

This manuscript is in press in Biological Cybernetics. I've added an explanatory paragraph on NMDA receptors for this class, and I've put some material in small print that I think you can afford to skip. Please feel free to ask questions about unfamiliar terminology at the start of class.

SBU

The instructive role of binocular vision in the *Xenopus* tectum

Susan B. Udin

Department of Physiology and Biophysics, Program in Neuroscience, State University of New York

tel: 716-829-3571

fax: 716-829-3575

sudin@buffalo.edu

Abstract:

This review presents the neurobiology underlying the development of the frog optic tectum, the brain structure where the two separate inputs from the two eye are combined into a single, integrated map. In the species *Xenopus laevis*, binocular visual information has a dramatic impact on axon growth and connectivity, and the formation of binocular connections in this system provides a rich basis for both theoretical and experimental investigations.

Keywords: acetylcholine, glutamate, binocular, nucleus isthmi, nitric oxide, NMDA, synapse

ACh: acetylcholine

EPSC: excitatory postsynaptic current

GABA: gamma-aminobutyric acid

NMDA: N-methyl-D-aspartate

STDP: spike timing dependent plasticity

Run-in heading: Binocular vision in developing tectal circuits

1. The problem for binocular vision circuits

Vertebrates have two eyes, and each eye independently views the world. Some of that view is binocular, i.e., part of the visual field is seen by both eyes. The two eyes' independent views have to be integrated in the central nervous system so that a single, orderly representation of the scene can be constructed. This challenge is met by multiple mechanisms that enable axons make connections in proper locations during normal development; gradients of molecules such as ephrins and eph receptors provide initial topographic order (Braisted et al., 1997; Mann et al., 2002), and visual activity refines the registration of the two maps (Udin and Grant, 1999).

The South African claw-toed frog *Xenopus laevis* offers a dramatic example of the role of visual experience in determining the pattern of axonal connections in the central nervous system. The projection that relays input from the eye to the tectal lobe on the contralateral side of the body has a minimal requirement for visual input; in fact, it develops quite well in the dark. In contrast, the projection that brings input from the other (ipsilateral) eye to the tectum is totally dependent upon binocular visual input in order to form a retinotopic map that matches the map from the contralateral eye. In fact, the dependence upon visual input in this species is so dominant that it can override molecular gradients, as described below. The model that will be presented below is based on the assumption that correlated firing of ipsilateral and contralateral axons triggers a sequence of events that stabilizes the ipsilateral connections. The search for the specific anatomical and physiological mechanisms of this phenomenon has revealed many surprises and posed an array of still unsolved problems.

2. The circuit in mature *Xenopus*

The circuit that we will be examining in this review is illustrated schematically in Figure 1A. Visual input reaches the *Xenopus* tectum from the contralateral eye directly, via the retinotectal projection. Visual input from the ipsilateral eye, however, reaches the tectum via an indirect route. We illustrate this indirect route with the projections that begin with ganglion cell b (open circle) in the left eye. This cell's axon projects to the right tectal lobe, where tectal cells relay the message to the nucleus isthmi, a midbrain structure. Some isthmic cells send a reciprocal projection back to that lobe but others project across the midline to the left tectal lobe, where they form the ipsilateral visual map (Gruberg and Udin, 1978). Under normal circumstances, this ipsilateral map is in register with the retinotectal contralateral map. For example, retinotectal cell B (stippled circle) and isthmotectal cell b (black circle), which both respond to a stimulus at visual position B, terminate at the same site in the left tectal lobe.

3. Plasticity

When one observes the normal development of *Xenopus*, it becomes obvious that the binocular system confronts a serious challenge, since the eyes of tadpoles are positioned laterally, with little binocular overlap, while the eyes of the adult are positioned dorso-frontally, with approximately 160° of overlap (Fig. 2). During this period, the direct retinal projection occupies the entire extent of the tectum, and the proportion that is binocular increases from a small sliver at the front to almost the entire lobe as the animals mature (Fig. 2). The isthmotectal that mediate the binocular maps adapt to this change by changing their connections during the months-long period of eye migration. Initially, they enter, grow far into the tectum, make synapses, but branch very little (Fig. 3A) (Udin and Fisher, 1985; Udin et al., 1992). Axons then begin to make arbors at the front of the tectum, establishing the first territory of the binocular map (Fig. 3B). As binocularity increases, these axons shift their arbors caudally, and their places are taken by arbors of other axons (Udin and Fisher, 1985).

What mechanisms guide the axons during this period? Do molecular cues help to lead the axons to proper locations, or do visual cues instruct the axons, or do both play roles? Of course, both are involved, with non-visual cues helping to target the axons initially, particularly along the mediolateral axis of the tectum, and binocular visual cues later determining the final positions of the isthmotectal arbors.

3.1 Eye rotation

A very clever method to distinguish when the axons are following molecular cues and when they are being governed by visual cues was pioneered by Keating and his colleagues (Gaze et al., 1970). They realized that rotating one eye would produce a mismatch in the visual inputs from the two eyes. One tectal lobe would have a normally-oriented retinotectal map and a rotated isthmotectal map, while the other lobe would have a rotated retinotectal map and a normally-oriented isthmotectal map. If the isthmotectal axons persisted in following normal molecular cues, then they would grow to their normal sites and the maps from the two eyes would develop with different orientations. This in fact is roughly what happens at first. Electrophysiological and anatomical experiments have demonstrated that isthmotectal axons initially grow towards their normal termination zones (Grant and Keating, 1992; Guo and Udin, 2000).

This pattern of behavior is illustrated schematically for one axon in Fig. 4B, where an isthmotectal cell that responds to visual stimuli at visual field B initially grows to its usual terminal zone, where it now encounters the retinotectal axon that -- due to the rotation of the right eye -- responds to stimuli at visual field A. The isthmotectal axon's morphology at this point is not greatly different from normal, as can be seen by a comparison of the drawings in the right panels of Fig. 4B and those of 4A, which shows one photograph and one drawing of normal isthmotectal axons. After several weeks, the mismatched visual input leads to abnormal patterns of growth. The axons begin to develop new trajectories and to find new termination zones that bring the maps from the rotated and unrotated eyes into register (Fig. 4c). Eventually, the axons lose their original connections, with their odd trajectories bearing witness to their original paths (Fig. 4d).

How are these remarkable changes accomplished? How do isthmotectal axons use visual input during normal development to shift their connections as the eyes move dorsally in the head, and how do the axons use visual input in the more extreme condition of eye rotation to establish connections in new zones of the tectum? Initially, we envisioned a typical NMDA receptor-dependent spike timing dependent plasticity (STDP) mechanism, with isthmotectal and retinotectal axons converging onto the same tectal cell dendrite; retinotopically matching connections would be reinforced, and mismatched connections would be weakened. The evidence, however, has forced us to consider a more complicated model involving paracrine interactions in which isthmotectal activity reinforces retinotectal activity via presynaptic nicotinic receptors, as will be explained below.

[NMDA receptors are molecules that are part of the family of glutamate receptors. They mediate synaptic transmission. When an axon releases glutamate, it can bind to a glutamate receptor on another neuron., The receptor then responds by changing shape and causing some sort of change in the postsynaptic neuron. In the case of NMDA-type glutamate receptors, they admit calcium into the cell. This effect is very important, because calcium can trigger many biochemical and structural changes. NMDA receptors also are interesting because they only admit calcium if the postsynaptic cell is already strongly activated. In engineering terms, they can be thought of as "and gates."]

4. NMDA receptors

First we will examine the pivotal role of NMDA receptors. A cornerstone of most models of STDP is the activation of NMDA receptors, and the isthmotectal system conforms splendidly in this respect.

4.1 Effect of blocking NMDA receptors

Reorganization of the isthmotectal projection after eye rotation is prevented by blocking NMDA receptors during the critical period (Fig. 5) (Scherer and Udin, 1989).

4.2 Acceleration of plasticity by NMDA application

Moreover, map reorganization can be accelerated by chronic application of NMDA itself during the early stages of the critical period (Fig. 6) (Bandarchi et al., 1994).

4.3. Restoration of plasticity by NMDA application

In addition, plasticity, which is normally lost in adults, can be restored by chronic application of NMDA (Fig. 7) (Udin and Scherer, 1990).

Thus, any model that is constructed to help understand this system must incorporate NMDA receptors. There is an substantial body of literature establishing that those receptors are located on *Xenopus* tectal cells (Wu et al., 1996) (Lin and Constantine-Paton, 1998). A fully adequate model needs to incorporate information on the specific amounts and types of NMDA receptor subunits that are present in the tectum at specific periods of maturation, since quantitative and qualitative changes in NMDA receptors are strongly associated with control of plasticity in other systems (Sawtell et al., 2003; Yoshii et al., 2003). This information is not yet available for *Xenopus* but is currently under investigation in the author's laboratory.

4.4 Downstream effects of NMDA receptor activation

One reasons that NMDA receptors are so central to plasticity mechanisms is that they generally do not allow passage of ions through their channel unless the membrane in which they reside is substantially depolarized; therefore, weak, asynchronous stimuli rarely activate NMDA receptor currents. In addition, significant amounts of calcium pass through open NMDA receptor channels, and the resulting changes in intracellular calcium trigger innumerable events ranging from phosphorylation of nearby molecules to alterations in gene transcription. One such class of events is the release of molecules that are termed "retrograde messengers" because of their ability to leave the postsynaptic cell and change the state of the presynaptic cell. We will return to this last topic later because it is central to our model of how binocular activity stabilizes isthmotectal axon branches.

5. *Timing*

Elegant experiments from investigators such as Poo (Zhang et al., 1998) have revealed crucial time windows, typically 20 msec in duration for the visual system, for spike-timing dependent plasticity (STDP) in several model systems, notably the early developing retinotectal projection and the neocortex (Markram et al., 1997): if an axon fires within 20 msec prior to a cell onto which it synapses, the synaptic connection will become stronger in the sense of producing a larger EPSC. Conversely, if the axon fires within 20 msec after the cell, the connection becomes weaker. More recent studies are refining this model, but the 20 msec window has shown up with remarkable robustness in a steadily increasing number of systems (Bi and Rubin, 2005).

5.1 *Timing in the retinotectal system*

A digression will help to clarify the STDP idea in the context of topographic map formation. The mechanism works well to explain how visually-evoked activity can promote refinement of the primary retinotectal map. In young *Xenopus*, retinotectal axons make errors in their initial connections; molecules such as Ephs and Ephrins ensure that most connections are in approximately the right location, but some branches find themselves in the wrong tectal locations (Fig. 8). Visual activity can refine this crude pattern. Visually-evoked firing can both stabilize the more numerous population of correct connections and destabilize the minority population of incorrect connections. When a visual stimulus activates the "correct" inputs to a tectal cell, their essentially simultaneous activity works cooperatively to fire the postsynaptic cell and thereby to trigger stabilization events. On the other hand, a topographically incorrect input has a different receptive field location and will therefore probably not be firing at the same time and will not be stabilized. Those stray connections will sometimes fire within 20 msec after the tectal cell has spiked; they will gradually be weakened and will retract.

5.2 *Timing in the binocular system*

This model does not work so well with the isthmotectal system of *Xenopus*. One major problem is the built-in time delay between the arrival of activity from the two eyes at the tectum. As Fig. 1 illustrates, the contralateral eye's input comes directly, via the retinotectal projection, but the ipsilateral eye's input comes indirectly, via a relay from the opposite tectal lobe through the nucleus isthmi. The ipsilateral response to a flashing stimulus is thus delayed by a minimum of 10 msec relative to the first contralateral spikes (Scherer and Udin, 1991). This is an appreciable period of time when put in the context of the 20 msec window described above. It is entirely possible that the tectal cell would have

spiked during that first 10 msec, so that the isthmotectal activity might arrive "too late." However, recent data and models indicate that pairs or triplets of spikes, some of which arrive after the tectal cells spikes, may promote events leading to plasticity (Rubin et al., 2005). Alternatively, the window may be greater than 20 msec. As the anatomical studies below reveal, though, this question becomes even more complicated when the detailed connectivity is considered.

6. *Convergence*

The assumption implicit in the discussion thus far is that isthmotectal axons make their final connections based on the correlation between their action potential activity and that of the retinotectal axons. In other words, we postulate that when a visual stimulus activates retinotectal axons and branches of isthmotectal axons in the same tectal locations, those isthmotectal branches will be stabilized. But how do those isthmotectal axons "know" that the retinotectal axons are firing at about the same time? What is the anatomical basis of this correlation process? We initially assumed that both sets of axons would simply synapse upon the same tectal cells, so that when they fired in a correlated manner, the summed inputs would strongly activate the target cell. That cell would, in turn, send a retrograde messenger to strengthen the isthmotectal axon. We were wrong.

6.1 *Anatomy: electron microscopy*

We used electron microscopy to determine whether isthmotectal and retinotectal axons converge onto the same tectal cell dendrites (Rybicka and Udin, 2005). We found no cases of convergence, indicating either that the two sets of inputs project to sufficiently spatially separated locations that we failed to identify their convergence, or they simply do not terminate onto the same sets of postsynaptic cells. The latter interpretation seems more likely, since there are many retinotectal synapses very close to the isthmotectal synapses: those retinotectal synapses just are not on the same dendrites as the isthmotectal synapses (Fig. 9A). To make matters worse, one cannot easily solve this problem by postulating that the retinotectal axons activate tectal cells that then synapse on the cells that get isthmotectal input. The reason adding this extra synapse does not work is that most of the synapses that converge with isthmotectal synapses are GABA-immunoreactive, i.e., inhibitory (Fig. 9B). Therefore, a cell that is excited by the retinotectal axons would inhibit the cell that receives input from the nucleus isthmi.

6.2 *Acetylcholine receptors*

How then do these two sets of axons interact, if not by terminating on the same target dendrites? We propose that one important route is via release of acetylcholine from the isthmotectal axons and excitation of the retinotectal terminals by that acetylcholine. Retinotectal axons have nicotinic

acetylcholine receptors (Sargent et al., 1989), and activation of those receptors causes increases in intraaxonal calcium levels (Dudkin and Gruberg, 2003; Edwards and Cline, 1999) and increased glutamate release (Titmus et al., 1999). We hypothesize that the additional glutamate release promotes additional opening of postsynaptic NMDA receptors and consequent release of a retrograde messenger that stabilizes the isthmotectal terminal (Fig. 10).

6.3 Retrograde messengers

According to the model that our data suggest, cells that receive retinotectal input (but not necessarily isthmotectal input) release a retrograde messenger when the cells are sufficiently activated to open their NMDA receptors. The threshold would be reached when the baseline level of retinotectal glutamate release is boosted by isthmotectal acetylcholine, and this confluence of events would occur only when the two sets of axons fire in a temporally correlated manner. That temporal correlation would generally be a result of the axons having overlapping visual receptive fields. Therefore, a visual stimulus would trigger transmitter release from both sets of axons, and the situation would promote release of the retrograde messenger.

What is the identity of this retrograde messenger? Among the molecules that have been examined for such a role in the tectum are brain-derived neurotrophic factor (BDNF) (Hu et al., 2005), arachidonic acid (Schmidt et al., 2004), and nitric oxide (Renteria and Constantine-Paton, 1999). In order for such molecules to have an impact on isthmotectal axons, the axons must have appropriate receptors or other molecules that can respond to the messengers. We have obtained preliminary results (unpublished) that support the observations of Allaerts et al (Allaerts et al., 1998) that tectal cells synthesize nitric oxide, and we have obtained preliminary results showing that isthmotectal axons express cGMP (Allaerts et al., 1998), a molecule that is synthesized in response to nitric oxide. Thus, nitric oxide is a viable candidate for further testing as a retrograde stabilizing messenger.

7. The time delay problem

The model that our data have suggested has unusual temporal characteristics. As mentioned above, there is a 10 msec delay between the initially arrival of the first spikes from the contralateral and ipsilateral eyes, but the hypothesis that the communication between the retinotectal and isthmotectal axons depends upon paracrine cholinergic transmission adds further complication to the story. Paracrine transmission is a type of neuronal communication that occurs by diffusion of transmitter through

relatively large volumes of extracellular space, beyond the normal confines of the synaptic cleft. As shown in Fig. 10, another time delay must now be added to allow for diffusion of acetylcholine to the retinotectal terminals from the isthmotectal axons.

7.1 Possible role of choline

A second aspect of the diffusion/timing problem is that the acetylcholine is vulnerable to breakdown by acetylcholinesterase, which is found in large amounts in the tectum (Contestabile, 1976; Udin and Fisher, 1985). However, one of the breakdown products, choline, is itself an agonist of the type of nicotinic acetylcholine receptors found in the tectum (Albuquerque et al., 1997; Papke et al., 1996). Therefore, either acetylcholine or choline can serve to communicate from the isthmotectal axon to the retinotectal terminal.

A third factor in the timing problem is therefore contributed by the ability of choline to act as a transmitter, since the duration of the message will be influenced by the time that is required for acetylcholine to be broken down and for choline to be taken up by neurons (Yamamura and Snyder, 1972). Neither of these parameters has been investigated in the tectum.

8. Volume vs point communication

Once this type of paracrine communication described above is added to the model, we have to start thinking in terms of volumes of contact rather than the points of contact that characterize conventional synaptic communication. Each point of acetylcholine (ACh) release could potentially influence multiple retinotectal axons, and one can envision a situation in the which several isthmotectal branches or axons would have synergistic effects if they fired in a correlated manner in a localized tectal volume; the combined ACh generated by multiple nearby sources could easily have qualitatively different effects from the ACh generated by a single, isolated branch. This possibility is particularly likely in light of recent observations that isthmotectal axons themselves have acetylcholine receptors (Yan et al., 2006). A cluster of isthmotectal axons with correlated activity might therefore create such a large pool of ACh that their terminals in turn would release additional ACh, in turn adding to the effect on the ACh receptors on the retinotectal terminals. However, until more is known about the specific subtypes of receptors in *Xenopus* and their desensitization characteristics, we cannot be certain about the temporal aspects of such effects.

8.1 Branch area

An important point to bear in mind is that these axons ultimately mature to form arbors with multiple branches occupying an area of about 100 x 200 square microns in a tectum that is only 1500 microns wide in an adult. (Udin, accepted for publication). Although they are quite flat and can be considered effectively two-dimensional, they certainly are not the dimensionless characters that populate so much of our thinking. If the 1500 micron width of the tectum is equivalent to 180° of visual field, then 100 microns of arbor might be thought of as spanning 12° of visual angle, but the situation is even less clear-cut, since each point on the tectum receives input from a cluster of retinal ganglion cells which respond to a substantial zone of the visual field. (The exact dimensions depend upon the specific lamina.)

8.2 Visual field area

Not only does each isthmotectal axon occupy an appreciable percentage of the tectum, but it also relays information about a substantial percentage of the visual field. Again, we need to deal with the fact that each axon does not respond to a point in the visual field but to a volume of the visual field, although for simplicity one may consider just the angular area of the cell's receptive field. For ipsilateral receptive fields, the size is quite large, averaging about 20° in diameter in juvenile *Xenopus* (Keating and Kennard, 1987). The retinotectal axons from which these axons are to get their topographic information have smaller receptive fields, averaging about 10° (Keating and Kennard, 1987). To add to the complexity is the fact that axons do not "tile" the tectum; instead, their arbors overlap, so axons with slightly different receptive fields will have arbors that are very similarly situated, with significant overlap. Thus, rather than a true point-to-point

concept, the true situation is area-to-area. A stimulus of non-zero dimensions activates a finite region of the retina, which in turn activates a substantial area of the tectum. The degree to which different axons in the tectum are activated depends in part on where the stimulus falls within their receptive fields, so in the untidy world of the physiologist, one finds that a stimulus produces strong firing in some axons, moderate firing in others, and just barely discernable firing in still others. We need to know whether these all contribute to stabilization of connections. Moreover, we need to incorporate the question of stimulus movement into our models, since most stimuli of relevance to the tectum are moving stimuli.

9. Two different questions: The size of an arbor versus the location of the arbor

When considering the forces that lead to formation of isthmotectal axon terminal arbors, there are three separable questions: 1) where does the arbor form? 2) how large is the arbor? and 3) how many branches does the arbor contain? As the eye rotation experiments show, binocular visual input plays a determining role in establishing where arbors ultimately form. However, in regard to question #2, we recently have found that visual input is not essential for isthmotectal axons to produce arbors of normal dimensions (Udin, accepted for publication). The arbors of the axons in dark-reared *Xenopus* are no different in size from those in normally-reared animals. Perhaps arbor size in dark-reared animals is controlled by the synergistic effects of acetylcholine described above: an action potential in an isthmotectal axon causes ACh release by a critical mass of synaptic sites; this ACh in turn influences the local retinotectal axons to increase their release of glutamate, in turn promoting NMDA receptor channel opening and eventual release of a retrograde stabilizing factor. Again, according to this model, some of the ACh also promotes further ACh via presynaptic receptors on the isthmotectal axons themselves. But how would these events be triggered when animals are kept in the dark?

One possible answer may be that in the dark, some classes of both isthmotectal and retinotectal axons can be expected to fire at substantial rates (Chung et al., 1974; Gaze and Keating, 1970). In addition, the reduced visually-induced activity is likely to trigger homeostatic responses that make the tectal tissue more responsive in dark-reared *Xenopus* than in normally-reared animals (Turrigiano and Nelson, 2004); a homeostatic response to diminished visual input that reduces a neuron's average firing rate might include changes in expression of potassium channels, glutamate receptors, and/or GABA receptors such that the cell would become more excitable and regain its previous average level of activity.

The size of the arbor would thus be governed by the maximum distance over which isthmotectal release sites could be distributed before their summed effects become too weak to sustain the most peripheral regions, a distance that would be raised by homeostatic adjustments in the dark, all other things being equal. But an important thing that is not equal is branch number, which is sharply reduced in dark-reared animals (Udin, accepted for publication), so each axon may well release less ACh per action potential than would a normal axon. This paucity of branches would tend to reduce the zone of effective ACh diffusion. Thus, homeostatic increases in responsiveness and reduced branching may counterbalance each other.

Reduced branch number in dark-reared animals presumably occurs because there is no correlated binocular activity to promote release of stabilizing retrograde messengers. For any branches to persist, either the homeostatic responses postulated above therefore would have to be sufficient to sustain a baseline level of branching, or some branches would have to be able to exist regardless of retrograde messenger release.

9.1 Nitric oxide. Another factor that compels us to deal with the system in terms of stabilization of areas is the possibility that nitric oxide is the retrograde messenger (Udin, unpublished observations). The small size and expected high mobility of this gas could allow it to spread from its point of manufacture

to a finite volume of isthmotectal terminals. The effective volume will be governed by the lifetime of the NO and unknown conditions that make the presynaptic elements receptive to the effects of the NO.

10. Analogies to long-term potentiation

Our model has similarities to models of long-term potentiation, notably the requirement for involvement of NMDA receptors and postulated release of a retrograde messenger. Another pivotal aspect of long-term potentiation in structures such as the hippocampus is protein synthesis [Kelly, 2000 #3697; Steward, 2003 #542]. This topic is as yet completely unexplored in the *Xenopus* binocular system. The cells that are likely to produce the retrograde messenger are the tectal cells, and the possible location of protein synthesis in the cell body or dendrites in response to correlated binocular input needs to be assessed.

11. Conclusion

The exploration of the mechanisms underlying formation of binocular maps in *Xenopus* tectum has benefited greatly from the great strides in understanding of development and plasticity in other model systems, notably the retinotectal projection. However, the circuitry of the binocular system poses new challenges for understanding how activity guides the formation of topographic maps. In particular, we are on the threshold of learning how indirect communication underlies the stabilization of connections of one set of axons by another.

References:

- Albuquerque EX, Alkondon M, Pereira EF, Castro NG, Schrattenholz A, Barbosa CT, Bonfante-Cabarcas R, Aracava Y, Eisenberg HM, Maelicke A (1997) Properties of neuronal nicotinic acetylcholine receptors: pharmacological characterization and modulation of synaptic function. *J Pharmacol Exp Ther* 280:1117-1136
- Allaerts W, De Vente J, Markerink-Van Ittersum M, Tuinhof R, Roubos EW (1998) Topographical relationship between neuronal nitric oxide synthase immunoreactivity and cyclic 3',5'-guanosine monophosphate accumulation in the brain of the adult *Xenopus laevis*. *J Chem Neuroanat* 15:41-56
- Bandarchi J, Scherer WJ, Udin SB (1994) Acceleration by NMDA treatment of visually induced map reorganization in juvenile *Xenopus* after larval eye rotation. *J Neurobiol* 25:451-460
- Bi GQ, Rubin J (2005) Timing in synaptic plasticity: from detection to integration. *Trends Neurosci* 28:222-228
- Braisted JE, McLaughlin T, Wang HU, Friedman GC, Anderson DJ, O'Leary DD (1997) Graded and lamina-specific distributions of ligands of EphB receptor tyrosine kinases in the developing retinotectal system. *Developmental Biology* 191:14-28
- Chung S-H, Bliss TVP, Keating MJ (1974) The synaptic organization of optic afferents in the amphibian tectum. *Proc R Soc Lond B* 187:421-447
- Contestabile A (1976) Comparative survey on enzyme localization, ultrastructural arrangement and functional organization in the optic tectum of non-mammalian vertebrates. *Experientia* 32:1223-1229
- Dudkin EA, Gruberg ER (2003) Nucleus isthmi enhances calcium influx into optic nerve fiber terminals in *Rana pipiens*. *Brain Res* 969:44-52
- Edwards JA, Cline HT (1999) Light-induced calcium influx into retinal axons is regulated by presynaptic nicotinic acetylcholine receptor activity in vivo. *J Neurophysiol* 81:895-907
- Gaze RM, Keating MJ (1970) Receptive field properties of single units from the visual projection to the ipsilateral tectum in the frog. *Q Jl expl Physiol* 55:143-152
- Gaze RM, Keating MJ, Székely G, Beazley L (1970) Binocular interaction in the formation of specific intertectal neuronal connexions. *Proc Roy Soc Lond B* 175:107-147

- Grant S, Keating MJ (1992) Changing patterns of binocular visual connections in the intertectal system during development of the frog, *Xenopus laevis*. III. Modifications following early eye rotation. *Exp Brain Res* 89:383-396
- Gruberg ER, Udin SB (1978) Topographic projections between the nucleus isthmi and the tectum of the frog *Rana pipiens*. *J Comp Neurol* 179:487-500
- Guo Y, Udin SB (2000) The development of abnormal axon trajectories after rotation of one eye in *Xenopus*. *J Neurosci* 20:4189-4197
- Hu B, Nikolakopoulou AM, Cohen-Cory S (2005) BDNF stabilizes synapses and maintains the structural complexity of optic axons in vivo. *Development* 19
- Keating MJ, Kennard C (1987) Visual experience and the maturation of the ipsilateral visuotectal projection in *Xenopus laevis*. *Neurosci* 21:519-527
- Lin SY, Constantine-Paton M (1998) Suppression of sprouting: An early function of NMDA receptors in the absence of AMPA/kainate receptor activity. *J Neurosci* 18:3725-3737
- Mann F, Ray S, Harris W, Holt C (2002) Topographic mapping in dorsoventral axis of the *Xenopus* retinotectal system depends on signaling through ephrin-B ligands. *Neuron* 35:461-473
- Markram H, Lubke J, Frotscher M, Sakmann B (1997) Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* 275:213-215
- Papke RL, Bencherif M, Lippiello P (1996) An evaluation of neuronal nicotinic acetylcholine receptor activation by quaternary nitrogen compounds indicates that choline is selective for the alpha 7 subtype. *Neurosci Lett* 213:201-204
- Renteria RC, Constantine-Paton M (1999) Nitric oxide in the retinotectal system: a signal but not a retrograde messenger during map refinement and segregation. *J Neurosci* 19:7066-7076
- Rubin JE, Gerkin RC, Bi GQ, Chow CC (2005) Calcium time course as a signal for spike-timing-dependent plasticity. *J Neurophysiol* 93:2600-2613
- Rybicka KK, Udin SB (2005) Connections of isthmotectal axons and GABA-immunoreactive neurons in *Xenopus* tectum: An ultrastructural study. *Visual Neuroscience* 22:305-315
- Sargent PB, Pike SH, Nadel DB, Lindstrom JM (1989) Nicotinic acetylcholine receptor-like molecules in the retina, retinotectal pathway, and optic tectum of the frog. *J Neurosci* 9:565-573
- Sawtell NB, Frenkel MY, Philpot BD, Nakazawa K, Tonegawa S, Bear MF (2003) NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. *Neuron* 38:977-985
- Scherer WJ, Udin SB (1989) N-methyl-D-aspartate antagonists prevent interaction of binocular maps in *Xenopus* tectum. *J Neurosci* 9:3837-3843
- Scherer WJ, Udin SB (1991) Latency and temporal overlap of visually-elicited contralateral and ipsilateral firing in *Xenopus* tectum during and after the critical period. *Devel Brain Res* 58:129-132
- Schmidt JT, Fleming MR, Leu B (2004) Presynaptic protein kinase C controls maturation and branch dynamics of developing retinotectal arbors: possible role in activity-driven sharpening. *J Neurobiol* 58:328-340
- Titmus MJ, Lima R, Tsai H-J, Udin SB (1999) Effects of choline and other nicotinic agonists on the tectum of juvenile and adult *Xenopus* frogs: a patch-clamp study. *Neurosci* 91:753-769
- Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. *Nat Rev Neurosci* 5:97-107
- Udin SB, Fisher MD (1985) The development of the nucleus isthmi in *Xenopus laevis*: I. Cell genesis and formation of connections with the tecta. *J Comp Neurol* 232:25-35
- Udin SB, Fisher MD, Norden JJ (1992) Isthmotectal axons make ectopic synapses in monocular regions of the tectum in developing *Xenopus laevis* frogs. *J Comp Neurol* 322:461-470
- Udin SB, Grant S (1999) Plasticity in the tectum of *Xenopus laevis*: binocular maps. *Prog in Neurobiology* 59:81-106
- Udin SB, Scherer WJ (1990) Restoration of the plasticity of binocular maps by NMDA after the critical period in *Xenopus*. *Science* 249:669-672
- Wu G, Malinow R, Cline HT (1996) Maturation of a central glutamatergic synapse. *Science* 274:972-976
- Yamamura HI, Snyder SH (1972) Choline: high-affinity uptake by rat brain synaptosomes. *Science* 178:626-628
- Yan X, Zhao B, Butt CM, Debski EA (2006) Nicotine exposure refines visual map topography through an NMDA receptor-mediated pathway. *Eur J Neurosci* 24:3026-3042
- Yoshii A, Sheng MH, Constantine-Paton M (2003) Eye opening induces a rapid dendritic localization of PSD-95 in central visual neurons. *Proc Natl Acad Sci U S A* 100:1334-1339
- Zhang LI, Tao HW, Holt CE, Harris WA, Poo M-m (1998) A critical window for cooperation and competition among developing retinotectal synapses. *Nature* 395:37-44

Figure Legends

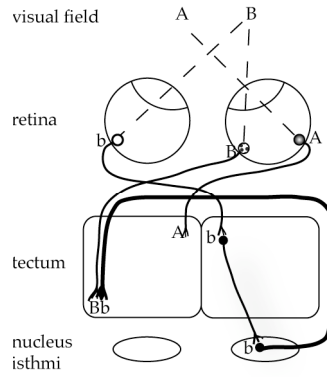


Fig. 1. Schematic view of the circuitry underlying binocular input to the left lobe of the optic tectum. Input reaches the left lobe directly from the right eye via the topographic retinotectal projection. That projection is represented here by two axons, relaying input from two ganglion cells, one (gray cell) with receptive field at position A and the other (stippled cell) at position B. Input from the left eye reaches the left lobe by an indirect pathway, illustrated by the connections that begin with ganglion cell b in the left eye. This cell has its receptive field at visual field B, the same as the receptive field of right eye cell B. Cell b projects to tectal cell b. The right lobe of the tectum projects topographically to the right nucleus isthmi (cell b), which in turn projects to the left tectal lobe. Under normal conditions, isthmial cell b terminates at the same site as retinotectal axon B.

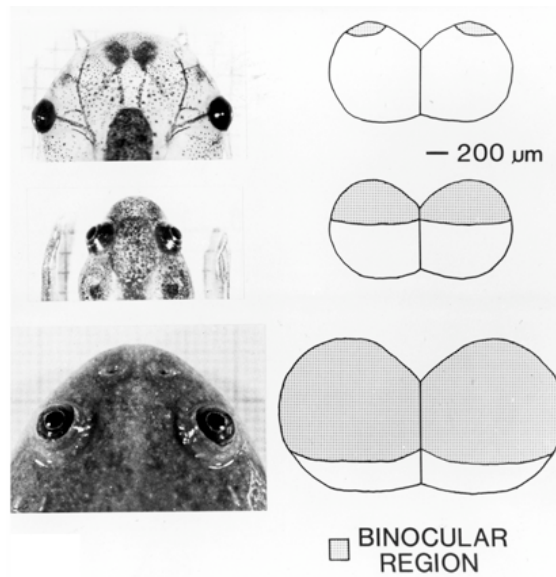


Fig. 2. Photographs of *Xenopus laevis* at 3 developmental stages, prior to eye migration (top), at the end of metamorphosis (middle), and in adult (bottom), and drawings of dorsal views of optic tectum showing proportion occupied by binocular zone of the retinotectal map.

a. Tadpole



b. Critical Period



Fig. 3. Examples of tracings of horseradish peroxidase-filled isthmotectal axons as they grow from rostral to caudal in the tectum. Dashed line represents rostral margin of tectum. a. In tadpoles prior to eye migration, the axons extend far into the tectum, beyond the retinotectal binocular zone, and make few branches. b. During the critical period in the first few months after metamorphosis, the axons make compact arbors, although some branches can be seen to be extending beyond the main terminal zone and some appear to be degenerating behind the main arbor.

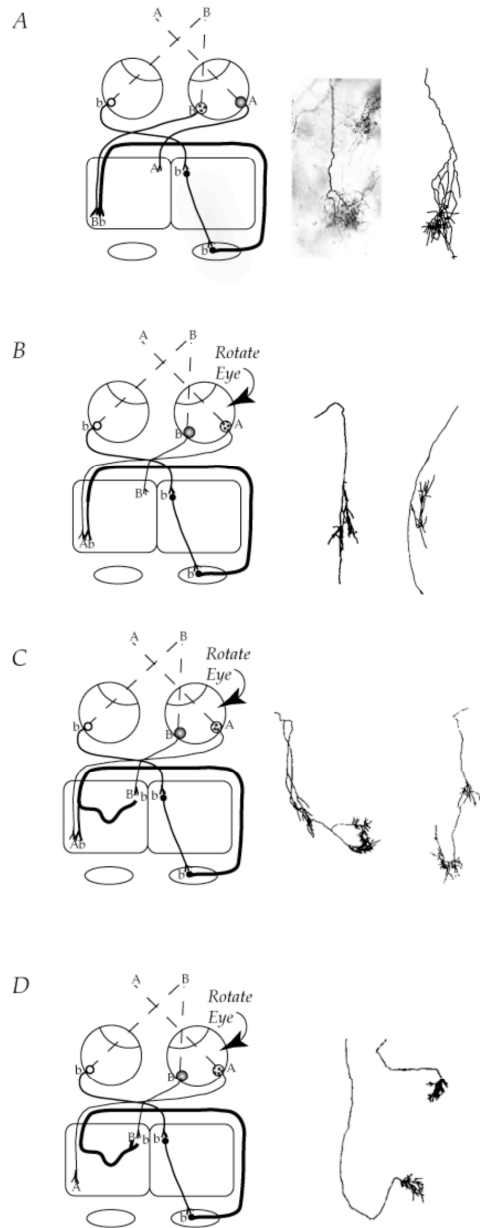


Fig. 4. Plasticity after rotation of one eye in a *Xenopus* tadpole. A. Normal connections. Right. Photograph and drawing of normal isthmotectal axons. B. During the first few weeks after an eye rotation, the isthmotectal axons initially take approximately normal trajectories and arborize at sites that would be appropriate along the mediolateral tectal axis and often correct along the rostrocaudal axis as well. However, because of the rotation of the eye (right eye, in this example), this growth behavior brings the axons to regions of the tectum that now have retinotectal input with different receptive fields. Thus, in this example, isthmotectal axon b now terminates at a site with a retinotectal axon that fires when there is a visual stimulus at field A. C. After another 1-2 months, the axons have begun to correct the topographic mismatch. Axons can have two distinct arbors, one at the original location and the other at a location where the isthmotectal axon's visual field matches that of the retinotectal axon (b and B, respectively). D. The original arbor tends to be retracted as the new one is consolidated.

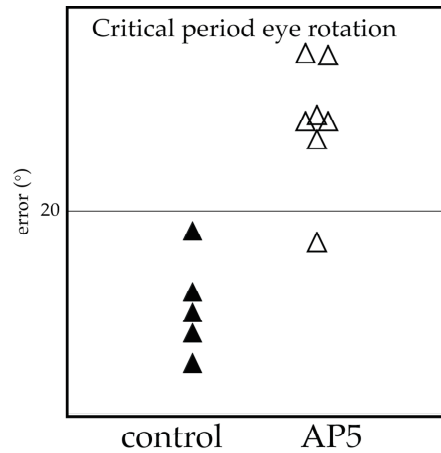


Fig. 5. Distance between centers of ipsilateral and contralateral receptive fields recorded at the same tectal location ("error") after eye rotation during the critical period. Each triangle represents the mean error in a single frog. Blocking NMDA receptors with AP5 during the critical period prevents reorganization of the ipsilateral map and results in much larger mean errors (open triangles) than in frogs that had had an eye rotation without blocking of the NMDA receptors (filled triangles).

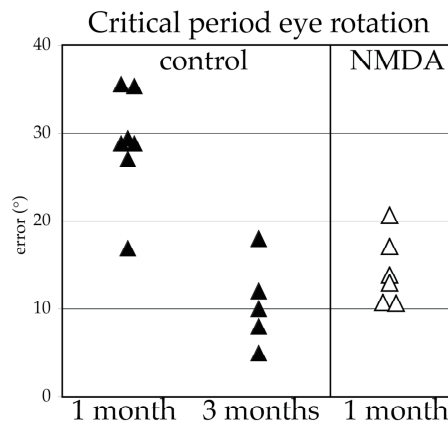


Fig. 6. After eye rotation in tadpoles, reorganization of the ipsilateral map requires several months to come to completion: the errors are much larger at 1 month post-metamorphosis than at 3 months post-metamorphosis (filled triangles). This process can be accelerated by treating the tectum with NMDA for 1 month; the error at 1 month post-metamorphosis is then reduced to the level normally not seen until 3 months (open triangles).

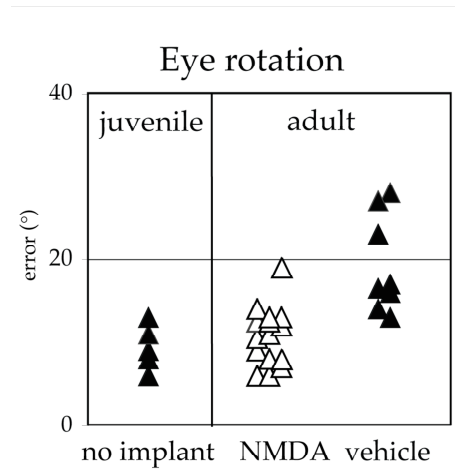


Fig. 7. After eye rotation in critical period frogs, the ipsilateral axons can reorganize, and the error is reduced to low levels (filled triangles on left), but after eye rotation in adults, the axons fail to reorganize and the error remains high (filled triangles on right). However, treatment of the tectum in adults with NMDA restores plasticity, so the axons are able to reorganize after eye rotation, and the error is reduced to the same level found in critical period animals (open triangles).

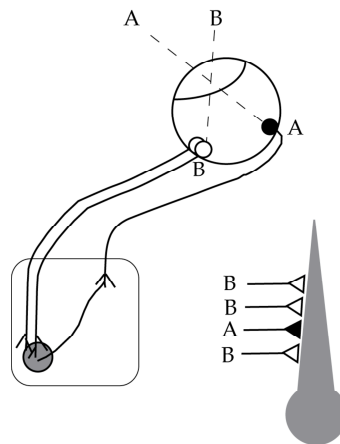


Fig. 8. The ganglion cell with receptive field A makes an incorrect connection at the part of the tectum that receives many correct inputs from ganglion cells with receptive fields at B. The tectal cell shown in gray will transiently receive synapses from both groups of axons. When there is a stimulus at visual field B, the cell will be strongly depolarized, NMDA receptors will open, and the terminals that were most recently active (B, open triangles) will be reinforced. Axon A is unlikely to be firing at the same time and will not be reinforced. When there is a stimulus at visual field A, the single input (A, filled triangle) is insufficient to open NMDA receptors and no reinforcement will occur. The input from axon A will eventually be withdrawn.

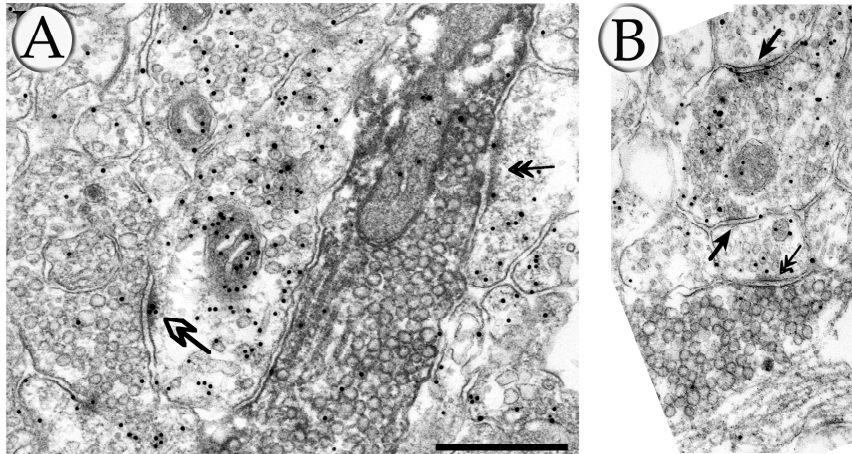


Fig. 9. A. Isthmotectal axons and retinotectal axons fail to converge on the same dendrites. The retinotectal axon terminal (left, pale mitochondrion) makes a synapse (open arrow) onto a GABA-immunoreactive dendrite, indicated by the immunogold reaction (black dots). The darkly stained horseradish peroxidase-labeled isthmotectal axon makes a synapse (double arrow) onto a different GABA-positive dendrite (right). Note the synapses in the dendrites, which are thus marked as part of the typical tectal dendrites that are not only post-synaptic but also are presynaptic to other dendrites. B. The majority of the identifiable inputs to dendrites that receive isthmotectal inputs are GABA-positive. The horseradish peroxidase-labeled isthmotectal axon makes a synapse (double arrow) onto a GABA-positive dendrite that also receives a synapse from another GABA-positive process, as indicated by the black immunogold deposits.

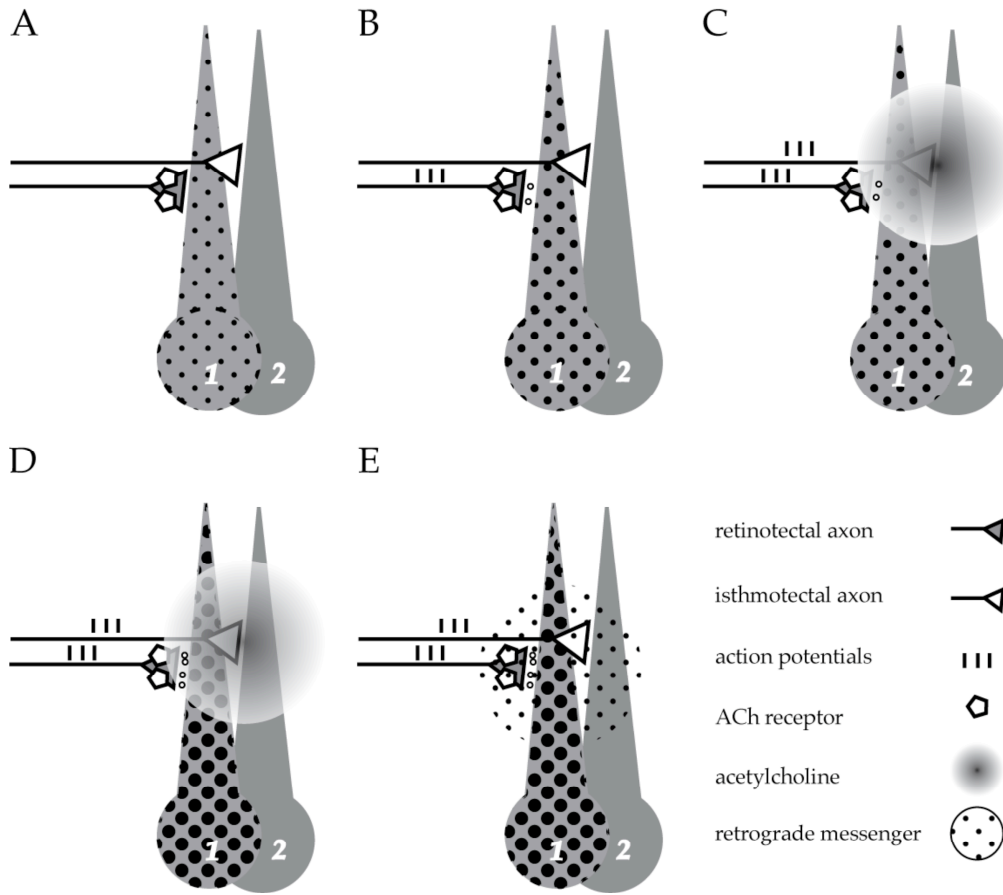


Fig. 10. Model of retinotectal-isthmotectal interaction. A. Retinotectal and isthmotectal axons terminate on different tectal cells. The low level of excitation of the cell that receives retinotectal input is indicated by the small stipples. Retinotectal cells have nicotinic acetylcholine receptors on their terminals. B. When a visual stimulus appears that activates the retinotectal axon, it releases glutamate, and cell #1 becomes more depolarized (larger stippling). C. After a delay of 10 msec, the visual stimulus activates the isthmotectal axon. It releases acetylcholine (gray cloud). Some of the ACh reaches the receptors on the retinotectal axons. D. The activation of the ACh receptors causes the retinotectal axons to release more glutamate. Cell #1 now is sufficiently excited (large stippling) and NMDA receptor channels open. E. Cell #1 releases a retrograde messenger that can stabilize the isthmotectal axon.