhibitory long-term potentiation underlies auditory conditioning of goldfish escape behaviour. *Nature* **394**, 182–185 (1998).
36. Moser, E. I., Krobot, K. A., Moser, M.-B. & Morris, R. G. M. Impaired spatial learning after saturation of long-term potentiation. *Science* **281**, 2038–2042 (1998).
37. Rioult-Pedotti, M. S., Friedman, D., Hess, G. & Donoghue, J. P. Strengthening of horizontal cortical connections following skill learning. *Nat. Neurosci.* **1**, 230–234 (1998).
38. Xu, L., Anwyl, R. & Rowan, M. J. Spatial exploration induces a persistent reversal of long-term potentiation in rat hippocampus. *Nature* **394**, 891–894 (1998).
39. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* **391**, 85–100 (1981).
40. Rae, J., Cooper, K., Gates, P. & Watsky, M. Low access resistance perforated patch recordings using amphotericin B. *J. Neurosci. Methods* **37**, 15–26 (1991).
41. Nieuwkoop, P. D. & Faber, J. *Normal Table of Xenopus Laevis* 2<sup>nd</sup> edn. (North Holland, Amsterdam, 1967).
42. Gaze, R. M., Keating, M. J. & Chung, S. H. The evolution of the retinotectal map during development in *Xenopus*. *Proc. R. Soc. Lond. B.* **185**, 301–330 (1974).
43. Holt, C. E. & Harris, W. A. Order in the initial retinotectal map in *Xenopus*: a new technique for labelling growing nerve fibres. *Nature* **301**, 150–152 (1983).
44. Edwards, J. A. & Cline, H. T. Light-induced calcium influx into retinal axons is regulated by presynaptic nicotinic acetylcholine receptor activity *in vivo*. *J. Neurophysiol.* **81**, 895–907 (1999).
45. Ben-Ari, Y., Khazipov, R., Leinekugel, X., Caillard, O. & Gaiarsa, J. L. GABA<sub>A</sub>, NMDA and AMPA receptors: a developmentally regulated 'ménage à trois'. *Trends Neurosci.* **20**, 523–529 (1997).
46. Lettvin, J. Y., Maturana, H. R., McCulloch, W. S. & Pitts, W. H. What the frog's eye tells the frog's brain. *Proc. Inst. Radio Eng.* **47**, 1950–1961 (1959).
47. Bi, G.-q. & Poo, M.-m. Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *J. Neurosci.* **18**, 10464–10472 (1998).
48. Engert, F. & Bonhoeffer, T. Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* **399**, 66–70 (1999).
49. Maletic-Savatic, M., Malinow, R. & Svoboda, K. Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* **283**, 1923–1927 (1999).