

# Unequal Representation of the Temporal and Nasal Retina in an Anomalous Projection to the Lateral Thalamus

FRANK SCALIA,\* DAVID SIMAI, BEN HARRIS, AND SHIRLEY EISNER

Department of Anatomy and Cell Biology, State University of New York Health Science Center at Brooklyn, Brooklyn, New York 11203

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## ABSTRACT

Study of an anomalously regenerated, nontopographically organized retinal projection in the frog olfactory cortex revealed that the temporal retina is the main source of this projection, suggesting the existence of specific temporal fiber-directed attractant or trophic influences. In the present study, we examined the organization of an anomalous retinal projection that forms in the frog thalamus after ablation of the optic tectum. The projections from different sectors of the retina were studied by means of the anterograde transport of biotinylated dextran-amine (BDA) delivered to incisions made across the nerve fiber layer in frogs surviving ablation of the contralateral tectal hemisphere for 13–46 weeks. The projections from nasal retinal sectors were always lightly constructed in the aberrant terminal field, whereas their projections to the lateral geniculate complex remained reasonably strong. In contrast, the projections from temporal retinal sectors, though also weak initially, in time became robust and filled the aberrant field over most of its extent. The specific amplification of the temporal fiber projection now observed in two foreign targets provides further evidence for the existence of target-based, attractant/trophic molecules with functional specificity for temporal retinal fibers. That such agents can exist or be inducible in a foreign area would suggest that they belong to a family of molecules having natural biological activity in normal development or regeneration. However, the possibility that the augmented role of the temporal retina in these projections is a result of experience-based plasticity is also discussed. *J. Comp. Neurol.* 416:213–223, 2000. © 2000 Wiley-Liss, Inc.

**Indexing terms:** neuronal specificity; retinotopic projections; topographic mapping; aberrant projections; frog; *Rana pipiens*

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Retinal ganglion cell axons can be induced to grow into and form synapses in certain nonoptic structures during optic nerve regeneration. This result has suggested that molecular mechanisms involved in attracting and stabilizing retinal fiber projections are present or can be expressed in parts of the central nervous system (CNS) outside of the normal optic pathway. One of the more thoroughly characterized examples of such an aberrant connection is the formation of a terminal field selectively in the frog olfactory cortex, which occurs when the optic nerve is severed from the optic chiasma and implanted into the caudal basal telencephalon (Scalia, 1987, 1992). The projection forms in the presence of an intact olfactory bulb and tract. More recent studies (Scalia et al., 1995, 1997a) have shown that this projection is much more stable than originally suggested, and may be permanent. The ability to induce this aberrant projection in adult *Rana pipiens* was viewed as an opportunity to study the mapping behavior of retinal fibers in an area presumably

lacking the appropriate topographic markers. The organization achieved by retinal fibers in a foreign area might reveal properties built upon or masked when interacting normally with the tectum and other normal optic nuclei. Subsequent electrophysiological recording and anatomical tracing studies (Scalia et al., 1995, 1997a) revealed that the bulk of the projection is established by the temporal retina, whereas the terminals of nasal retinal axons are confined to a narrow strip along the anterior edge of the projection field. When the retinal ganglion cells involved in this projection were labeled by means of retrograde transport, the preponderance of labeled neurons was found on

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\*Correspondence to: Dr. Frank Scalia, Department of Anatomy and Cell Biology, State University of New York Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, NY 11203.

Received 13 April 1999; Revised 12 August 1999; Accepted 23 September 1999

the temporal side of the optic disc.<sup>1</sup> Beyond this level of organization, there was no further topographic order.

The partial projections of nasal and temporal retinal fibers to more or less separate but adjacent subfields lacking any internal topological order suggested to us that the overall projection had been established by factors addressing the retina as two differentially identifiable hemiretinal blocks or compartments (Scalia et al., 1997). On the assumption that the molecules controlling this abnormal retinal projection have a more general biological significance, we presented the topography of the aberrant retinotectal projection as evidence of the probable existence of compartment mapping factors in the tectum as well. To support this argument further, we noted that the temporal fiber-repellent molecules in posterior tectal membrane (for reviewed see O'Leary et al., 1999) appear insensitive to the level of origin of the fiber along the horizontal axis and could, therefore, only be expected *in vivo* to be capable of restricting temporal fiber endings to the anterior half of the tectum as an unsorted block. A recent study using the striped membrane model has led to a similar conclusion (Rosentreter et al., 1998).

The earlier findings suggested further that the agents responsible for establishing the anomalous projection to olfactory cortex may be positively biased toward the temporal retina. This bias could mean that attractants or trophic agents may exist or be inducible both in the tectum and in the olfactory cortex that are addressed selectively to temporal retinal fibers. Evidence that nasal fiber-specific attractants may exist in the tectum during the development of the retinotectal projection has been presented in studies demonstrating that nasal retinal fibers mistarget to anterior tectal sites overexpressing the engrailed homologues *en-1* or *en-2* (Friedman and O'Leary, 1996; Itasaki and Nakamura, 1996), whose expression is usually confined to the posterior end of the tectum (Itasaki and Nakamura, 1992). Therefore, the underrepresentation of the nasal retina in the anomalous projection might be accounted for by an absence of the specific nasal fiber attractants controlled by the *en* genes. By the same logic, however, the presence of a substantial representation of temporal fibers in the foreign target would lend weight to the probable existence of temporal fiber-specific attractants, and, if such agents are present in foreign targets, it seems likely, as noted above, that they would also exist in the tectum. However, no molecule with attractant or trophic activity specific to temporal retinal fibers has yet been identified. The only guidance molecules known to have an effect on the targeting of temporal retinal fibers, i.e., RGM (Stahl et al., 1990) and the Eph receptor ligands ephrin-A2 and ephrin-A5, appear to be repellents (Walter et al., 1990; Cheng et al., 1995; Drescher et al., 1995; Nakamoto et al., 1996). The likely absence of ephrin-A2/ephrin-A5 expression in the foreign target could permit the temporal retinal fibers to spread their arbors throughout the neuropil, but the question would remain, are the temporal and nasal retinal fibers differentially attracted to and/or supported by the foreign target? When the winged helix transcription factors CBF-1 and CBF-2, which are normally expressed in the nasal and temporal retina, respectively, are misexpressed in the opposite

halves of the retina during development, fibers from each half of the retina form terminal arbors in the wrong half of the tectum (Yuasa et al., 1996). This effect would be expected if CBF-1 and CBF-2 control attraction-mediating receptors specific to the nasal and temporal retina, although the interpretation actually given proposed that CBF-2 may up-regulate and CBF-1 down-regulate receptors activated by the repellent ligands.

One approach to examining this question further would be to study the representation of the retina in other examples of anomalous retinal projections. Anomalous retinal projections form in the thalamus of neonatal mammals following various schedules of brain lesions (Frost, 1981). In particular, retinal fibers form a terminal field in the lateral posterior nucleus (LP) after ablation of the superior colliculus (Schneider, 1973; Baisinger et al., 1977). The anomalous projection to LP has been shown in the hamster to have elements of a crude retinotopic order, with a strong asymmetry in which it is the ventral retina that is heavily overrepresented (Frost, 1981). An analogous projection has been described in nonmammalian vertebrates.

In adult fish and amphibians, a retinal projection forms in the ipsilateral dorsal thalamus when the optic fibers entering the midbrain tectum are severed and the tectal hemisphere of that side is ablated (Sharma, 1973, 1981; Kicliter et al., 1974; Cantore and Scalia, 1987). The cell group in which the novel projection appears is normally the main recipient of the ascending tectothalamic projection (Rubinson, 1968; Lazar, 1969; Masino and Grobstein, 1990; Montgomery and Fite, 1991). This structure, which in the frog is a complex formed by the central, anterolateral, and posteroventrolateral (C/AL/PVL) nuclei (Neary and Northcutt, 1983), does not receive a retinal projection during optic nerve regeneration in the presence of intact tecta, nor at any time during development (Stelzner et al., 1981; Hoskins and Grobstein, 1985). The normal tectal projection to the lateral neuropil of C/AL/PVL is not topographically organized and, therefore, presumably not retinotopically labeled (Montgomery and Fite, 1991).

The present study examines the topographic organization of this anomalous retinotectal projection in *Rana pipiens*. The results establish a second example in which temporal retinal fibers appear to develop an augmented role relative to nasal fibers in the innervation of a foreign area. This result further supports the likelihood that positive, target-based factors contribute to the differential projections of temporal and nasal retinal fibers. However, insofar as prey insects that are successfully captured and swallowed are usually imaged in the binocular field immediately preceding the attack, the firing patterns of temporal and nasal retinal fibers in the aberrant projection are not equally correlated with feeding activity. The possibility that the closer correlation of temporal fiber activity with food intake may lead to stabilization of the temporal in preference to the nasal fiber terminals must, therefore, be considered as an alternative or supplementary explanation for the asymmetry of the aberrant projections.

## MATERIALS AND METHODS

This study is based on a series of 15 adult frogs (*Rana pipiens*, 3–3.5 in. body length) surviving surgical ablation of the right tectal hemisphere. Surgery was performed with methane tricaine sulfonate (MS-222) anesthesia (0.2 mg/g sc). A skin flap was made over the midbrain, the cranium was opened with a dental burr, the meninges

<sup>1</sup>The distribution of retrogradely labeled ganglion cells was reminiscent of the asymmetric distribution of the TRAP antigen, which, as studied in the chick, is expressed almost exclusively in temporal retinal axons (McLoon, 1991).

TABLE 1. Experimental Subjects: Tracer Placements and Survival Times

| Frog no. | Retinal sector | Postoperative survival (weeks) |
|----------|----------------|--------------------------------|
| 1        | Nasal          | 13                             |
| 2        | Nasal          | 13                             |
| 3        | Ventrotemporal | 13                             |
| 4        | Temporal       | 16                             |
| 5        | Ventral        | 20                             |
| 6        | Ventral        | 23                             |
| 7        | Dorsal         | 23                             |
| 8        | Ventronasal    | 31                             |
| 9        | Nasal          | 32                             |
| 10       | Dorsonasal     | 33                             |
| 11       | Dorsotemporal  | 36                             |
| 12       | Ventrotemporal | 37                             |
| 13       | Ventral        | 40                             |
| 14       | Dorsotemporal  | 40                             |
| 15       | Ventral        | 46                             |

were incised, and the tectal plate was removed unilaterally by aspiration. Care was taken to avoid damaging the pretectal region and underlying torus semicircularis. The cranial wound was closed with a square of millipore filter, the skin was sutured, and the frog was returned to its holding pen. The frogs were maintained in an aquarium provided with running water and floating platforms and were fed crickets. They usually resumed feeding the day after operation. All procedures followed appropriate institutional animal care guidelines.

Sampling was begun at 13 weeks survival and continued through 46 weeks (see Table 1). The partial projections from defined regions of the left retina were studied by application of biotinylated dextran amine (BDA) to retinal axons severed intraocularly. As described in more detail elsewhere (Scalia et al., 1997b), the sclera was surgically exposed over a quadrant of the retina with the frog under MS-222 anesthesia and punctured with a curved stylus. The stylus was drawn across the vitreal surface of the retina to cut retinal axons derived from a particular sector. The stylus was withdrawn, and the eye was injected with 4–5  $\mu$ l of frog Ringer's containing 10–20% BDA (Molecular Probes, Eugene, OR; 3,000 mw), 20  $\mu$ Ci L-proline 5-<sup>3</sup>H (25–36 Ci/mmol; Amersham, Arlington Heights, IL), and 20  $\mu$ Ci L-leucine 4,5-<sup>3</sup>H (47 Ci/mmol; Amersham). This was done to map the projection of the cut axons derived from a truncated sector of the retina against a background provided by the transport of the tritium label in the intact axons derived from the remainder of the retina. Three or four days later, the frog was reanesthetized and killed by transcardial perfusion with Ringer's, followed by 4% glutaraldehyde in 0.12 M phosphate buffer, pH 7.2. The brains and eyes were dissected out and saved.

The brains were cryoprotected by equilibration with 30% buffered sucrose and sectioned frozen at 50  $\mu$ m in the horizontal plane. Two alternating series of sections were collected. One set was reacted freely floating for BDA, mounted on glass slides, dehydrated, cleared, and covered. The other set was mounted and processed by autoradiography. The procedures for BDA histochemistry and autoradiography were described previously (Scalia et al., 1997b). Additional material from previous studies on the normal optic projections and the aberrant retinothalamic projection was also available for examination.

## RESULTS

Figure 1 is provided to illustrate the form and location of the aberrant retinothalamic projection in relation to the normal retinal projection to the contralateral diencephalon. The abnormal projection (Fig. 1A,C,E) lies just deep to

the optic tract and occupies the external half or more of the superficial neuropil of the C/AV/PVL complex. This lateral thalamic neuropil is interposed between the neuropil of the lateral geniculate complex ("nucleus" of Bellonci and "corpus" geniculatum thalamicum) anteriorly and the posterior thalamic "nucleus" of the pretectum posteriorly, each of which receives a dense, retinotopically organized projection from the retina both in the normal (Fig. 1B,D,F) and in the experimental frog. In the normal frog, the lateral neuropil does not contain any part of the retinal projection (Fig. 1B,D,F), although some fascicles of retinal axons pass through the field on the way to the pretectum and tectum (Cantore and Scalia, 1987).

### Projections from the temporal retina

The density of the partial projections contributing to the aberrant field varied according to the location of the tracer placements and the survival time. The strongest projections were obtained from placements in the temporal retina in frogs surviving 36 or more weeks postoperatively (Nos. 11, 12, and 14). In contrast, the projections observed in Nos. 3 and 4, which survived only 13 and 16 weeks, was much less dense, giving the impression that the temporal fiber projection had not yet fully formed. Among the stronger projections, a typical pattern is illustrated in Figure 2A, in a case having a ventrotemporal retinal incision (No. 12). As expected, the BDA label in the nucleus (neuropil) of Bellonci is confined to its posterior rim at this horizontal level, and the label in the posterior thalamic nucleus (neuropil) of the pretectum is also confined to the posterior half of the profile. This pattern is consistent with earlier descriptions of the organization of the retinal projections to the lateral geniculate complex and pretectum (Muntz, 1962; Lazar, 1971; Scalia and Fite, 1974; Montgomery and Fite, 1989). Correspondingly, the autoradiographic silver (Fig. 2B) is prominent anteriorly in both structures, and clear defects appear in the positions occupied by the BDA-labeled field components. In contrast with this retinotopically differentiated pattern, the BDA label is distributed evenly along the anteroposterior dimension of the lateral neuropil of C/AV/PVL, but there is no defect present in the autoradiogram. As illustrated at a series of horizontal levels in Figure 3A–E, the lateral neuropil in this animal (No. 12) contained a smooth distribution of label along most of its dorsoventral extent (A is most dorsal). However, the label became attenuated toward the ventral end of the neuropil. This pole is strongly labeled in the adjacent autoradiograms (not shown). Similar results were observed in a case (No. 11) showing the projection from the dorsotemporal quadrant of the retina (Fig. 3F–J). The projection in the lateral geniculate complex and pretectum was organized in the expected pattern. The BDA and autoradiographic labeling in the lateral neuropil were exactly coextensive and showed no preferential distribution along the anteroposterior dimension. In this case, however, the BDA label became attenuated toward the dorsal end of the neuropil, which was strongly labeled in the adjacent autoradiograms.

Taken together, the two cases illustrated demonstrate some degree of topographic differentiation along the dorsoventral axis of the lateral neuropil in the sense that axons from the ventral part of the temporal retina, which normally run in the dorsal half of the optic tract, fail to reach the ventralmost end of the lateral neuropil. Similarly, fibers from the dorsal part of the temporal retina, which normally run in the ventral half of the optic tract, fail to

reach the dorsal pole of the lateral neuropil. In contrast, there is little or no differentiation in the anteroposterior dimension; the temporal retinal axons in either case are spread uniformly across this axis and also throughout the thickness of the lateral neuropil.

### Projections from the ventral retina

In four other cases (Nos. 5, 6, 13, and 15), the retinal incision was placed ventrally across a vertical meridian passing through the optic disc. In three of these frogs (Nos. 5, 6, and 15), the BDA-labeled component of the projection to the lateral geniculate complex and posterior thalamic nucleus was, as reported in earlier studies, confined to

their most dorsal poles on the contralateral side of the brain, and there was a significant ipsilateral thalamic component to this projection (see, e.g., Scalia and Fite, 1974). There was no projection to the lateral neuropil in the BDA-labeled sections, although the adjacent autoradiograms confirmed the presence of a fully developed aberrant projection. In the fourth frog (No. 13), the retinal incision was much longer than in the others and extended both nasally and temporally almost to the level of the horizontal meridian. The BDA label in this case filled the entire profile of the lateral neuropil across its anteroposterior and dorsoventral dimensions. The results in these cases suggest that very little of the aberrant projection originates near the ventral pole of the retina.

### Projections from the nasal retina

In five cases, the retinal incision was placed on the nasal side of the optic disc. The partial projections to the lateral geniculate and pretectal complexes were typically well developed and retinotopically organized in each case (Fig. 4). Fibers arising in the nasal retina terminated anteriorly in the nucleus of Bellonci and corpus geniculatum thalamicum, anterior to the lateral neuropil, and also in the anterior part of the posterior thalamic nucleus, posterior to the lateral neuropil. In contrast, the projections to the lateral neuropil were consistently weak. In each case, the BDA-labeled fiber plexuses were loosely composed, but extended along most of the dorsoventral axis of the lateral neuropil. Near the ventral pole, the BDA-labeled fibers occupied the deeper region of the neuropil and seemed to extend anteriorly from the labeled fiber system entering the pretectum. In the middle and dorsal end of the lateral neuropil, the fibers tended to shift more superficially and posteriorly to maintain contiguity with the field of label in the pretectum. In the three cases (Nos. 8–10) studied at 31–33 weeks (see Fig. 4A,C), the contribution of the nasal

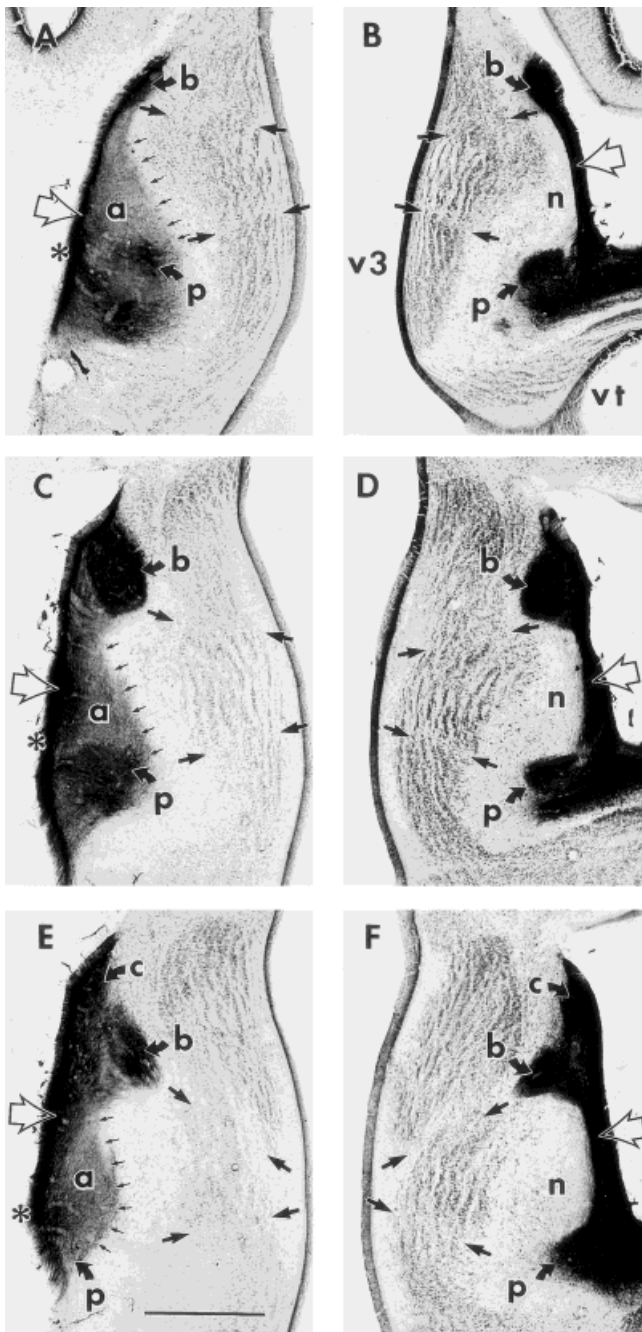


Fig. 1. Topographic relationship between the aberrant retinal innervation of the lateral neuropil, as induced by ablation of the tectum (A,C,E), and the normal retinal innervation of the lateral geniculate complex and pretectum (B,D,F) at three comparable levels in horizontal sections of the brain of *R. pipiens*. The projections of the retina were displayed in both frogs by the anterograde migration of horseradish peroxidase instilled into the contralateral optic nerve. The left and right half-sections are oriented with their medial sides facing inward, i.e., toward the third ventricle (v3), as though both experiments had been done in a single frog. Anterior is to the top. At the most dorsal level (A,B), the aberrant field (a) of retinal fiber terminals in the lateral thalamic neuropil (n) forms a crescentic lamina that spans the interval between the nucleus of Bellonci (b; a major component of the neuropil of the lateral geniculate complex) and the posterior thalamic nucleus (p; a major component of the pretectal neuropil). The inner border of the aberrant field is marked by a row of small arrows. The lateral neuropil (n) contains the dendrites of the subjacent neurons that form the C/AL/PVL complex mentioned in the text. The anterior and posterior borders of this cell mass are defined by the large arrows. The optic tract (open arrow) runs across the lateral surface of the diencephalon. The tectal ventricle (vt) is present only on the right, and an asterisk (\*) marks the original anterior edge of the ablated tectal hemisphere on the left. At an intermediate level (C,D), at which b is more prominent, the aberrant field is more or less triangular in outline and continues to span the interval between b and p. At the most ventral level (E,F), the dorsal end of the so-called corpus geniculatum thalamicum (c; another component neuropil of the lateral geniculate complex) appears lateral to b, which has shifted to a deeper location. The aberrant field is somewhat fusiform in outline at this level, although the most posterior corner of this profile belongs to p. Scale bar = 0.5 mm.

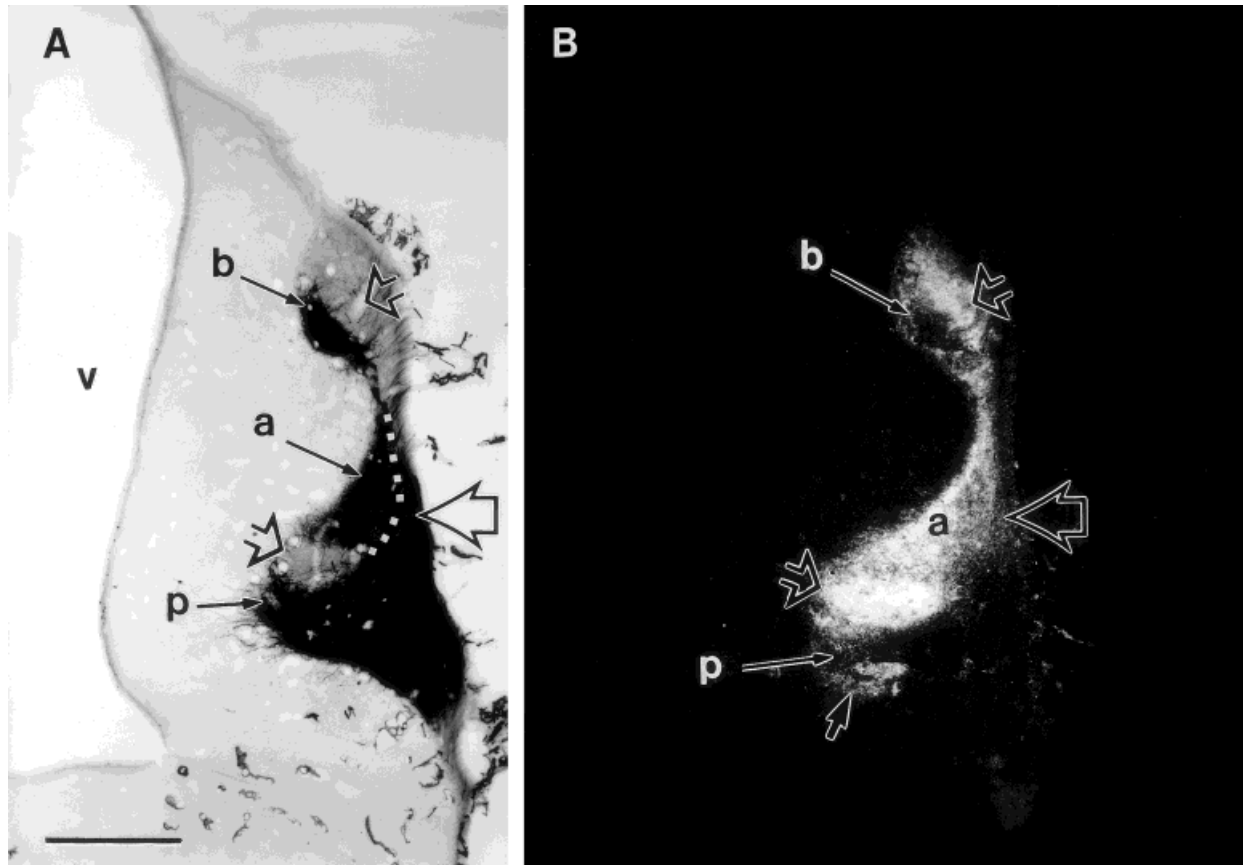


Fig. 2. Relative absence of retinotopic organization in the aberrant field (a) compared to the normal retinotopic innervation of the lateral geniculate complex and pretectum. Brightfield display (A) of the localization of BDA transported in ventrotemporal retinal axons to terminals in b, a, and p is shown alongside an adjacent autoradiograph (B) displaying in darkfield format the tritium label transported from the remainder of the retina, including the spared central apex of the ventrotemporal sector. The BDA-containing retinal axons terminating in the posterior part of the nucleus of Bellonci and posterior thalamic nucleus are labeled b and p, respectively. These terminal zones are deficient in autoradiographic silver in the adjacent section. The part of the optic tract (hollow arrow) present at this level is also marked with

BDA, but not with tritium. In a complementary pattern, the anterior parts of the nucleus of Bellonci and posterior thalamic nucleus (open-tailed arrows) are densely labeled with autoradiographic silver, but not labeled at all with BDA. In contrast, the BDA and tritium label in a are exactly coextensive, indicating that transected (BDA-carrying) and intact (tritium-labeled) retinal axons terminate together in this profile. In B, the failure of the temporal axons to transport the tritium label unmasks the nucleus lentiformis mesencephali (solid arrow), another component of the pretectal region. Horizontal sections. Anterior is to the top; lateral is to the right; v, third ventricle. Scale bar = 0.5 mm.

sectors to the aberrant field was virtually nonexistent compared to the somewhat greater projection in the two cases (Nos. 1 and 2) examined at 13 weeks survival (as in Fig. 4B). In these two frogs, the density of the projection from the nasal retina was comparable to that of the temporal retina when observed at the shorter survival times. These changes in the density of the partial projections may mean that such projection fields as are formed initially by the nasal retinal fibers are not maintained over the longer survival periods, while the temporal fiber projection becomes increasingly amplified.

#### Projections from the dorsal retina

Four cases had incisions placed on the dorsal side of the retina (Nos. 7, 10, 11, and 14). In one case (No. 7), the incision included tissue on both sides of the vertical meridian. The projections to the lateral geniculate complex and pretectum in this case were localized toward their ventral ends, and, as expected, there was a small ipsilateral projection owing to involvement of the temporal

retina. The projection to the lateral neuropil was well developed but was most concentrated near its ventral pole. In the remaining three cases, the incision, although dorsal, was confined to either the nasal or the temporal side of the vertical meridian. Case No. 10 is illustrated in Figure 4C, and case No. 11 is illustrated in Figure 3F–J. In case No. 14, the incision was confined to a small area in the dorsotemporal retina very close to the vertical meridian. The appropriate pattern of contralateral and ipsilateral projection was found in the lateral geniculate complex and pretectum, and the BDA-labeled projection to the lateral neuropil was densely matted and spread throughout its extent. These results indicate that the contribution of the dorsal retina to the anomalous innervation arises mainly from the temporal side of the vertical meridian.

#### Recrossed innervation of the intact tectum

With prolonged survival times, increasing numbers of retinal fibers grow across the midline through available

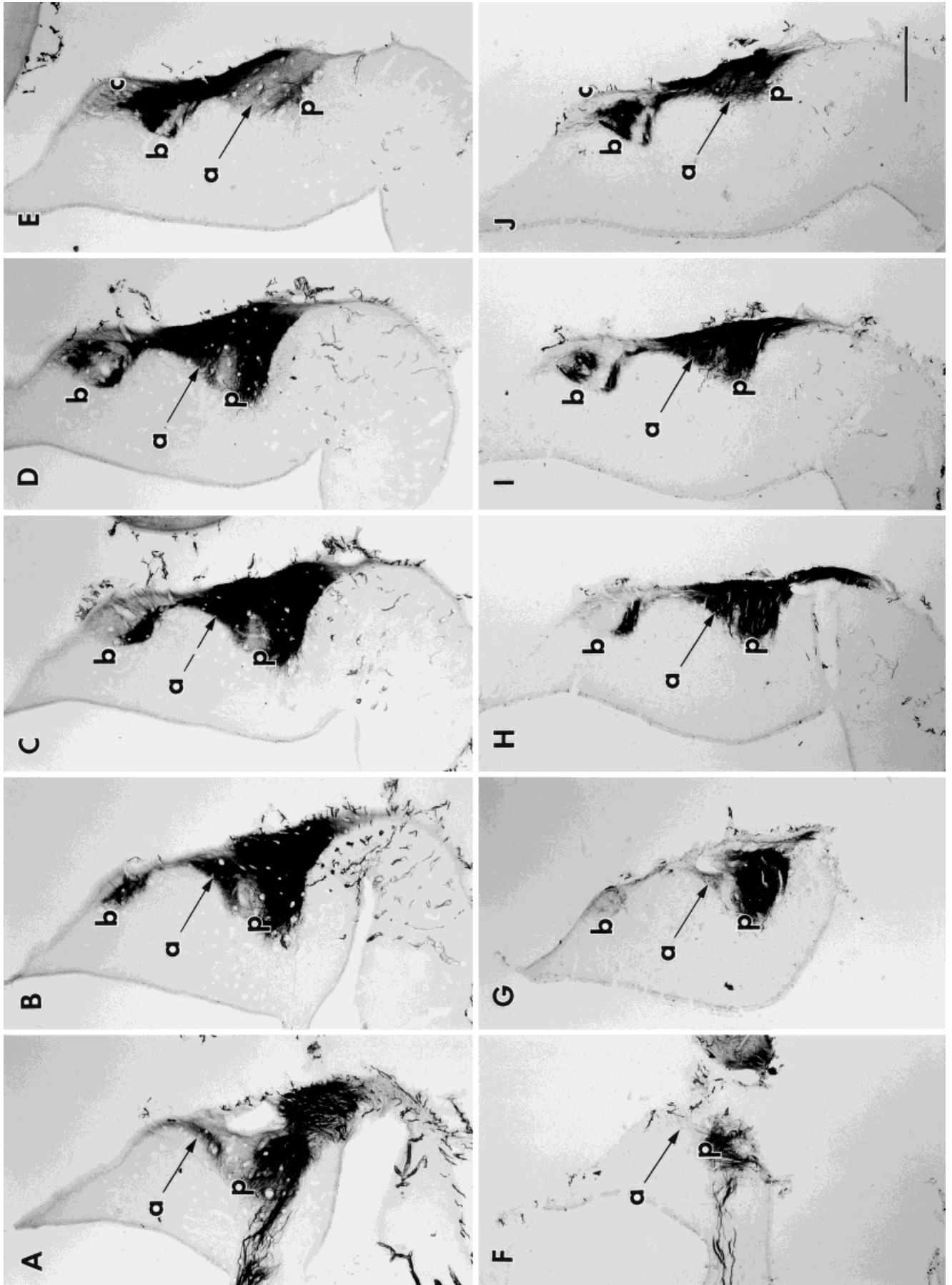


Figure 3

tissue bridges and innervate the intact tectum (Kicliter et al., 1974; Misantone et al., 1974). The size of the recrossed population was examined qualitatively in relation to the placement of the retinal incisions, to determine whether differences in the strength of innervation of the lateral thalamic neuropil might be inversely correlated with the size of the recrossed populations. There was no evidence in the BDA-labeled sections that nasal retinal fibers had recrossed in greater numbers than the temporal fibers at any survival time. This is further borne out in the autoradiographs of frogs 5, 6, and 15, all of which had ventral incisions sparing most of the temporal and nasal retina. In these cases, the relative density of autoradiographic silver marking the projections from intact temporal and nasal axons was approximately equivalent in the anterior and posterior regions of the intact tectum.

## DISCUSSION

The results of this study indicate that the aberrant retinothalamic projection established in the adult frog after tectal ablation is formed mainly by fibers from the temporal retina. In the experimental animals surviving for more than 16 weeks postoperatively in which placements of tracer (BDA) were made in the nerve fiber layer of the dorsotemporal or ventrottemporal quadrants of the retina, the tracer labeled densely a large fraction of the aberrant field, occupying nearly its complete outline. Placements of tracer in the nasal half of the retina revealed weaker projections formed by smaller populations of fibers at all survival times, although the same placements labeled substantial projections in the lateral geniculate complex and pretectum.

A notable outcome of the present study is the similarity revealed in the organization of the anomalous projections of retinal fibers to two anatomically unrelated brain areas, the olfactory cortex and lateral neuropil. This similarity suggests certain inferences that would be less compelling had the projections to either of the nonoptic targets been shown to arise equally from both halves of the retina or to arise mainly from the nasal retina. The common asymmetry of the projections immediately suggests two hypotheses: 1) Specific chemoattractant or trophic factors exist that are selectively targeted to temporal retinal fibers. 2) Reinforcing stimuli, as may occur during successful attacks on prey insects, stabilize the misplaced temporal fiber terminals in preference to the nasal fiber terminals. However, before discussing these possible determinants, the extent to which the present results might have been influenced by incidental neuroanatomical relationships and other nonspecific variables should be considered.

### Nonspecific determinants

First, it is possible that some aspects of the organization of the aberrant retinothalamic projection are influenced by

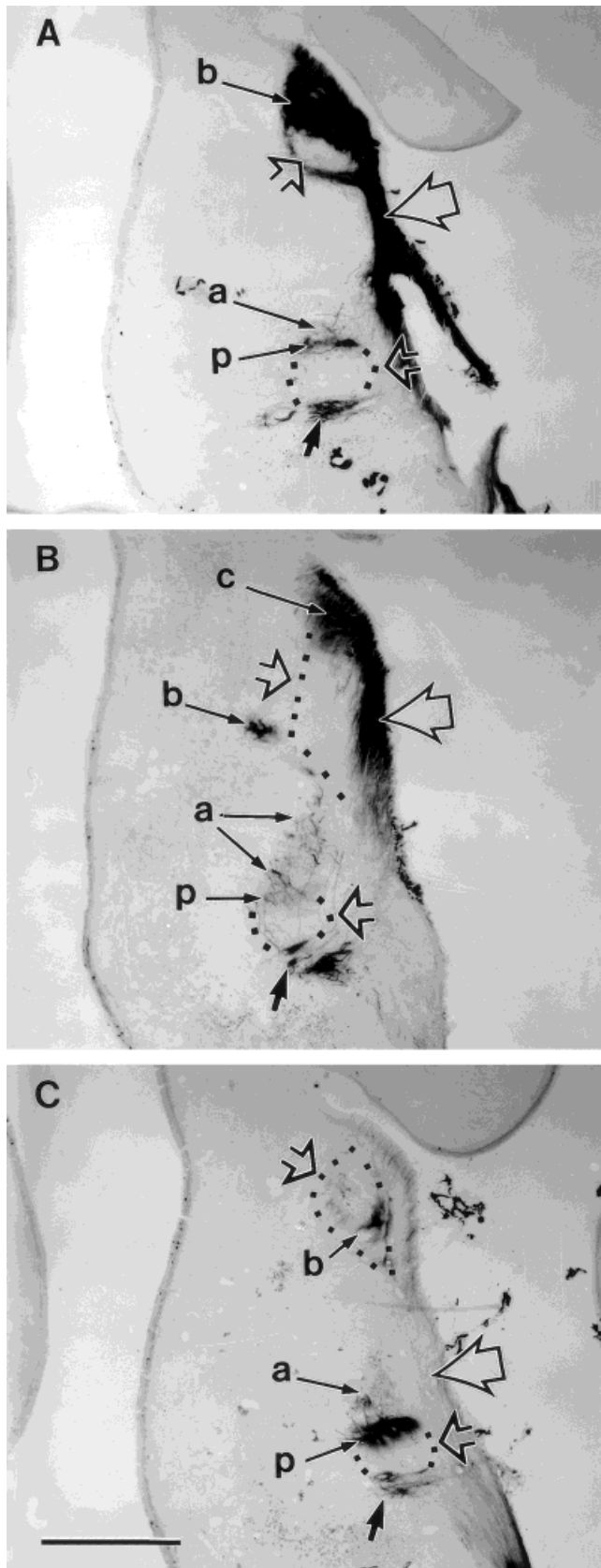
the topographic geometry of the normal optic tract. For example, although the subfields labeled by dorsal and ventral placements of tracer in the temporal retina were broadly overlapping, an indication of poor retinotopic differentiation, the overlap was not absolute. Fibers originating in the dorsal or ventral parts of the temporal retina tended not to reach into, respectively, the ventral and dorsal poles of the aberrant field. This might reflect only a failure of tectal ablation to disturb the normal organization of the optic tract. That is to say, because fibers from the ventral retina normally run in the dorsal half of the optic tract, whereas fibers from the dorsal retina run in its ventral half (Fujisawa et al., 1981), the persistence of this arrangement may tend to focus the regenerating fiber terminals from the dorsal and ventral halves of the retina into regions of the lateral neuropil more proximate to the location of their parent axons in the optic tract. However, this would not explain the final distribution of temporal and nasal fiber terminals. Nasal retinal fibers normally course within both the dorsal and the ventral margins of the optic tract in the diencephalon, whereas the temporal fibers occupy the middle stream (Fujisawa et al., 1981). If the normal fiber topography had determined the distribution of temporal and nasal fiber terminals, the nasal terminals would have accumulated in the dorsal and ventral poles of the lateral neuropil, and the temporal fiber terminals would have concentrated at midlevel. However, this was not observed.

Second, it seems reasonable to assume that possible topographic markers in the foreign targets may interact with the misplaced retinal fibers. Although the olfactory cortex does not normally receive a retinal projection, the lateral neuropil does have second-order retinally derived input by way of the tectum. However, this projection, which arises from all regions of the ipsilateral tectum, is not tectotopically organized (Montgomery and Fite, 1991). Therefore, the lateral neuropil does not appear to contain at the outset any vestige of a retinotopic map with which to influence the organization of the novel projection.

Third, the format of the retinal projection in areas adjacent to the lateral neuropil is also not likely to have influenced the organization of the aberrant terminal field. One might suppose, for example, that the new terminals might become arranged in a pattern consistent with the mapping of the retinal terminals in the established contiguous areas of the thalamus and pretectum. However, although the lateral neuropil is bordered anteriorly by a temporal retinal projection field in the nucleus of Bellonci and corpus geniculatum, it is also bordered posteriorly by a nasal projection field in the posterior thalamic nucleus (Montgomery and Fite, 1989). Because the temporal retinal component of the aberrant projection extends the full anteroposterior width of the lateral neuropil, it becomes contiguous with the nasal projection field in the posterior thalamic nucleus.

Finally, the possible influence of plastic/regenerative responses in other fiber systems damaged during ablation of the tectum might warrant consideration. In particular, fibers from the contralateral nucleus isthmi are transected along the anterior border of the tectum at the time of surgery. These fibers might recover and innervate the lateral neuropil along with the injured retinal fibers. Because the contralateral nucleus isthmi represents the

Fig. 3. Overall projection patterns obtained from ventrottemporal (A-E) and dorsotemporal (F-J) sectors of the retina in two frogs surviving unilateral tectal ablation for 37 and 36 weeks, respectively, as observed in horizontal sections at comparable levels. Anterior is to the top; lateral is to the right. A and F are most dorsal. Although the patterns are broadly overlapping, there is some degree of retinotopic order along the dorsoventral axis of the aberrant field (a); the ventrottemporal fibers do not reach into the ventral pole of a (as in E), whereas the dorsotemporal fibers fail to innervate its dorsal pole (as in F). Labeling as in Figure 1. Scale bar = 0.5 mm.



temporal retina (Gruberg and Udin, 1978; Gruberg and Lettvin, 1980; Grobstein and Comer, 1983), such rerouting of the isthmal fibers could in some way influence the organization of the retinal projection. However, although the ability of injured isthmoctectal fibers to regenerate has never been studied, it has been shown that tectal axons do not regenerate in the frog (Lyon and Stelzner, 1987). This suggests that, with the exception of the optic nerve, frog CNS axons have only a limited capacity to regenerate.

### Molecular specificity

If attractant or trophic factors are responsible for the formation, and particularly for the asymmetry, of the experimentally induced aberrant projections, it seems reasonable to infer that such agents would have a natural function in the normal targets of the retinal projection. However, with respect to the development of the retinotectal system, there is little agreement on whether any aspect of the organization of the retinal map, or even the targeting of the tectum per se, is controlled by specific attractant/trophic factors. Evidence has been presented against the existence of long-range chemical attractants (Harris et al., 1985; McFarlane et al., 1995), while others have given theoretical arguments suggesting the need for target-based, short-range chemoattractants to provide a positive factor in the innervation of the tectum (Taylor, 1990; O'Leary et al., 1999).

In one model (O'Leary et al., 1999), a gradient of a receptor on retinal fibers, running from high in the most temporal fibers to low in the most nasal fibers, is matched with a ligand in the tectum, graded from low anteriorly to high posteriorly. An attractant function, expressed as the induction of interstitial branching behind (anterior to) the growth cone of the advancing fiber, is activated at all points at which a threshold value in the product of receptor and ligand concentration is exceeded. This would establish along the anteroposterior axis of the tectum a critical coordinate for each fiber at which its most anterior branches could appear. A parallel gradient of repellent activity would inhibit branching in the region posterior to the critical coordinate so that synaptic terminals that result from the branching would be confined to the critical region. The model does not invoke different attractant systems for

Fig. 4. Projection patterns obtained from the nasal retina reveal its minimal involvement in establishing the aberrant terminal field. Horizontal sections from levels at which maximal densities of BDA labeling were observed in the aberrant field (a) in frogs having ventronasal (A), midnasal (B), and dorsonasal (C) placements of tracer show significant innervation of the lateral geniculate complex and pretectum but only weak projections to the lateral neuropil. Anterior is to the top; lateral is to the right. Optic tract is indicated by the hollow arrows. In A and C, the parts of the nucleus of Bellonci (dotted outline in C) in which the BDA-containing fibers terminate are labeled b, and the open-tailed arrows point to the complementary zone. The nucleus of Bellonci (b) also appears in B, in which the label c identifies the anterior region of the corpus geniculatum thalamicum targeted by the nasal retinal fibers. The remainder of this neuropil is outlined by dots. Similarly, in A-C, p points to the anterior region of the posterior thalamic nucleus containing the BDA-labeled terminal zone (it is very light in B), and the open-tailed arrows point to the complementary unlabeled region (dotted outline). The posterior side of this neuropil is bordered by the nucleus lentiformis mesencephali (solid arrows), which contains a knot of BDA-labeled fibers. The strongest projection of nasal retinal fibers to a appears in B, but all examples are weak compared to the dense projections from the temporal retina illustrated in Figures 2 and 3. Scale bar = 0.5 mm.

temporal and nasal retinal fibers, only one system activated over a spatial gradient. To explain the asymmetry noted in the anomalous projections in terms of this model, it would only be necessary to assume that the foreign targets express the attraction-activating ligand only at a single concentration throughout the neuropil. That concentration would determine how much of the temporonasal axis could be effectively represented, because it would be sufficient to promote the branching of all axons from the temporal side meeting or exceeding a critical level of receptor, but not enough to activate the more nasal population.

Alternatively, the attractants involved in the targeting of temporal and nasal retinal fibers may belong to separate molecular systems. This possibility is supported by several lines of evidence indicating that nasal fibers have a specific affinity for posterior tectum that is mediated by a mechanism not shared with temporal fibers. First, it has been reported that nasal fibers growing *in vitro* have a tendency to prefer and to survive longer on substrates containing posterior tectal membrane components, whereas temporal fibers are repelled by posterior membrane and survive better on anterior membrane (Boxberg et al., 1993). The repellent effect is believed to be activated by the Eph receptor ligands ephrin-A2 and ephrin-A5, which are expressed at high levels in the posterior tectum of many species. These members of the ephrin family appear to be regulated by *en-1* and *en-2*, homologues of *Drosophila engrailed* (Itasaki and Nakamura, 1992; Shigetani et al., 1997). Experimental misexpression of the *en* genes in the anterior tectum during the development of the retinotectal projection disrupts the targeting of both nasal and temporal fibers. Temporal retinal fibers tend to stall in the optic tract at the anterior border of the tectum, whereas nasal fibers tend to branch ectopically in the anterior tectum, sometimes forming terminal arbors in areas of high *en* misexpression (Friedman and O'Leary, 1996; Itasaki and Nakamura, 1996). Thus, the *en* gene appears to mediate contrasting effects of attraction in nasal fibers and repulsion in temporal fibers. It is not known whether these effects are graded along the temporonasal axis of the retina or expressed as a step function. Either way, the reversal evidently occurs somewhere near the middle of the retina, and, insofar as the functional consequence is to create an attractant specific for nasal fibers, it seems reasonable to suggest that a separate system is needed to activate attraction specific to temporal fibers.

In any event, the hypothesis that retinal fiber-specific factors are involved in establishing the aberrant projections raises the question of why such factors would be expressed in nonoptic areas of the brain. One possibility is that these agents are induced by the presence of retinal fiber endings, an idea proposed earlier in a more general model of the retinotectal projection (Wilshaw and van der Malsburg, 1979). The induction process may take place over a protracted period; the abnormal projections appear to develop gradually over several months postoperatively. However, the protracted amplification of the temporal fiber projection may also reflect the gradually realized impact of behaviorally relevant events on the morphology of the retinal terminals, as discussed below.

### Behavioral reinforcement

It is possible that the asymmetric organization of both aberrant projections is consolidated, or even driven, by a

process of activity/experience-dependent plasticity associated with feeding behavior after the misplaced terminals become functionally integrated into the normal synaptic networks of the telencephalon and lateral thalamus. The retinal terminals in the olfactory cortex may be conscripted into assuming some of the roles of the normal olfactory tract terminals whose territory they have invaded. Visually evoked activity may then supplement true odorant-elicited signals during olfactory learning. Similarly, retinal terminals in the lateral neuropil may take on the role of the tectal endings they have replaced, which may involve them in processes normally related to, for example, the learning of environmental cues important for effective prey capture. In their new roles, the retinal terminals may undergo morphological changes appropriate to the long-term storage of associations between visual signals and feeding. However, the terminals would provide signals meaningfully correlated with the capture of prey insects only when the prey has been imaged in the binocular field, as described below.

The frog's total visual field consists of lateral subfields that are monocular and a frontal binocular subfield (Fite, 1973). Careful study of prey-catching behavior in *R. pipiens* (Ingle, 1970) has revealed that the prey insect is almost always captured by means of a direct frontal attack. In other words, the frog does not usually flick its tongue to the side to capture prey in the lateral visual field but turns bodily to face the victim prior to or at the time of capture. This maneuver places the prey in the frontal binocular field, if only momentarily. Thus, when a prey insect is detected in the lateral field of the normal eye, which is imaged on the nasal retina, the frog may turn toward it to view it in the frontal field. This orienting response repositions the image onto the temporal retina of both eyes. Because the normal eye remains connected to the contralateral tectum (the entire retina projects contralaterally to the tectum), the frog may lunge forward in the normal way and capture the insect (the snapping response). At the same time, the temporal fiber terminals in the aberrant projection will also be active, although probably without behavioral consequence. If, as a result of prey capture a reward-associated fiber system is activated, the normal function of which would be to potentiate the recently active synapses of exteroceptive systems firing in temporal correlation with feeding, the long-term effect of the repeated food reinforcement may lead to stabilization and proliferation of the temporal in preference to the nasal fiber terminals of the aberrant projection. On the other hand, when a prey insect moves in the lateral field of the abnormal eye, there is either no behavioral response or an inappropriate one, in which the frog orients toward the mirror-symmetric locus in the opposite lateral field. Mirror-symmetric orienting occurs as a result of the recrossed innervation of the intact tectum, as observed previously (Misantone and Stelzner, 1974). In either case, there would be no reward associated with this activity. The nasal fiber terminals of the abnormally connected eye that are located in the foreign target (olfactory cortex or lateral neuropil) would be less frequently reinforced than the temporal fiber endings and may ultimately be retracted.

Many studies have presented evidence that changes in various aspects of synaptic structure can accompany learning and memory formation (for review see Bailey and Kandel, 1993), but only a few have provided information on the effects of learning on the size of the terminal arbor.

It is known that the number of varicosities and terminal branches of identified sensory nerve terminals in *Aplysia* increase after long-term sensitization of gill withdrawal and decrease with its habituation (Bailey and Chen, 1988). Although such correlations might appear to provide supportive evidence, an important difference between the model studied in *Aplysia* and the proposal discussed above is that sensitization and habituation are considered to be forms of nonassociative learning (Bailey and Kandel, 1993), but the hypothetical process considered here is associative. In contrast to the findings in *Aplysia*, a reduction in arbor size was observed in a study of associative learning in *Hermissenda* (Alkon et al., 1990). Thus, appropriate evidence to support the above proposal is not presently available. In any event, the possible contribution of behavioral reinforcement may be tested by reexamining the topographic organization of the aberrant retinohalamic and retinotelencephalic projections formed under conditions that eliminate binocular imaging during prey capture.

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