
This is the published version of the paper.

This version of the publication may differ from the final published version.

Permanent repository link: http://openaccess.city.ac.uk/3912/

Link to published version:

Copyright and reuse: City Research Online aims to make research outputs of City, University of London available to a wider audience. Copyright and Moral Rights remain with the author(s) and/or copyright holders. URLs from City Research Online may be freely distributed and linked to.

City Research Online: http://openaccess.city.ac.uk/ publications@city.ac.uk
Synchronizing Retinal Activity in Both Eyes Disrupts Binocular Map Development in the Optic Tectum

Stephen G. Brickley,1 Elizabeth A. Dawes,1 Michael J. Keating,1 and Simon Grant1,2

1Division of Neurophysiology, National Institute for Medical Research, London NW7 1AA, United Kingdom, and 2Department of Sensorimotor Systems, Division of Neuroscience, Imperial College School of Medicine, London W6 8RF, United Kingdom

Spatiotemporal correlations in the pattern of spontaneous and evoked retinal ganglion cell (RGC) activity are believed to influence the topographic organization of connections throughout the developing visual system. We have tested this hypothesis by examining the effects of interfering with these potential activity cues during development on the functional organization of binocular maps in the Xenopus frog optic tectum. Paired recordings combined with cross-correlation analyses demonstrated that exposing normal frogs to a continuous 1 Hz of stroboscopic illumination synchronized the firing of all three classes of RGC projecting to the tectum and induced similar patterns of temporally correlated activity across both lobes of the nucleus. Embryonic and eye-rotated larval animals were reared until early adulthood under equivalent stroboscopic conditions. The maps formed by each RGC class in the contralateral tectum showed normal topography and stratification after stroboscopic illumination synchronized the firing of all three classes of RGC projecting to the tectum and induced similar patterns of temporally correlated activity across both lobes of the nucleus. Embryonic and eye-rotated larval animals were reared until early adulthood under equivalent stroboscopic conditions. The maps formed by each RGC class in the contralateral tectum showed normal topography and stratification after

Activity contributes importantly to the organization of synaptic connectivity at many levels of the developing visual system. For example, topographic projections from the retina and between different visual centers undergo activity-dependent refinement during development, in which connections most appropriate for visual information processing become stabilized and misplaced inputs are withdrawn (for review, see Udin and Fawcett, 1988; Constantine-Paton et al., 1990; Rauschecker, 1991; Goodman and Shatz, 1993). It is generally proposed that synchronous activity between convergent inputs reinforces developing connections, whereas asynchrony leads to their removal. Two distinct sources of afferent activity, with topographical origins needed to mediate these processes, now have been identified; correlated spontaneous firing of neighboring retinal ganglion cells (RGCs) may drive structural refinements of primary retinal maps, and correlated visually evoked activity in the two eyes appears to be necessary for organizing binocularly convergent projections.

Although these proposals enjoy considerable theoretical support, the experimental evidence remains indirect. Previous work on the formation of topographic projections from the contralateral and ipsilateral eyes onto the frog midbrain tectum typifies this problem. Activity clearly influences the organization of retinal and crossed isthmotectal connections underlying these projections (see Fig. 1), because the refinement and proper alignment of the two maps are disrupted when afferent activity or synaptic activation of tectal NMDA receptors is reduced (Cline and Constantine-Paton, 1989, 1990; Grant and Keating, 1989b). These procedures also block more extreme forms of connectional plasticity in the two pathways under conditions in which between-eye activity correlations are assumed to be altered significantly, such as when two retinal projections form eye-specific stripes in one tectum (Reh and Constantine-Paton, 1985; Cline et al., 1987), and the ipsilateral map changes orientation after early eye rotation in Xenopus (Keating and Feldman, 1975; Scherer and Udin, 1989; Grant and Keating, 1992). However, the main treatment effects in these studies were on the overall level or balance of neural activity, rather than on its spatiotemporal pattern or degree of synchrony, so the connectional disruptions cannot be attributed specifically to interference with correlated activity-based mechanisms.

To test this possibility more directly, we generated abnormal spatiotemporal patterns of afferent activity in the developing Xenopus tectum by stimulating animals under constant stroboscopic light. Previous studies have reported connectional disorders after similar stimulation of developing (Berman and Payne, 1985; Grigonis and Murphy, 1991; Schmidt and Buzzard, 1993) and regenerating (Schmidt and Eisele, 1985; Cook, 1987) visual pathways, but the studies provided no evidence to support the assumption that activity was correlated in the species used. We demonstrate that during stroboscopic illumination RGC axons fire in synchronized bursts, which correlate spontaneous and
Figure 1. Schematic illustration of the topographic projections from the two eyes onto the frog tectum and predicted effects of stroboscopic stimulation on spatiotemporal firing patterns. 

MATERIALS AND METHODS

Rearing and recording procedures. Xenopus embryos were obtained after injection of human chorionic gonadotrophin into the dorsal lymph sac of adult breeding pairs. Embryos derived from each mating were divided at hatching (stage 35/36; Nieuwkoop and Faber, 1967) into two groups: one group was reared normally under natural diurnal lighting conditions, and the other was raised in an environment consisting of continuous stroboscopic illumination (strobe-reared). Postembryonic animals of each of these RGC classes should respond consistently to the chosen temporal frequency of stroboscopic illumination and that it should alter the spontaneous discharges of each. In preliminary experiments (data not shown) we monitored the single-unit responses of each RGC class to brief (5–10 min) presentations of a range of strobe frequencies (between 0.1 and 10 Hz). These recordings indicated that frequencies of ≤0.5 and of ≥2.5 Hz were unsuitable for rearing purposes; all unit classes responded to low-frequency strobe flashes, but the interstimulus intervals were too long to prevent the return of spontaneous activity; at the higher frequencies, only event units were capable of consistent responses. Another requirement was that the strobe-induced RGC firing should activate postsynaptic tectal cells. Because we are unable to record from these with our techniques, this was assessed indirectly by examining the selective outflow of their activity via the tecto-isthmic and crossed isthmo-tectal pathways (Fig. 1.4). In these experiments frogs were enucleated monocularly and multunit recordings made from just below the pupil surface in the tectum ipsilateral to the remaining eye, where the arbors of fine-caliber crossed isthmo-tectal axons terminate (Udin, 1989). Ipsi-lateral units are known to habituate during repetitive stimulation in anes-thetized frogs, but successively, the animals were allowed to recover fully and then either were returned to their original environment or were strobe-reared, as above, until the recording experiment.

Because these observations suggested that a 1 Hz frequency might confer optimal conditions for strob rearing, a more detailed series of evoked activity right across the tectum, rather than just locally as under more natural stimulus conditions. Electrophysiological mapping assays demonstrated that the precision and plasticity of retinotectal and crossed isthmo-tectal connections were disrupted significantly in normal and early eye-rotated frogs reared under our experimental conditions. The disruptions, although generally less severe, are similar in nature to those observed when activity in these developing pathways is reduced.
control experiments was undertaken to determine whether this strobe frequency could synchronize and correlate RGC firing during much longer exposures. In these experiments two microelec trodes were used for simultaneous paired recordings in the same or opposite tectal lobes, with their depths and positions carefully adjusted to record multiunit activity from the same class of RGC having response fields that were either partially overlapping or completely nonoverlapping in visual space. Then the strobe unit was moved into position \( -33 \) cm from the animal, either within the region of RF overlap or at a strategic distance between the two nonoverlapping fields that optimized their firing to test flashes. Spontaneous discharges (in the dark) were monitored at the two recordings sites for 5–15 min before the strobe was activated, and responses to constant stroboscopic illumination (10 \( \mu \text{s} \) duration at 1 \( \text{Hz} \)) were recorded for a further period of 2–6 hr. Toward the end of the session the response or responses of one or both multiunits to a 5, 10, or 20° black disk introduced into their RF or RFs also were recorded to evaluate any modulatory effects of visual contrasts on the strobe-induced firing pattern.

Signals from the two recording sites were processed through separate channels and logged for the entire course of the experiment with a CED 1401 (Cambridge Electronic Design, Cambridge, UK) laboratory interface on which on-line and subsequent analyses could be performed in an IBM microcomputer. After the recording the temporal pattern of RF firing were examined by depicting the data from each channel as raster plots showing the time of occurrence of individual spikes in relation to the strobe flash for every 1 sec epoch of the experiment. Changes in firing rates were calculated from the number of spikes per second after each strobe flash. Cross-correlation (CC) analyses were performed on the simultaneously recorded responses. For this purpose one channel was treated as “reference” and for each space of changes in single-unit RF size, which any spikes occurred in the other channel during the interval \( 0 \pm 500 \text{ msec} \) were computed, after dividing this channel into 2 msec bins. The time-averaged CCs determined in this way from selected periods in each recording session were plotted as histograms showing the temporal phase and relative strengths of the correlations that were present.

To provide a quantitative indication of the degree of coincident firing occurring under the different conditions of these recordings, which subsumed differences in firing rates associated with them, we divided the average number of events occurring in the CC histograms at time \( 0 \pm 10 \text{ msec} \) by the average number of noncoincident events (i.e., in the remaining bins at \( t = \pm 10–500 \text{ msec} \)) over the same sampling period. This measure was termed the “coincidence index” (CI\( \text{lo} \)); increasing values \( >1.0 \) on this index indicate increasing degrees of in-phase, synchronous activity at the two recording sites (while disregarding any anti-phase or asynchronous relationships). As an indication of any broader temporal correlations that were present, a “correlation index” (CI\( \text{hi} \)) also was calculated from the data by similarly comparing the events occurring at \( t = 0 \pm 50 \text{ msec} \) with those in the remaining bins. Our reason for selecting these particular intervals is that 10 and 50 msec correspond, respectively, to the average initial delay and the overall in the normal Xenopus tectum by ON/OFF stimuli at corresponding positions in the contralateral and ipsilateral eyes (Scherer and Udin, 1991). Either or both intervals thus may have physiological relevance for stabilizing the developing visual connections under investigation, as well as falling within known integration times for inducing long-term potentiation of synaptic strength after stimulating paired inputs (i.e., associative LTP) in the adult hippocampus (Levy and Steward, 1983; Gustafsson and Wigstrom, 1986). In these analyses we made no attempt to separate direct stimulus-induced correlations in firing from those arising via “effective connectivity” (Aertson et al., 1989) between the paired recording sites. The reason for this is that effective connectivity attributable to common input (e.g., on邻居 classes of RGC's) or to direct (e.g., intertectal) connections is more likely to exist at corresponding than at noncorresponding sites, and so the analysis should properly reflect this difference by including all possible sources of coincident or correlated activity. In fact, the completed analyses revealed no meaningful differences between the two indices used, so only the data relating to CI\( \text{lo} \) are shown.

Effects of stroboscopic illumination on binocular map development. Visual inputs to the tectum were mapped at multiple recording sites in normal and stroboscopically illuminated frogs by conventional von Frey presentations against the uniform background of the perimeter arc and RFs plotted onto polar coordinate charts for future analyses. Initial efforts focused on the direct retinotectal projection from the contralateral eye. To assess its topographic order, we made stepwise electrode penetrations into the tectum, separated by 100–200 \( \mu \text{m} \) across its surface dimensions and by 25–50 \( \mu \text{m} \) in depth while plotting minimum response fields and classifying the sequence of units that were encountered. In some of these experiments and in later ones dedicated to the purpose, the RF sizes of single- and multiunit recorded at different depths were evaluated quantitatively, using computer-controlled methods of visual stimulation and data capture (Keating et al., 1986). These measures can provide an index of the detailed precision of the retinotopic map; enlarged multiunit RFs (MURFs) in the absence of changes in single-unit RF size, indicate that the RGCs projecting to a given tectal site arise from a wider territory than normal and/or that their arbors are more diffuse. To minimize other variables that can affect these measures, we used the same set of low-impedance electrodes for all of the recordings in both groups of frogs, and window discrimination of the amplified signals was always set at 100% above the noise level. RFs were plotted on a large television screen positioned 38 cm from the stimulated eye, and covered an effective stimulus area of 64.5° in the nasotemporal axis and 34.5° in the supero-inferior axis of its visual field. The visual stimulus (a 6° black square) was moved either horizontally or vertically in 1.5° steps across the screen at a constant speed of 3°/sec. Spikes occurring during each traverse were timed and stored on computer. The direction of stimulus movement was interleaved randomly between runs, with at least a three sets of traverses completed for each possible direction. For each MURF plotted, electrode depth was set to ensure that only one RGC class was being sampled and that the response contained at least three separate units, as revealed by differences in their spike height and width. To analyze the data, we produced twodimensional matrix representations of the screen, in which both the stimulus radius was divided into 1.5° × 1.5° pixels and the number of spikes per pixel was presented as an element of the matrix. The RF size was measured by pooling matrices: the horizontal diameter by combining matrices from the two vertical directions of stimulus movement, the vertical diameter from its two horizontal directions of movement, and its area from the outline of the combined matrices. To avoid inclusion of elements arising from occasional spontaneous or artificial bursts of spikes, we considered only pixels containing \( >10% \) of the maximum number of spikes per pixel to be genuine components of the RF.

In other animals of both groups, including those with early eye rotations, the visual projections from both eyes to one tectum were mapped to examine the topographic order of the crossed isthмотopic map from the ipsilateral eye and to assess its spatial alignment with the direct retinotop map. In these experiments we plotted minimum response fields in the contralateral and ipsilateral eyes independently on the polar coordinate system while alternatingly covering the nontested eye with an opaque shield. The center of each RF was defined as the geometric center of the response field, a position typically approximated the point of maximal activation. Spatial alignment between the RF map was quantified by calculating the degree (in degrees) between the binocular RF centers at each tectal site receiving input from the two eyes. Disparity was expressed both by its absolute value and by its horizontal (nasotemporal) component. Data derived from the strobe-reared frogs were compared with the controls from the present investigation and also with those previously obtained from age-matched dark-reared frogs in which these features were found to be disrupted systematically (Grant and Keating, 1989b). These and other statistical comparisons were conducted with the software package SIGMA STAT.

RESULTS

The results are presented in two sections. First, we provide evidence that exposure to 1 Hz of stroboscopic illumination interferes with the temporal pattern of RGC firing, in support of the rationale for the rearing experiments (Fig. 1B). Second, we examine the consequences of development under these conditions for the organization and plasticity of binocular maps in the optic tectum.

Control experiments: correlated firing in the tectum

Simultaneous recordings were undertaken in 12 normal frogs to monitor the effects of prolonged 1 Hz of stroboscopic illumination on RGC firing patterns. The recordings were obtained from pairs of sustained \( (n = 4) \), event \( (n = 6) \), or dimmer \( (n = 2) \) RGC classes, comprising six pairs from corresponding sites with spa-
Table I. Summary of control recording data

<table>
<thead>
<tr>
<th>L.D.</th>
<th>Type</th>
<th>Location</th>
<th>Firing rate (spikes/sec) mean ± SD</th>
<th>Coincidence index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spont.</td>
<td>Strobe onset</td>
</tr>
<tr>
<td>str-01</td>
<td>Sustained</td>
<td>L, R</td>
<td>7 ± 1</td>
<td>69 ± 12</td>
</tr>
<tr>
<td>str-02</td>
<td>Event</td>
<td>R, R</td>
<td>5 ± 2</td>
<td>70 ± 24</td>
</tr>
<tr>
<td>str-03</td>
<td>Sustained</td>
<td>R, R</td>
<td>8 ± 1</td>
<td>66 ± 10</td>
</tr>
<tr>
<td>str-04</td>
<td>Dimmer</td>
<td>R, R</td>
<td>5 ± 3</td>
<td>89 ± 19</td>
</tr>
<tr>
<td>str-05</td>
<td>Sustained</td>
<td>L, R</td>
<td>11 ± 4</td>
<td>77 ± 43</td>
</tr>
<tr>
<td>str-06</td>
<td>Event</td>
<td>R, R</td>
<td>4 ± 1</td>
<td>65 ± 15</td>
</tr>
<tr>
<td>str-07</td>
<td>Dimmer</td>
<td>R, R</td>
<td>4 ± 2</td>
<td>89 ± 24</td>
</tr>
<tr>
<td>str-08</td>
<td>Sustained</td>
<td>R, R</td>
<td>12 ± 2</td>
<td>58 ± 36</td>
</tr>
<tr>
<td>str-09</td>
<td>Event</td>
<td>R, R</td>
<td>15 ± 1</td>
<td>90 ± 19</td>
</tr>
<tr>
<td>str-10</td>
<td>Event</td>
<td>R, R</td>
<td>15 ± 2</td>
<td>119 ± 36</td>
</tr>
<tr>
<td>str-11</td>
<td>Event</td>
<td>L, R</td>
<td>15 ± 1</td>
<td>90 ± 19</td>
</tr>
<tr>
<td>str-12</td>
<td>Event</td>
<td>L, R</td>
<td>16 ± 2</td>
<td>82 ± 9</td>
</tr>
</tbody>
</table>

Noncorresponding sites

Str-03 Sustained R, R 8 ± 1 66 ± 10 57 ± 2 96 ± 13 0.9 1.2 1.8 1.3
Str-04 Dimmer R, R 15 ± 2 119 ± 36 44 ± 5 73 ± 13 1.2 1.9 2.2 1.7
Str-05 Sustained L, R 11 ± 4 77 ± 43 73 ± 3 143 ± 23 1 2.6 2.9 1.5
Str-06 Event R, R 12 ± 2 58 ± 36 23 ± 2 47 ± 8 1.4 1.9 2.4 1.9
Str-07 Dimmer R, R 13 ± 3 102 ± 25 91 ± 5 140 ± 17 0.9 2.8 3 2.6
Str-08 Sustained R, R 4 ± 1 43 ± 28 56 ± 5 87 ± 30 2.7 2.7 2.6 2.6
Str-09 Event R, R 15 ± 1 89 ± 19 86 ± 3 n.r. 1.5 2.8 2.6 x
Str-10 Event R, R 16 ± 2 82 ± 9 70 ± 6 n.r. 1.5 2.8 2.6 x

R, L, Right, left tectum; n.r., no response to visual stimulation; x, unit not stimulated.

Spontaneous activity generally was maintained at low levels regardless of RGC class, both in the light (Fig. 2) and in the dark (Fig. 5), with just occasional bursts of spikes detected. Nonetheless, the spontaneous discharges occurring at neighboring locations in the same tectum consistently exhibited a degree of synchrony (CI < 1.0; Table 1) presumably arising via effective connectivity, whereas random activity correlations were present among most of the other paired sites (compare Fig. 2). Stroboscopic illumination entrains the firing of all three RGC classes All 24 sustained, event, or dimmer unit types that were examined acquired and maintained similar patterns of entrained firing during prolonged exposure to 1 Hz of stroboscopic illumination. The raster plots in Figure 3 illustrate the time course of this entrainment effect for representatives of each RGC class. Initial responses to the strobe usually were vigorous but variable in rate (Table 1) and temporal pattern. With continued exposure the firing rate became more stable, and a distinctly triphasic response pattern emerged, comprising short, middle, and long latency bursts of periodic activity with silent or near-silent periods in between, each element of which was reproduced after successive

```
spontaneous firing at other sites were random (CI = 1 ± 0.1).
```
strobe flashes. The triphasic latency profile was remarkably similar across RGC classes (see also Fig. 5). The early and middle components of the response were most prominent at 100–150 msec and at 300–500 msec poststimulus, respectively, and the late component began at 700 msec, usually to be curtailed only by the next strobe flash in the sequence. Moreover, the entrained firing patterns remained essentially invariant and without obvious contamination by spontaneous discharges until the recording was interrupted. These results thus imply that stroboscopic illumination causes RGC axons in all regions and depths of the \textit{Xenopus} tectum to fire in synchrony.

The response of normal RGCs to 1 Hz of stroboscopic illumination has been examined previously during several minutes of continuous recording from the goldfish tectum and consisted of just a single short latency burst, ~50–200 msec in duration, followed by quiescence (Schmidt and Eisele, 1985). The more intermittent bursting activity in \textit{Xenopus} almost certainly results from differences in intraretinal processing between frogs and fish, a difference that begins in the photoreceptor layer, which is known to be rod- and cone-dominated, respectively, in these species. Indeed, intermittent periodic bursts of activity, lasting for as long as 1 sec poststimulus, also appear to characterize the firing patterns of frog RGCs responding to the onset and offset of bright
light flashes (25–1000 msec duration), which are confined to their excitatory RF [Rana, Stiles et al. (1985); Xenopus, S. Brickley and S. Grant, unpublished data]. The main difference between these responses and those elicited by (more transient and diffuse) strobe flashes is that the earliest component (up to 250 msec poststimulus) is more periodic and begins at a shorter (50–75 msec) latency. The strobe-entrained firing pattern of Xenopus RGCs thus appears to be a variant on their normal temporal response to abrupt changes in illumination.

**Stroboscopic illumination induces temporal correlations in firing at all tectal locations**

Representative data showing the effects of stroboscopic entrainment on correlations in firing at corresponding and at noncorresponding tectal locations are presented in Figure 4. CC histograms computed from all of the paired recording sites in positions of tectal correspondence (Fig. 4A), whether in the same or opposite sides of the brain, exhibited a sharp (20–50 msec) peak of coincident activity centered at \( t = 0 \) as soon as the strobe flashes began within their region of RF overlap (Table 1). With more prolonged exposure and consequent entrainment of firing at the two recording sites, this correlated firing usually improved but was accompanied by the emergence of secondary peaks of anticorrelated activity (at \( t > \pm 50 \) msec), the number and distribution of which depended on subtle phase differences in the periodic activity of the two strobe-entrained responses. At paired noncorresponding tectal locations, correlations in firing were closer to random at strobe onset, but a similar pattern of correlated and anticorrelated firing emerged during strobe entrainment (Fig. 4B). Most strikingly, a sharp peak of coincident activity, at \( t = 0 \pm 10 \) msec, appeared in all of the CC histograms computed from these paired recordings once entrainment was well established (Table 1). These results indicate, therefore, that stroboscopic synchronization of RGC firing induces similar temporal patterns of correlated and anticorrelated activity at all tectal locations.

**Effects of visual contrasts on the rate and temporal pattern of stroboscopically entrained firing**

Although we took steps to limit visual contrast information in the strobe-rearing environment, some potential sources of this (e.g., the air holes in the animal container lids and food supplied) could not be excluded. To evaluate their possible effects on strobe-induced firing patterns, we introduced visual stimuli (black disks) into the RF or RFs of each unit pair once stable strobe entrainment and activity correlations had become established. Figure 5, A and C, shows segments of the raster plots for unit pairs of each RF condition for the period immediately before, during, and after visual stimulation of both (overlapping) or one of their (nonoverlapping) RFs. The visual contrast significantly increased the rate of firing of the stimulated units (by 50–100%; Table 1), most prominently in the interval between 200 and 700 msec after each strobe flash. As indicated in the raster plots of Figure 5 and confirmed by analyses of cumulative poststimulus time histograms compiled from these recording periods (data not shown), this contrast-evoked activity was superimposed on the middle component of the strobe-entrained firing and on the silent periods on either side of it, with little effect on the early or late components of the entrained response.

This increased firing would be expected to impact on the pattern of activity correlations, because the added activity presents more opportunities for temporal coincidences and noncoincidences to be established. However, CC histograms computed for the period of visual contrast stimulation, although differing markedly from those obtained for comparable stimulation under normal viewing conditions (see Fig. 2A), showed remarkably similar patterns of correlated and anti-correlated activity to those derived from the strobe-entrained periods immediately before and afterward. Indeed, the main difference appeared to be in the height of the peaks, all of which were elevated in the CCs computed from the responses during contrast stimulation, suggesting that absolute levels of both coincident and noncoincident activity were increased. This was confirmed by subtracting one CC from another (e.g., during contrast stimulation minus before). As typified in Figure 5, B and D, the residual histograms were flat for both overlapping and nonoverlapping RF conditions, indicating that contrast-evoked correlations in firing were essentially random (compare Fig. 2B) during stroboscopic entrainment.
To determine the effects of contrast-evoked firing on the relative levels of correlated and anti-correlated activity, which might provide a basis for distinguishing corresponding from noncorresponding tectal locations, we calculated coincidence indices for the period of stimulation and compared them with those for preceding periods of stable strobe entrainment. As shown in Table 1, these comparisons revealed that during visual stimulation the CI was generally reduced, and by a similar degree, at both corresponding and noncorresponding sites. These findings strongly suggest that the additional firing induced by visual contrast produces an equivalent net loss in the probability of coincident activity at all tectal locations. The explanation for this rests with the temporal profile of the contrast-evoked RGC firing, which occurred sporadically over a long (500 msec) period after each strobe flash, rather than in synchrony with elements of the entrained response.

**Effects of strobe rearing on binocular map development and plasticity**

Organizational features of the maps formed by the retinotectal and crossed isthmotectal (intertectal) projections were examined in 41 normal and 48 strobe-reared frogs.

The topography of the retinotectal map is normal in strobe-reared frogs but may lack detailed precision

The topographic order of the retinotectal projection in animals strobe-reared from embryonic stage 35/36 (see Figs. 6, 8) was indistinguishable from normal; stepwise microelectrode penetrations along the rostrocaudal or mediolateral tectal axes yielded similar nasotemporal or superoinferior progressions in RF position through the contralateral eye in both groups of frog. These features of normality after strobe rearing were shared by all three classes of RGC projecting to the tectum, as was their relative depth of termination within the superficial neuropil. In 89 penetrations made into the tectum of these animals (n = 14) in which two or three classifiable unit types were encountered, a complete sustained-event–dimmer sequence was obtained from superficial to deep in 31, and combinations of two unit classes in the same appropriate sequence were obtained in the remainder: that is, a sustained unit first and an event (n = 38) or a dimmer unit deeper down (n = 5) or just an event–dimmer (n = 15) combination. Similar proportions of classifiable unit types and sequences were recorded in 65 penetrations in control (n = 10) animals (χ² = 8; df = 7; p > 0.3). In addition, as indicated in Figure 6, RFs of the different units encountered in any given penetration were, in large part, overlapping in space, especially in recordings from central tectum in which such penetrations should be near perpendicular to its surface. The scatter in the RF centers of different unit classes mapped sequentially at such central tectal sites was also comparable in strobe-reared (mean = 6.0 ± 3.0°, SD; n = 66) and normally reared frogs (mean = 5.5 ± 2.7°, SD; n = 57; Student’s t test, p > 0.4). These results indicate that all three RGC classes in strobe-reared *Xenopus* are able to deploy their arbors with appropriate topographic order in the opposite tectum, both across its surface and in depth.

In these recordings the response fields of the multiunits that were plotted were typically 20–40° in diameter (Fig. 6), irrespective of rearing conditions. However, to obtain a more accurate
index of this aspect of the mapping fidelity, RF sizes in the retinotectal projection were measured by quantitative methods. Single-unit (class III) RF diameters were identical in the two groups of frog, averaging 19° in both nasotemporal and superoinferior dimensions (n = 10/group). MURF sizes, measured for each of the three RGC classes, are presented in Table 2. Illustrative three-dimensional matrix plots of the data that were obtained are shown in Figure 7. The average MURF diameters and their areas increased significantly with depth in both groups of frog (i.e., sustained < event < dimmer; Student’s t tests, p < 0.05), but all equivalent dimensions were larger in the strobe-reared animals (n = 10) than in the controls (n = 12). The increases were statistically significant for sustained multiunits and for some of the event MURF dimensions.

Probing these differences further, it was evident from the matrix plots that the MURF profiles fell into two categories: those possessing a single, central peak of maximal activation (Fig. 7A,B) and others possessing two or more regions of peak activity (Fig. 7C,D). This latter category of multiunit response could imply that the RGCs contributing to it had terminal arbors of normal size but cell bodies that were spatially separated on the retina, rather than immediate neighbors, and might be an expected trait of strobe-induced disorder in the retinotectal map (Cook and Rankin, 1986; Cook, 1987). Such responses were, however, recorded with similar frequency in the normal (10 of 61; 16%) and strobe-reared animals (14 of 61; 23%). We believe, therefore, that the consistent trend toward MURF enlargement across RGC classes after strobe rearing more likely reflects a general increase in retinal arbor size, so that our sampling was obtained from a more diffuse set of active terminal branches. This interpretation is consistent with recent findings that retinal MURFs and arbor sizes are enlarged in the tectum of goldfish reared under a mixed strobe/dark cycle (Schmidt and Buzzard, 1993).

**Table 2. Comparison of multiunit receptive field (MURF) dimensions (mean ± SD) in normal and strobe-reared frogs**

<table>
<thead>
<tr>
<th>RGC class</th>
<th>MURF dimensions</th>
<th>Normal frogs</th>
<th>Strobe-reared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sustained</td>
<td>Nasotemporal</td>
<td>23.0 ± 4.9°</td>
<td>27.2 ± 4.1°</td>
</tr>
<tr>
<td></td>
<td>Superoinferior</td>
<td>19.0 ± 2.4°</td>
<td>21.5 ± 3.5°</td>
</tr>
<tr>
<td></td>
<td>Area (degrees²)</td>
<td>345 ± 105</td>
<td>484 ± 116***</td>
</tr>
<tr>
<td>Event</td>
<td>Nasotemporal</td>
<td>28.4 ± 5.9°</td>
<td>32.2 ± 6.5°</td>
</tr>
<tr>
<td></td>
<td>Superoinferior</td>
<td>24.1 ± 4.4</td>
<td>24.6 ± 5.7</td>
</tr>
<tr>
<td></td>
<td>Area (degrees²)</td>
<td>544 ± 175</td>
<td>634 ± 163*</td>
</tr>
<tr>
<td>Dimmer</td>
<td>Nasotemporal</td>
<td>31.8 ± 5.6°</td>
<td>35.7 ± 6.0°</td>
</tr>
<tr>
<td></td>
<td>Superoinferior</td>
<td>27.2 ± 3.5°</td>
<td>28.3 ± 3.0°</td>
</tr>
<tr>
<td></td>
<td>Area (degrees²)</td>
<td>693 ± 149</td>
<td>829 ± 166</td>
</tr>
</tbody>
</table>

All values are mean ± SD (n = number of animals). t test, p < 0.05*: p < 0.01**: p < 0.005***.

The topographic order and alignment of the crossed isthmotectal map is disrupted in strobe-reared frogs

In 18 strobe-reared frogs the binocular inputs to one or both tectal lobes were mapped in sufficient detail to evaluate the order and alignment of the crossed isthmotectal projection. Two classes of result were obtained. In 10 animals the ipsilateral map or maps appeared completely normal, displaying good topographic order and spatial alignment with the map from the contralateral eye on the same tectal lobe. In the other frogs, an example of which is shown in Figure 8, the ipsilateral map or maps displayed abnormal features reminiscent of dark-reared *Xenopus* (Keating and Kennard, 1987; Grant and Keating, 1989b). Their overall topography showed some order, with sequences of electrode penetrations across the rostrocaudal or mediolateral axes of the tectum usually revealing appropriate temporonasal or superoinferior shifts in RF position. However, there were also signs of misalignment with the contralateral map, which were manifest at some recording sites (e.g., 4, 7, and 27 in Fig. 8) by abnormally large spatial disparities between the RFs in the two eyes and manifest at others on the margins of the map (e.g., 1–3 and 31–33) by
systematic inward shifts in ipsilateral relative to contralateral RF positions, giving the maps a compressed appearance. At some of these recording sites (33 of 207; 16%), ipsilateral multiunit response fields were almost double the normal size, ranging from 60 to 90° in diameter.

Quantitative analyses of the strobe-reared ipsilateral maps were undertaken to establish more firmly the nature and severity of the abnormalities present and to better assess their similarity with those known to exist in *Xenopus* that were dark-reared to the same age. For these purposes, data from the strobe-reared frogs with apparently “unaffected” and “abnormal” maps were treated separately. The spatial alignment between the ipsilateral and contralateral maps, expressed by their mean absolute and nasotemporal binocular RF disparities, is given in Table 3. These latter disparities were grouped by mediolateral row of tectal recording sites and the mean values computed for each such row along the rostrocaudal axis. On our conventions, indications that the ipsilateral map is systematically compressed are revealed by large positive nasotemporal disparities rostrally and by large negative disparities caudally. There were no significant disparity differences between the control and the unaffected strobe-reared animals: absolute disparities averaged \( \pm 10° \), and nasotemporal disparities were within \( \pm 5° \) of zero for all rows, as previously reported in normal *Xenopus* at 1 year PM (Grant and Keating, 1989a). In the abnormal strobe-reared frogs, absolute disparities were significantly larger than normal, as were the positive and negative nasotemporal disparities in the most rostral and caudal tectal rows (outcomes that also were obtained after pooling these data with those from the “unaffected” animals). However, all of these disparity values were significantly smaller than in dark-
reared frogs. Taken together, these results show that strobe rearing can lead to disorders in the crossed isthmotectal map, which are qualitatively similar to, but less severe than, those resulting from total visual deprivation.

Only limited intertectal plasticity occurs after larval eye rotation in strobe-reared frogs

The much larger scale reorienting of this map that usually follows early eye rotation also was affected by strobe rearing. In a control group of eye-rotated frogs reared under natural lighting conditions (n = 4), the binocular inputs mapped at one or both tectal lobes were found to be aligned spatially, despite the eye rotation present (which ranged from 60° to 180°). Absolute binocular RF disparities in these frogs (mean = 8.7 ± 4.8°; SD; n = 128) were no different from those in normal age-matched animals (Table 3), indicating that the reoriented map formed by crossed isthmotectal arbors in eye-rotated Xenopus indicating that the reoriented map formed by crossed isthmotectal arbors in eye-rotated Xenopus was qualitatively similar to, but less severe than, those resulting from total visual deprivation.

The results obtained from the strobe-reared frogs (n = 8) differed markedly from those of the controls; the ipsilateral maps showed both disorder and compression and no or very little plasticity. In five frogs with eye rotations of between 120° and 180°, showed some evidence of intertectal plasticity. As partial map alignment indicates that a cohort of crossed isthmotectal arbors had reorganized in these animals, almost compensating for the rotated visual input. A similar partial intertectal plasticity is known to occur during the initial process of map reorientation in Xenopus, but it has never been seen in eye-rotated animals reared in total darkness (Keating and Feldman, 1975; Grant and Keating, 1992; Keating and Grant, 1992).

DISCUSSION

The results show that (1) stroboscopic illumination induces high rates of entrained firing in Xenopus RGCs, masking any local correlations in their spontaneous discharges and synchronizing activity in the two eyes, and (2) in frogs reared under these conditions the precision, alignment, and plasticity of visual maps in the tectum are disrupted. We attribute these disruptions to strobe-induced levels of correlated afferent activation sufficient to obscure temporal differences between the pattern of activity at topographically appropriate and inappropriate synapses.

Strobe rearing was initiated mainly at embryonic hatching, when all retinal neuron classes are present in Xenopus, and just before the normal onset of photoreceptor function at stage 39/40 (Witkovsky et al., 1976). During the next 10–12 d (up to midlarval stage 49/50), RGC responses to visual stimulation, including transient light flashes, usually become increasingly reliable, coinciding with the emergence of distinct plexiform layers (Chung et al., 1975) and synaptic inputs from bipolar and amacrine cells onto discrete morphological classes of RGC resembling those of the adult retina (Fisher, 1976; Sakaguchi et al., 1984). This early period is also a key stage in the formation of the Xenopus retinotectal map. Previous studies have shown that retinal axons first form terminal arborizations in the tectum at stage 39 and that they undergo substantial refinements, especially along the rostrocaudal axis, until the appearance of well ordered topography at stage 49/50 (Gaze et al., 1974; Holt and Harris, 1983; O’Rourke and Fraser, 1986; Fujisawa, 1987). The arbor refinements have been visualized in situ and shown to be influenced by synaptic activity at NMDA receptors (O’Rourke et al., 1994). Whether the retinal origin of this activity is generated spontaneously, as occurs in other vertebrate classes while translaminar retinal inputs are being assembled (Maffei and Galli-Resta, 1990; Meister et al., 1991; Wong et al., 1993; Sernagor and Grzywacz, 1996), or is light-evoked remains unknown. Regardless, we doubt that strobe entrainment of RGC activity would have begun until these midlarval stages, when the retinal circuitry needed to drive their responses to light becomes fully established and the early map refinements are completed.

Major adjustments in retinotectal connectivity would be required during subsequent development, however, when activity is
synchronous under strobe conditions. New RGCs are added constantly to the retinal periphery throughout later larval and juvenile postmetamorphic life in *Xenopus*, unmatched by the pattern of tectal cell addition (Gaze et al., 1979; Grant and Keating, 1986b). To preserve the topography of the map during this period, resident retinal arbors are forced to shift in an orderly manner across the tectum, changing postsynaptic partners as they go. It is also in this period that arbors belonging to the different RGC classes normally become sorted into distinct strata of the superficial tectum (Chung et al., 1975). The lack of effect of correlating activity on either the topography or the stratification of RGC class-specific projections strongly suggests that the underlying shifts in arbor position are achieved in *Xenopus* by activity-independent mechanisms, such as graded molecular affinities and selective adhesion in the target (see Sanes, 1993; Yamagata et al., 1995). This conclusion accords with reports that

---

**Figure 9.** Binocular maps on the tectum of an eye-rotated, strobe-reared frog showing no evidence of intertectal plasticity. In this animal the left eye was rotated counterclockwise by 180° at larval stage 56. All conventions are as in Figure 8, except that the contralateral and ipsilateral maps on both tectal lobes are shown with the animal positioned so that its rotated eye is centered on the origin of the coordinate system. Both visual projections recorded through this operated eye are rotated 180° counterclockwise, and both visual projections through the unoperated right eye are in a normal orientation. As a result, the binocular maps on each tectum are out of alignment by the degree and direction of the eye rotation present (e.g., compare RF positions 1-3 in the two eyes). The ipsilateral maps also show signs of disorder and compression.
correct retinotectal arbor position, including lamination by RGC type, can be established independently of patterned activity in other amphibians (Harris, 1980) and in teleost fish (Stuermer et al., 1990; Schmidt and Buzzard, 1993); it also is in accord with the specific finding of Fraser et al. (1984) that brief application of neural cell adhesion molecule antibodies to early juvenile *Xenopus* tectum distorts the existing retinal map and almost doubles its MURF sizes.

A small, but consistent, increase in MURF sizes was also present after synchronizing RGC activity under strobe conditions. As in other frogs and in goldfish, *Xenopus* RGC arbors continue remodeling as they shift across the tectum, retracting older branches and synaptic contacts while extending new ones that are necessary to preserve their detailed point-to-point maps (Constantine-Paton et al., 1983; Easter and Stuermer, 1984; Fujisawa, 1987). The implication that these arbors are enlarged after
strobe rearing suggests that the proper elimination of older branches and synapses may be repressed when their activity is highly correlated with that of more topographically appropriate arbors converging on the same tectal neurons. Such a temporary stabilization of coactive, although inappropriate, synapses supports the hypothesis that associative reinforcements of coactive retinal afferents normally contribute to maintaining their mapping precision (Cline and Constantine-Paton, 1989, 1990; Schmidt and Buzzard, 1993).

We can be confident that strobe-induced activity correlations were present throughout the development of the ipsilateral map. Crossed isthmotectal axons normally invade the *Xenopus* tectum after stage 50 and only form terminal arborizations at later metamorphic stages (Udin, 1989), coinciding with the appearance of visually driven, topographically organized ipsilateral units (Grant and Keating, 1989a). During subsequent postmetamorphic development in both normal and eye-rotated frogs, crossed isthmotectal arbors shift their position under the influence of visually evoked activity (Grant and Keating, 1986a, 1989a,b, 1992). These shifts are protracted, serving to maintain the topography of the map and match its RFs to corresponding retinal inputs in normal animals in which eye positions are changing continuously or to reacquire such spatial correspondence after eye rotation. In normal animals this process probably is accomplished by systematic remodeling of existing arbor structures, whereas eye rotation demands radical alterations in their trajectory, apparently involving a random search strategy (Udin, 1983). Both processes, however, are thought to be mediated by common mechanisms involving associative reinforcements or the elimination of crossed isthmotectal connections on the basis of their temporal activity correlations with convergent retinal afferents that activate postsynaptic NMDA receptors (Scherer and Udin, 1989).

Strobe rearing led to defective ipsilateral maps, and it blocked their complete reorganization in eye-rotated animals. We conclude that both systematic and radical shifts in crossed isthmotectal arbor position were disrupted because they had difficulty in distinguishing between retinal coactivity at inappropriate versus appropriate sites for binocular convergence. Importantly, these defects have been shown to resemble those that follow visual deprivation during the same developmental period, although they are generally less severe and less consistent. There are two likely explanations for this. First, because ipsilateral units are capable of responding to 1 Hz strobe flashes, they probably are activated with a degree of synchrony in the rearing environment, and it is known that correlated visual activity between neighboring crossed isthmotectal arbors can preserve their mapping order even when mismatched with rotated retinal input (Grant and Keating, 1992; Keating and Grant, 1992). Second, visual contrasts in the strobe environment may have provided additional spatio-temporal information for some relatively minor or gradual reorganizations to occur, such as remodeling of the normal map, but not for the larger scale plasticity required to compensate fully for an eye rotation. The nature of the partial plasticity observed in some of the eye-rotated frogs supports this possibility. As we have noted before (Grant and Keating, 1992), because crossed isthmotectal arbor dimensions average ~200 μm (Udin, 1989), ipsilateral map reorganization in central tectum representing the axis of the rotated eye and in the opposite rostral tectum (Fig. 10) could be achieved by remodeling the existing arbor framework without the need for changes in trajectory.

Although we have no explanation for why this information apparently was used more by some normal and eye-rotated animals than by others, a more interesting question concerns its content. Analyses of contrast-evoked responses during established strobe entrainment showed that they reduced the relative level of synchronous activity at all tectal sites while increasing noncoincident activity over intervals of ± 500 msec, some of which presumably were translated into asynchronous activation of crossed isthmotectal synapses. By analogy with the limits of temporal coincidence (up to 500 msec) between the activity of paired inputs capable of inducing associative LTP in the adult hippocampus (Levy and Steward, 1983; Gustafsson and Wigstrom, 1986), these conditions would seem to be inadequate for reinforcing appropriate crossed isthmotectal synapses. Indeed, given the persistent associative depression of weaker inputs reported in these and other hippocampal studies after asynchronous (by ±100 msec) afferent activations (Stanton and Sejnowski, 1989; Otani and Connor, 1995), they might favor their elimination.

The temporal constraints within which developmental processes of "coincidence detection" actually operate, however, are unknown, and so the possibility remains that afferent activity correlations over hundreds, rather than tens, of milliseconds are of considerable significance. In fact, this possibility is consistent with evidence that NMDA receptor-mediated synaptic currents are this prolonged during periods of structural synaptic plasticity in the developing visual system (Carmignoto and Vicini, 1992; Hestrin, 1992) and that binocular visual stimulation needs to be asynchronous by at least 500 msec to induce ocular dominance shifts in kitten cortex (Blasdel and Pettigrew, 1979; Altmann et al., 1987). The present demonstration that the detailed temporal patterning of neural activity within and between the two eyes influences their synaptic connectivity adds urgency to future characterization of the coincidence sensitivity of developing visual neurons.

REFERENCES


Cook JE (1987) A sharp retinal image increases the topographic preci-


