Enhanced Visual Activity In Vivo Forms Nascent Synapses in the Developing Retinotectal Projection

Carlos D. Aizenman1,2 and Hollis T. Cline1

1Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; and 2Department of Neuroscience, Brown University, Providence, Rhode Island

Submitted 29 April 2006; accepted in final form 22 January 2007

INTRODUCTION

Sensory experience is required for the normal development and plasticity of central sensory systems within the brain. The effect of sensory experience on brain development is particularly well studied in the visual system where visual stimuli can be controlled and the central projections are relatively well understood. For instance, visual experience is required for the formation of organized topographic projections of axons within the visual system (Crair 1999). Dendritic arbor structure is also sensitive to changes in sensory experience (Kossel et al. 1997; Sin et al. 2002; Tieman and Hirsch 1982).

Brief periods of visual stimulation in Xenopus tadpoles increase dendritic arbor growth rates and result in increased branch length and branch tip numbers in the arbor (Sin et al. 2002). Expression of green fluorescent protein (GFP)–tagged PSD95 within optic tectal neurons of zebrafish neurons indicates that PSD95-GFP puncta are distributed widely throughout the dendritic arbor and in particular are located at most branch points. Time-lapse images of PSD95-GFP puncta distribution during dendritic arbor growth suggest that newly added branches also form synapses rapidly after the formation of the branch (Niell et al. 2004). These data suggest that neurons in animals that receive bouts of visual experience may have a new population of relatively immature synapses. Furthermore, brief periods of visual stimulation affect retinal axon arbor development by stabilizing axon arbor structure and increasing the brightness of presynaptic cyan fluorescent protein (CFP)–tagged synaptophysin puncta (Ruthazer et al. 2006). These data suggest that visual stimulation may increase the strength of retinotectal synaptic transmission.

A similar visual stimulation protocol that enhances dendritic arbor development also affects tectal cell excitability and responses to subsequent stimuli (Aizenman and Cline 2003; Aizenman et al. 2002). These changes occur through mechanisms requiring intracellular polyamines, where an activity-dependent increase in polyamine synthesis results in an overall decrease of transmission mediated by polyamine-sensitive Ca2+-permeable α-amino-3-hydroxy-5-methyl-4-propiononic acid receptors (AMPARs). Although modulation of AMPA receptors by intracellular polyamines provides a rapid and transient mechanism to normalize synaptic transmission after enhanced activation, it is not clear whether this type of visual stimulation results in other types of synaptic plasticity.

We carried out electrophysiological studies to test whether in vivo visual stimulation strengthens retinotectal synapses and increases synaptogenesis. Studies in the tadpole optic tectum and other experimental systems indicate that immature synapses are characterized by transmission mediated principally by N-methyl-D-aspartate receptor (NMDAR) and that AMPAR are added to the synapse as it matures (Crair and Malenka 1995; Durand et al. 1996; Liao et al. 1995; Wu et al. 1996). Synapses with only NMDAR are “silent” at resting potentials as a result of the voltage-dependent block of the channel by magnesium ions; the addition of AMPAR to synapses renders them functional at resting potentials. Similarly, the fraction of silent synapses, in which transmission is mediated solely by NMDAR, is higher in early stages of synapse formation and decreases as synapses and neurons mature, resulting from the insertion of AMPAR at synaptic sites. Consequently, the fraction of silent synapses and the ratio of AMPA- to NMDA-receptor–mediated transmission can be used as indicators of synaptic maturity. Further strengthening of synapses occurs by insertion of additional AMPARs.

Acknowledgments: We thank C. F. Niell and oligodendrocyte glycoprotein (ODG) antibodies and their expressers were generously provided by K. Kitamura and C. C. Gage, respectively.

Address for reprint requests and other correspondence: H. Cline, Cold Spring Harbor Laboratory, 1 Bungtown Rd./Beckman Building, Cold Spring Harbor, NY 11724 (E-mail: cline@cshl.org). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Developing glutamatergic synapses may be distinguished by several other features: First, some immature synapses show greater facilitation in response to a pair of stimuli (paired-pulse facilitation (PPF)) compared with more mature synapses (Akaneya et al. 2003; Gasparini et al. 2000), presumably because immature synapses have relatively poorly developed vesicle release machinery (Mohrmann et al. 2003; Vaughn 1989), which results in a low probability of release (Pr). In addition, presynaptic Ca$^{2+}$ dynamics change during development, resulting in changes in PPF (Mori-Kawakami et al. 2003). However, this is not the case for all types of synapses. Different types of synapses were previously reported to undergo either no change or a decrease in Pr over development (Chen and Regehr 2000; Lu and Constantine-Paton 2004; der gas 2005), relatively poor envelopment of nascent synapses by glia (Constantine-Paton and Cline 1998; Correa-Gillieron and Caveltier 2003). However, this is not the case for all types of synapses. Different types of synapses were previously reported to undergo either no change or a decrease in Pr over development (Chen and Regehr 2000; Lu and Constantine-Paton 2004; Rohrbough and Spitzer 1999). This may be explained by inadequate clearance of transmitter from the synaptic cleft (Diamond 2005), relatively poor envelopment of nascent synapses by glia (Constantine-Paton and Cline 1998; Correa-Gillieron and Caveltier 1999), or synaptic scaling produced by neural activity (Desai et al. 2002).

We compared these features of retinotectal synaptic transmission in animals that did or did not receive enhanced visual stimulation to determine the effect of visual activity on retinotectal synaptic development.

METHODS

Visual stimulation

All animals were reared under approximately 12-h dark/12-h ambient light conditions, together with other tadpoles of the same brood. Freely swimming stage 48 albino Xenopus laevis tadpoles were treated with a 4-h period of enhanced visual stimulation as described in Aizenman et al. (2002). This was done using a custom-built light chamber consisting of four rows of three green light-emitting diodes (LEDs, λ-max 567 nm; Allied Electronics, Fort Worth, TX). The rows of LEDs were flashed in sequence at 1 Hz, thus creating a simulated motion stimulus. Tadpoles were put in 12-well plates with rearing solution and allowed to swim freely during the 4-h stimulation period. Brains were prepared for electrophysiology immediately after the visual stimulation and were kept for recording for a maximum of 4 h. Control animals were kept in ambient light conditions for the 4-h period before preparing the brains for recording.

Electrophysiology

For whole brain recordings, stage 47–48 albino Xenopus tadpole brains were prepared as described in Wu et al. (1996). Animals were anesthetized in 0.01% MS-222 and brains were cut along the dorsal midline to expose the ventricular surface and dissected into HEPES-buffered extracellular saline (containing, in mM: 115 NaCl, 2 KCl, 3 CaCl$_2$, 3 MgCl$_2$, 5 HEPES, 10 glucose, 0.1 picrotoxin; pH 7.2, osm 255). In some experiments CaCl$_2$ was substituted by 3 mM SrCl$_2$ so that the final CaCl$_2$ concentration was 0.1 mM. Brains were mounted in a submerged perfusion chamber and maintained at room temperature. A bipolar stimulating electrode (FHC) was placed in the optic chiasm and was used to evoke synaptic stimuli. Cells were visualized using a Nikon E600FN light microscope with ×60 water-immersion objective. Recordings were limited to the caudal third of the tectum. Whole cell voltage-clamp recordings were made using glass microelectrodes (8–12 MΩ) filled with intracellular saline (containing, in mM: 100 K-glucosone, 8 KCl, 1.5 MgCl$_2$, 20 HEPES, 10 EGTA, 2 ATP, 0.3 GTP; pH 7.2, osm 255). In some experiments (Figs. 2 and 3) 100 μM spermine was added to the intracellular solution. Typical input resistances averaged around 1.7 GΩ and series resistances averaged 40–60 MΩ (Aizenman et al. 2002; Wu et al. 1996). Signals were measured with an Axopatch 2-D amplifier and digitized using a Digidata 1200 A-D board (both from Axon Instruments, Union City, CA). Traces were recorded using pClamp 8 software and digitized at 10 kHz. Ifenprodil and NBQX were obtained from Tocris; all other chemicals were obtained from Sigma.

Data analysis

AMPA/NMDA ratios were calculated by comparing evoked synaptic responses at −60 mV to measure AMPAR-mediated excitatory post synaptic currents (EPSCs) and at +55 mV to measure the NMDAR-mediated EPSC. The peak AMPA response was used to calculate the AMPAR component and the amplitude between 15- and 25-ms postresponse onset was used to calculate the NMDA component. This time window was chosen because the AMPA component has decayed to <10% of the peak value. Although calculating the NMDA component at this later time window may underestimate the size of the peak response, this method still allows us to calculate relative changes in the AMPA/NMDA ratio. This method was successfully used to detect developmental changes in the AMPA/NMDA ratio in this and other preparations (Cantallops et al. 2000; Isaac et al. 1997; Wu et al. 1996). To calculate the percentage of silent synapses we used minimal stimulation at −60 and +55 mV. Failure rates at both holding potentials were measured and used to calculate the percentage of silent synapses present as described in Liao et al. (1995) and Wu et al. (1996). In paired-pulse experiments an interstimulus interval (ISI) of 50 ms was used unless otherwise specified.

For Sr$^{2+}$ experiments we used a template-based analysis to detect evoked miniature (m)EPSCs (Aizenman et al. 2002; Clements and Bekkers 1997). Templates were determined for every experiment from averaged events. The fit criteria used to identify events are sufficiently stringent that minis that are obviously overlapping would not be detected by the template. Events were captured starting ≥20 ms after the onset of the stimulus to avoid the remaining synchronous response. Events were discarded if they could not be temporally resolved (i.e., occurred within 10 ms of each other). Consequently, most of the events were extracted from the tail end of the response to minimize the possibility that captured events would contain two overlapping mEPSCs. Captured events were then averaged to obtain the average evoked mEPSC amplitude or plotted as cumulative distributions to compare across cells. Data were analyzed using Axograph software (all from Axon Instruments). Mann–Whitney U tests were used for most statistics, unless otherwise specified, and were calculated using InStat by GraphPad Software. All error bars are SE.

All animal experiments were done in accordance with approved IACUC protocols.

RESULTS

To test whether visual stimulation results in formation of new retinotectal synapses and/or maturation of existing ones, we exposed stage 48 tadpoles to a 4-h persistent visual stimulus using a custom-made stimulation chamber (see Aizenman et al. 2002; Sin et al. 2002). We then performed whole cell recordings from tectal neurons using an open brain preparation (Wu et al. 1996) and compared visually stimulated to control animals.
Effects of visual stimulation on preestablished retinotectal synapses

One typical characteristic of developing glutamatergic synapses is an increase in the amount of current carried by AMPAR versus NMDAR (Chen and Regehr 2000; Isaac et al. 1997; Lu and Constantine-Paton 2004; Wu et al. 1996). To test whether visual stimulation increased the maturation of retinotectal synapses, we measured the AMPA/NMDA ratio of evoked retinotectal synaptic responses. We found no significant difference in the AMPA/NMDA ratio between control and visually stimulated tadpoles (control: 2.2 ± 0.3, n = 30; stimulated: 2.7 ± 0.5, n = 27, P = 0.8; Fig. 1A).

Nascent glutamatergic synapses are believed to contain mainly NMDARs (Isaac et al. 1997; Liao et al. 1995; Wu et al. 1996) and to incorporate AMPARs as they mature (Petralia et al. 1999). We looked for changes in the number of NMDA-only synapses after visual stimulation by recording EPSCs evoked by minimal stimulation of one or a few retinotectal axons. We compared the relative failure rate of responses recorded at −60 and at +55 mV and estimated the percentage of activated synapses that contain only NMDARs (Liao et al. 1995). Figure 1B shows two representative sets of recordings. Figure 1C shows the distribution of AMPAR- and NMDAR-mediated EPSCs across all cells while recording under minimal stimulation conditions. Although we did not detect any difference in the percentage of NMDA-only synapses between visually stimulated (32 ± 4%, n = 21) and control (28 ± 4%, n = 28) tadpoles, we did detect an increase in the size of both AMPAR-mediated (control: 7.7 ± 0.2 pA vs. visual stimulation: 10.11 ± 0.3 pA, P < 0.001) and NMDAR-mediated (control: 5.44 ± 0.1 pA vs. visual stimulation: 7.7 ± 0.2, P < 0.001) EPSCs evoked by minimal stimulation (Fig. 1D). This indicates that retinotectal synaptic drive evoked by minimal stimulation is strengthened after visual stimulation.

Visual stimulation results in the formation of nascent synapses

As a consequence of an immature presynaptic release machinery, some immature synapses have a low Pr and therefore display more PPF (Akaney et al. 2003; Gasparini et al. 2000). To test whether this is true for the Xenopus retinotectal synapse, we compared the amount of PPF between developmental stage 42/43 tadpoles and the more mature stage 48 tadpoles at a range of ISIs. At the earlier developmental stages, retinal axons have recently innervated the optic tectum and are establishing new synapses, so the majority of stimulated retinotectal synapses are likely to be immature. Stage 42/43 tadpoles show significantly greater PPF at a range of ISIs than their more mature, stage 48 counterparts (P < 0.05 at 30- to 90-ms ISIs; Fig. 2), consistent with a developmental increase in Pr.

We used a paired-pulse protocol to test whether visual stimulation induced the formation of a nascent population of retinotectal synapses, by taking advantage of the lower Pr in immature synapses. By comparing the synaptic responses to the first stimulus (which we propose mainly activates established synapses with a higher Pr) with responses to the second stimulus of the pair (which would activate both established synapses and low Pr synapses) we were able to compare properties of established versus nascent synapses. We measured the amount of PPF at a range of ISIs in control and visually stimulated tadpoles. Overall, visually stimulated animals show significantly more PPF (Fig. 2; control: 1.66 ± 0.1 vs. visual stimulation: 2.41 ± 0.28, P = 0.036). Although the maximum PPF was achieved at different ISIs for different cells, on average, the greatest facilitation was observed at an ISI of 50 ms (control: 1.46 ± 0.12 vs. visual stimulation: 2.18 ± 0.22, P = 0.007), so we used a 50-ms ISI for subsequent experiments.

J Neurophysiol • VOL 97 • APRIL 2007 • www.jn.org
If the increase in PPF after visual stimulation arises from recruitment of a population of nascent synapses with low Pr, one prediction is that the synaptic responses to paired stimuli should have other characteristics of immature synaptic contacts, such as a low AMPA/NMDA ratio. We compared AMPA/NMDA ratios evoked by the first and second stimuli in control and visually stimulated tadpoles. We measured responses to the first and second stimuli taken sequentially at \( +55 \) and \( -60 \) mV in control animals and those with 4-h visual experience (Fig. 3A). When the responses at \( +55 \) mV are normalized to those recorded at \( -60 \) mV, we find that the NMDAR responses are significantly larger in the paired response compared with the first response in neurons from visually stimulated animals (Fig. 3A). That is, the AMPA/NMDA ratio of responses evoked by a paired stimulus is significantly smaller than the AMPA/NMDA ratio of responses evoked by a single stimulus in visually stimulated, but not control, animals (Fig. 3B, C; \( P < 0.05 \)). This is not attributed to an unblocking of \( \text{Ca}^{2+} \)-permeable AMPAR by elevated levels of polyamines (and therefore an increase in AMPAR amplitudes) because recordings were taken with a saturating spermine concentration in the pipette (Aizenman et al. 2002, 2003).

**Quantal properties of nascent synapses**

Our minimal stimulation experiments show an increase in AMPA response amplitudes after visual stimulation. Furthermore, during early development of *Xenopus* tadpoles, synapses in the optic tectum and spinal cord reportedly have large quantal size (Pratt and Aizenman 2005; Rohrbough and Spitzer 1999). We therefore tested whether visual stimulation affects quantal size of preexisting synapses and nascent synapses that form after visual experiences. To specifically record individual synaptic events from the retinotectal pathway, we substituted external \( \text{Ca}^{2+} \) with \( \text{Sr}^{2+} \) and stimulated the optic chiasm. \( \text{Sr}^{2+} \) desynchronizes synaptic vesicle release, enabling one to study individual quantal events evoked from a specific pathway (Oliet et al. 1996; Xu-Friedman and Regehr 2000). Retinotectal synaptic events evoked in the presence of 3 mM \( \text{Sr}^{2+} \) in control and visually stimulated animals appear as asynchronous release of individual evoked mEPSCs (Fig. 4A, left traces). The amplitudes of the desynchronized evoked mEPSCs are comparable to the amplitudes of spontaneous mEPSCs (Aizenman et al. 2002). We detected a small but significant decrease in the amplitudes of evoked mEPSCs after visual stimulation (control: \( 4.5 \pm 0.1 \) pA, \( n = 13 \); visual stimulation: \( 3.7 \pm 0.1 \) pA, \( n = 14, P = 0.05 \); Fig. 4B), consistent with the decrease in spontaneous mEPSC amplitude previously observed after visual stimulation, which is caused by an activity-dependent increase in polyamine synthesis (Aizenman et al. 2002).

The decrease in evoked mEPSC amplitude after visual stimulation provides additional support to the idea that the increase in EPSCs evoked by minimal stimulation reported in Fig. 1 is likely the result of an overall increase in the number of activated synapses from a given axon.

To study the quantal properties of nascent synapses that emerge after visual stimulation, we compared mEPSCs evoked by the first synaptic stimulus versus those evoked by the paired synaptic stimulus (50-ms ISI; Fig. 4A, right traces). In control tadpoles, the first and paired stimuli evoked mEPSCs that had very similar amplitude distributions (Fig. 4C; average ampi-
tude first 4.4 ± 0.3 pA vs. paired 4.5 ± 0.2 pA, n = 13, P = 0.5, Wilcoxon). In visually stimulated tadpoles mEPSCs evoked by paired stimuli were larger than those evoked by the first stimulus (Figs. 4D and 5A, left; average amplitude first 3.6 ± 0.2 pA vs. paired 4.4 ± 0.4 pA, n = 14, P = 0.0017, Wilcoxon).

To test whether the increase in amplitude of the mEPSCs evoked by the paired-pulse stimulus was sufficient to account for the increase in PPF after visual stimulation, we estimated the total number of events released by the first and paired stimuli. We first calculated the absolute area under the curve of the total asynchronous response induced by single and paired stimuli. This measure also showed more PPF after visual stimulation [Fig. 5A, middle; PPF (area) = 6.7 ± 2 control vs. 11.1 ± 2 visual stimulation, P = 0.019]. Next we expressed PPF in terms of the number of quanta contained in the total.

FIG. 3. Visual stimulation enhances AMPA/NMDA ratios of paired responses. A: averaged retinotectal responses (10 traces) recorded at +55 and −60 mV from control and visually stimulated animals. Amplitude of the paired response has been normalized to the first response at −60 mV. NMDA component in the paired response is relatively larger in visually stimulated animals. B: AMPA/NMDA ratios for first and paired responses in control and visually stimulated animals. Dots represent individual neurons; triangles are averages. C: average change in AMPA/NMDA ratios between first and paired responses for both conditions. *P < 0.05, Mann–Whitney.

FIG. 4. Visual stimulation enhances PPF of mEPSCs evoked in the presence of Sr2+. A: sample traces of retinotectal responses in 5 mM Sr2+ evoked by the first (left) and paired (right) stimuli. Paired stimuli recorded from visually stimulated animals result in a large increase in the number and size of mEPSCs evoked. Insets: average amplitudes (control: n = 13; visual stimulation: n = 14). B: cumulative probability histogram of all mEPSCs evoked by single stimuli in controls (solid line) and visually stimulated (dotted line) animals. Events are smaller in visually stimulated animals. C and D: cumulative probability histogram of all mEPSCs evoked by the first (solid line) and paired (dotted line) stimuli in controls (C) and visually stimulated animals (D). Events evoked by paired stimuli are larger than events evoked by single stimuli in visually stimulated animals but not in controls. Insets, C and D: averages of 13 and 14 traces, respectively.
asynchronous response. To do this we divided the area under the curve of the total asynchronous response by the average area of the evoked mEPSCs (for a given cell and stimulus conditions) to get an estimate of the total number of events in the response. We found a significant increase in facilitation measured as the estimated number of events evoked by the second stimulus compared with the first stimulus (Fig. 5A, right; PPF = 6.1 ± 2% control vs. 10.1 ± 2% visual stimulation, \( P = 0.022 \)). This number was essentially comparable to the PPF calculated by the absolute areas, suggesting that a majority of the increase in facilitation seen in visually stimulated tadpoles can be explained by an increase in the number of synaptic events evoked by paired-pulse stimulation.

To test whether the inputs that express the largest increase in mEPSC amplitude are also the ones that overall have more PPF, we compared these two parameters and found that they were positively correlated (Fig. 5B; Spearman \( r = 0.513, p < 0.01 \)). This correlation means that in cells where we see an increased number of events evoked by paired stimuli, we also see bigger events evoked by paired stimuli, further supporting the idea that these bigger events represent a separate population of synaptic inputs that emerges after visual stimulation and is preferentially activated by paired stimuli. The histograms shown in Fig. 5C are normalized amplitude histograms of all the evoked mEPSCs shown in Fig. 4, comparing single and paired-pulse evoked responses. The graph below the histogram is the change in the number of events of a given amplitude evoked by paired versus single stimuli. In control and visually stimulated tadpoles the peak of the histogram remains the same, yet after visual stimulation there is a greater number of large events evoked by paired-pulses. Our favored interpretation is that this effect represents an unmasking of a subpopulation of immature synapses with a low Pr and larger quantal size.

**Discussion**

Our data suggest that 4 h of in vivo visual stimulation results in the emergence of a new population of synapses, which have a lower AMPA/NMDA ratio, a larger quantal size, and a lower Pr than those of more mature synapses. The AMPA/NMDA ratio of established synapses remains unaffected, despite an overall increase of synaptic strength in these synapses. This conclusion is based on two important experimental assumptions.

The first assumption is that immature retinotectal synapses have low Pr. This is supported by data from stage 42/43 tadpoles that show a greater degree of PPF. After visual stimulation, the overall Pr decreases because we are now activating a mixed synaptic population consisting of established synapses with higher Pr and nascent synapses with low Pr, PPF was previously proposed to reflect release probability (Zucker and Regehr 2002), although this interpretation may be clouded by some confounding factors. One possible confound could be that visual stimulation increases polyamine synthesis, which increases polyamine-dependent unblocking of Ca\(^{2+}\)-permeable AMPARs and increases facilitation by use-dependent unblocking of AMPAR (Aizenman et al. 2002). To rule out a contribution of activity-dependent polyamine effects, the PPF experiments were done in the presence of saturating concentrations of intracellular polyamines. A second possible confound is that the facilitation of the AMPA response is underestimated as a result of AMPAR desensitization. This would create the appearance that NMDAR-mediated currents facilitate less than AMPAR-mediated currents and that the
responses to the second stimulus would therefore have a lower AMPA/NMDA ratio. If desensitization was a factor we would expect larger EPSCs to have less PPF than smaller EPSCs. In our data, the size of the initial EPSC was not significantly correlated with the level of PPF (Spearman $r = -0.24$, $P = 0.27$). Furthermore, if AMPAR were desensitized during PPF after visual stimulation, we would expect that after visual stimulation the cells with more PPF would have overall smaller AMPA/NMDA ratios in response to the second stimulus. Across this population of cells, the amount of PPF in a given cell did not correlate to the AMPA/NMDA ratio evoked by the second pulse (Spearman $r = 0.35$, $P = 0.11$). Taken together, it seems unlikely that AMPAR desensitization accounts for our observations, although we cannot completely rule out AMPAR desensitization, particularly at ISIs <50 ms, where the change in PPF does not reach statistical significance (see Fig. 2).

The second assumption is that in response to pairs of stimuli, the second stimulus activates a greater proportion of immature synapses than the first. If Pr is low in immature synapses, then responses to the second stimulus will include a greater contribution from immature synapses. This follows from the classical interpretation of PPF, in which the first stimulus results in accumulation of residual Ca$^{2+}$, which then facilitates release to the second stimulus (Zucker and Regehr 2002). This would mean that if a certain subset of stimulated axons contains immature synapses, these are more likely to fail during the first stimulus than during the second stimulus, whereas axons with more mature synapses would release during both stimuli. Therefore the response to the second stimulus contains a greater proportion of low Pr synapses than does the response to a single stimulus. This is validated by the paired-pulse data collected in the presence of Sr$^{2+}$ (Fig. 4), where the response to paired stimuli contains a greater number of events than the response to a single stimulus. After visual stimulation the amplitude distribution of these responses is significantly different, suggesting that paired stimuli recruit a novel set of low Pr synapses. In this study, tadpoles we have shown that immature synapses have both low Pr (Fig. 2) and larger quantal amplitude (Pratt and Aizenman 2005), consistent with the interpretation that, after visual stimulation, the novel pool of synapses activated by paired pulses consists of immature synapses.

Another possible confound is whether the increase in PPF in visually stimulated tadpoles could be the result of an increase in fiber excitability in response to the second stimulus. Although this possibility is not ruled out by our data, it would not explain why the mEPSCs evoked by the second stimulus are different (i.e., larger) than those evoked by the first stimulus, unless a selective enhancement of a different subclass of axons occurs. This would still be consistent with the unmasking of a novel synaptic population. However, at these early developmental stages, differences in retinal ganglion cell (RGC) axon classes are yet to become clearly defined (Chung et al. 1975), making this interpretation less favorable.

Comparison to other immature synapses

Our data show that the novel population of retinotectal synapses induced by visual stimulation has a low Pr and a low AMPA/NMDA ratio, suggesting that they are immature. These findings are consistent with features of other nascent populations of synapses. Studies in neonatal hippocampus showed that new synapses have very low Pr, often making them appear as functionally silent (Gasparini et al. 2000; Renger et al. 2001). Furthermore, in visual cortical slices, silent synapses were also found to have very low release probabilities (Akaneya et al. 2003). The low Pr is consistent with lower numbers of synaptic vesicles (Jones 1983) and incomplete vesicle cycling seen in immature presynaptic terminals (Mohrmann et al. 2003). As the presynaptic release machinery matures, the number of docked vesicles increases and release probability increases (Renger et al. 2001). Immature synapses are also known to have a low AMPA to NMDA ratio, arising from the fact that initial transmission in most glutamatergic synapses is believed to be mediated mainly by NMDAR and as the synapse matures AMPARs become incorporated (Isaac et al. 1997). Although alternative possibilities exist, such as the development of synapses without NMDAR (Carroll and Zukin 2002; Gomperts et al. 2000) or the presence of a population of unstable AMPAR in nascent synapses (Rumpel et al. 2004; Xiao et al. 2004), a sequence in which transmission at new synapses is mediated principally by NMDAR followed by the addition of functional AMPAR to synaptic sites appears to occur in the optic tectum and at the majority of glutamatergic synapses.

The fact that evoked mEPSCs in nascent synapses are large is consistent with studies on developing spinal interneurons in Xenopus tadpoles and in immature visual cortex of rats, which show that mEPSCs are larger early in development (Desai et al. 2002; Rohrbough and Spitzer 1999). This is also consistent with the observation that retinotectal synapses in stage 42 Xenopus tadpoles, a stage where retinal axons are initially innervating the tectum, mEPSCs are consistently larger than at later developmental stages when the tadpoles have been raised under controlled light conditions (Pratt and Aizenman 2005).

Our observation stands in contrast to other studies (Chen and Regehr 2000; Lu and Constantine-Paton 2004) in which no change in Pr is seen in retinogeniculate and retinocollicular synapses after eye opening. We propose that one key difference is that our experimental manipulation caused the sudden addition of many nascent synapses at one time, allowing us to study their electrophysiological properties, whereas the population of synapses in the other developmental studies was more heterogeneous. The fact that we do not detect features of immature synapses when we compare single and paired-pulse responses in control tadpoles suggests that this nascent stage of synapse formation is very transient and normally represents a small proportion of synapses in the retinotectal system of the tadpoles used in our studies. Our data, however, cannot be fully reconciled with a study of thalamocortical synapses that suggests that silent synapses have a higher Pr than that of functional ones, despite the fact that PPF of AMPAR-mediated currents decreases with age (Yanagisawa et al. 2004). This suggests that synapses in different brain structures may develop with different presynaptic dynamics.

Activity-dependent effects on retinotectal cell structure and function

These experiments, together with previously published work (Aizenman et al. 2002, 2003; Ruthazer et al. 2006; Sin et al. 2002), indicate that in vivo visual stimulation has multiple...
ACKNOWLEDGMENTS

We thank members of the Cline and Aizenman laboratories for helpful discussions and comments on the manuscript.

REFERENCES

Constantine-Paton M, Cline HT. LTP and activity-dependent synaptogenesis: the more alike they are, the more different they become. Curr Opin Neurobiol 8: 139–148, 1998.

GRANTS

This work was supported by the National Eye Institute and the National Institutes of Health Directors Pioneer Award to H. T. Cline and an Epilepsy Foundation grant to C. D. Aizenman.


