Development 123, 439-450
Printed in Great Britain © The Company of Biologists Limited 1996
DEV3373

Mutations disrupting the ordering and topographic mapping of axons in the retinotectal projection of the zebrafish, *Danio rerio*

Torsten Trowe[1,§], Stefan Klostermann[1,*], Herwig Baier[1,†], Michael Granato[2], Alexander D. Crawford[1], Barbara Grunewald[1], Heike Hoffmann[1], Rolf O. Karlstrom[1], Stefan U. Meyer[1], Bernhard Müller[1], Sandra Richter[1,‡], Christiane Nüsslein-Volhard[2] and Friedrich Bonhoeffer[1]

[1]Abteilung Physikalische Biologie and [2]Abteilung Genetik, Max-Planck-Institut für Entwicklungsbiologie, Spemannstrasse 35, 72076 Tübingen, Germany

*Present address: Abteilung TB-D, Boehringer Mannheim, Nonnenwald 2, D-82377 Penzberg, Germany
†Present address: Department of Biology 0366, University of California, San Diego, La Jolla, CA 92093, USA
‡Present address: Zoologisches Institut der Universität Basel, Rheinsprung 9, CH-4051 Basel, Switzerland
§Author for correspondence (e-mail: trowe@mpib-tuebingen.mpg.de)

SUMMARY

Retinal ganglion cells connect to their target organ, the tectum, in a highly ordered fashion. We performed a large-scale screen for mutations affecting the retinotectal projection of the zebrafish, which resulted in the identification of 114 mutations. 44 of these mutations disturb either the order of RGC axons in the optic nerve and tract, the establishment of a topographic map on the tectum, or the formation of proper termination fields. Mutations in three genes, *boxer*, *dackel* and *pinscher*, disrupt the sorting of axons in the optic tract but do not affect mapping on the tectum. In these mutants, axons from the dorsal retina grow along both the ventral and the dorsal branch of the optic tract. Mutations in two genes, *nevermind* and *who-cares*, affect the dorsoventral patterning of the projection. In embryos homozygous for either of these mutations, axons from dorsal retinal ganglion cells terminate ventrally and dorsally in the tectum. In *nevermind*, the retinotopic order of axons along the optic nerve and tract is changed in a characteristic way as well, while it appears to be unaffected in *who-cares*. Two mutations in two complementation groups, *gnarled* and *macho*, affect the anteroposterior patterning of the projection. In these mutants, nasodorsal axons branch and terminate too soon in the anterior tectum. In 27 mutants belonging to six complementation groups, retinal axons do not form normal termination fields. Some implications for models concerning the formation of topographic projections are discussed.

Key words: retinotectal projection, topographic mapping, zebrafish, *Danio rerio*, visual system

INTRODUCTION

Most sensory inputs to the central nervous system are topographically arranged (reviewed by Udin and Fawcett, 1988), that is, the spatial order of neurons is reflected by the order of their axon terminals in the target organ. Such order is well illustrated in the extensively studied retinotectal projection of lower vertebrates, where axons of dorsal retinal ganglion cells (RGCs) project to the ventral (lateral) tectum while ventral axons project to the dorsal (medial) tectum. Likewise, nasal RGC axons project to posterior (caudal) tectum while temporal axons project to the anterior (rostral) tectum (Fig. 1). Thus a doubly inverted map of the retina is created on the tectum.

Several mechanisms have been proposed to account for the establishment of this highly ordered projection. Roger Sperry suggested the existence of graded cues in the tectum and in the retina that could serve as positional markers (Sperry, 1963). Other models propose that interactions between fibers give rise to the observed topography (e.g. Hope et al., 1976; Horder and Martin, 1978). Still other models assume that synaptic activity is used to order an initially random or crudely sorted projection (Willshaw and von der Malsburg, 1976; Whitelaw and Cowan, 1981).

However, these mechanisms on their own are unable to explain certain features of regeneration and regulation shown by the projection (Hope et al., 1976; Fraser, 1980; Fraser and Perkel, 1990; Whitelaw and Cowan, 1981; Gierer, 1983). If, for example, the temporal retina is ablated, the anterior tectum will remain devoid of retinal innervation. The remaining nasal RGC axons will pass the anterior tectum and still project correctly to the posterior tectum, demonstrating that they can distinguish between both (Sperry, 1963). Given sufficient time, however, the nasal arbors will expand to cover the entire tectum (Schmidt, 1978; Schmidt et al., 1978; Yoon, 1972). If the tectum, or a piece of it, is rotated prior to innervation by retinal axons, the resulting projection is also rotated, demonstrating the existence of stable positional cues (Yoon, 1973, 1975). If the posterior tectum is ablated prior to innervation by the retinal axons, the nasal axons will innervate the posterior part of the remaining anterior tectum

and, together with the other RGC axons, form a compressed but topographic map on the remaining tectal half (Gaze and Sharma, 1970). Finally, if axons of two eyes are forced to innervate the same tectal lobe, they will terminate in alternating stripes, reminiscent of ocular dominance columns (Constantine-Paton and Law, 1978; Levine and Jacobson, 1975). The formation of these columns is an activity-dependent process (Meyer, 1982).

To explain most or even all of the known properties of the projection, a model has to incorporate several mechanisms. Most models combine global positional cues, as proposed by Sperry, with one or more additional mechanisms, such as repulsion between fibers (Fraser, 1980; Fraser and Perkel, 1990; Gierer, 1983), activity-dependent strengthening of synapses (Whitelaw and Cowan, 1981), or all of the above (Fraser and Perkel, 1990).

Up to now, several laboratories have identified molecules with a graded expression (reviewed by Sanes, 1993), thus strengthening the idea that graded cues are used to set up the projection, but strict evidence for their involvement is still lacking. The best candidate molecules for positional signals are probably two recently cloned ligands for receptor tyrosine kinases of the EPH family, RAGS and ELF-1 (Drescher et al., 1995; Cheng et al., 1995; reviewed by Orike and Pini, 1996), and RGM, an antigen not yet characterised at the molecular level (B. Müller, unpublished observations). All three are expressed in gradients increasing from anterior to posterior tectum. There is evidence from in vitro studies that retinal axons read two of these graded molecules, RAGS and RGM, as repulsive signals.

One suitable organism in which to study the retinotectal projection is the zebrafish, *Danio rerio*. The development of its projection has been investigated in detail (Burrill and Easter, 1995; Kaethner and Stuermer, 1992; Stuermer, 1988; Stuermer et al., 1990). Unlike other vertebrates, the zebrafish offers the possibility of a genetic approach. In the hope of gaining new insights into the mechanisms that lead to the establishment of an ordered neuronal connection, we participated in a large-scale screen for mutations affecting the retinotectal projection of the zebrafish. Mutations were induced in male zebrafish by treatment with a point mutagen. The resulting mutations were made homozygous by inbreeding (Haffter et al., 1996). In the third generation, the retinotectal projections of the fish were analyzed. In order to make the retinal axons visible, one of the eyes of the fish was injected with two lipophilic fluorescent dyes at two specific positions (see Baier et al., 1996, for a detailed description of the method). The dyes are taken up by the RGCs and diffuse within the membranes of the axons, labeling them along their entire length. The pattern of the labeled axons is very characteristic. Mutations that resulted in a deviation from that pattern could be detected by visual inspection with a fluorescence microscope.

We identified 114 mutations that affect different aspects of the development of the projection. This paper will concentrate on those mutations that affect the sorting of axons into the two branches of the optic tract, the topography of the projection, and the formation of termination fields. Mutations affecting the pathfinding of RGC axons are described by Karlstrom et al. (1996).

MATERIALS AND METHODS

General

Fish were raised and kept under standard conditions, as previously described (Mullins et al., 1994). Embryos were kept at 28.5°C in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 33 mM $MgSO_4$). Mutant carriers were identified by random intercrosses. To maintain a stock, identified carriers were outcrossed to wild-type fish. For complementation analysis, carriers of different mutations were crossed to each other. If the embryos of such a cross did not show a mutant phenotype, the mutations were considered to complement, and were assigned to different complementation groups.

Labeling of RGCs

Dye injections were done as described by Baier et al. (1996). Briefly, fish were fixed 5 days postfertilisation in 4% paraformaldehyde. After a minimum fixation time of 12 hours, they were mounted at defined positions in an agarose gel (1.2% in PBS diluted 1:3 in water). Axons of RGCs were labeled by intraocular injections of DiI (1,1′dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) and DiO (3,3′-dioctadecyloxacarbocyanine perchlorate). The brain was labeled by incubating the fish in DAPI (4,6-diamidino-2-phenylindole) (5 mg/l) overnight.

Photoconversion of DiI was done as described by Westerfield (1993).

For sectioning, fish were dehydrated through an alcohol series. Afterwards, they were first transferred to dry acetone, then to diluted araldite (50% acetone, 50% araldite). The acetone was allowed to evaporate overnight at room temperature, and the fish were transferred to pure araldite. After polymerization of the araldite (60°C, 48 hours), the fish were sectioned using a Reichert-Jung microtome (Reichert-Jung 2050). The thickness of the sections was 7 μm.

RESULTS

Retinotectal projection of the wild type

In zebrafish, the first RGC axons leave the eye about 34 hours postfertilization (Burrill and Easter, 1995; Stuermer, 1988). 3 days after fertilization, the first nasal axons arrive at the posterior tectum. In zebrafish, the mapping of RGC axons is precise from the onset.

To inspect the retinotectal projection, we injected two dyes, DiI and DiO, into the temporoventral and the nasodorsal quadrant of the retina, respectively. In a 5-day-old wild-type zebrafish, this yields the following pattern:

Nasodorsal axons and temporoventral axons project through the optic nerve and tract to the contralateral tectal lobe (Figs 1, 2). In the optic nerve, the two labeled axon populations occupy separate portions. Axons of neighboring cells grow next to each other (Stuermer, 1988) (Figs 1, 2). The optic tract is split into a dorsal and a ventral branch. Dorsal and ventral axons segregate into the ventral and the dorsal branch of the tract, respectively (Figs 1, 2). Nasodorsal axons, upon arriving at the tectum, continue their ventral trajectory and terminate in the posteroventral tectum (Figs 1, 2). Temporoventral axons terminate in the anterodorsal tectum (Figs 1, 2). The size of the labeled termination fields varies, depending on the number of axons labeled by the injection.

During the screen, we could not see the outline of the tectum. To judge whether the labeled axons terminated retinotopically, we investigated the position of the labeled axons relative to each other. Nasodorsal axons of wild-type fish always

terminate posterior and ventral to the temporoventral axons (Fig. 2C).

Mutants

We keep a stock of 114 mutants. They can be grouped according to their phenotypes into four different classes: pathfinding mutants in which RGC axons have abnormal trajectories, mutants that are affected in the sorting of axons in the optic tract, mutants in which the RGC axons do not map retinotopically on the tectum and mutants showing aberrant arborization patterns in the target. This paper will focus on the last three classes. The pathfinding mutants are described by Karlstrom et al. (1996).

The mutants described in this paper are listed in Table 1, and schematic drawings of their retinotectal phenotypes are shown in Fig. 3.

Mutations affecting the sorting of axons in the optic tract

We found 14 mutations in four loci, *boxer* (*box*), *dackel* (*dak*), *pinscher* (*pic*) and *nevermind* (*nev*), which affect the sorting of axons in the optic tract. All show a similar phenotype: dorsal axons grow through both the ventral and the dorsal branches of the optic tract, instead of selectively using the ventral branch (Fig. 4). Mapping is not affected in *box*, *dak* and *pic*. The misrouted axons are able to locate their retinotopic target area in the tectum. Mapping of retinal axons is affected in *nev*. This mutation is therefore discussed in detail with the mapping mutants. All of these mutants have defects in other organs as well. *box* and *dak* mutants have reduced pectoral fins, and *box*, *dak* and *pic* mutants have jaw and brain defects.

boxer and *dackel*

In fish with mutations in either *box* or *dak*, nasodorsal axons grow into both branches of the optic tract (Fig. 4B). The nasodorsal axons that enter the tectum through the ventral branch grow in a wild-type manner along the ventral tectal side and terminate retinotopically in the posteroventral tectum (Fig. 4C,E). Nasodorsal axons entering the tectum through the dorsal branch at first continue their dorsal trajectory. Near the posterior edge of the tectum they turn and grow to their retinotopic posteroventral target area (Fig. 4C,E). In *box*, the ventral location of the termination field has been confirmed by analysis of transverse araldite sections (Fig. 5B).

The nasodorsal axons that grow along the inappropriate dorsal side of the tectum are more fasciculated than the ones on the ventral side (Fig. 4E). Some misrouted axons leave the tectal neuropil (Fig. 4C, arrowhead). These axons invariably grow toward the ipsilateral tectal lobe. If they reach it, they enter it from the dorsal side, grow to its posterior edge, turn, and terminate retinotopically posteroventrally (data not shown).

The sorting of ventral axons is apparently not affected in *box* and *dak* mutants. Temporoventral axons grow mostly along the dorsal branch. We analyzed the behaviour of nasoventral axons in *box* fish to determine if they behave differently from temporoventral axons. In 33% of *box* mutant fish, nasoventral axons grow exclusively along the dorsal branch of the optic tract (n=14). This number is very similar in wild-type animals (25%, n=119). In the remaining *box* and wild-type fish, ventral axons were also seen in the ventral branch of the optic tract. In contrast, when dorsal axons are labeled in wild-type fish, they grow in 97% of cases in the ventral branch only (118 injections). That the sorting of ventral axons appears to be less precise than that of dorsal axons might simply reflect a weakness of our injection method. Using our apparatus, the nasoventral retina is more difficult to inject than the nasodorsal retina.

Dorsal axons that enter the tectum through the dorsal branch of the optic tract have two possible ways to reach their ventral target area. One is to grow around the periphery of the tectal lobe, and the other is to grow directly across it to the appropriate site. To distinguish between these two possibilities, we labeled middorsal axons (see Fig. 1). Like nasodorsal axons, middorsal axons grow into both branches of the optic tract. But instead of growing to the posterior margin of the tectal lobe, most ectopic middorsal axons make an abrupt turn in the middorsal lobe (Fig. 4D, arrow). They grow across the lobe directly to their retinotopic midventral target area.

In the optic nerve, the order of axons appears to be normal.

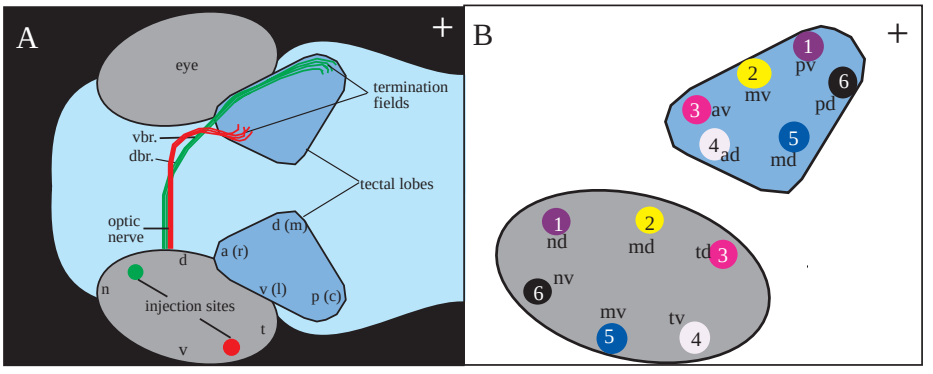

Fig. 1. (A) Schematic drawing of the retinotectal projection of a wild-type zebrafish, dorsal view. In this and all following figures, anterior is to the left. Only nasodorsal axons (green) and temporoventral axons (red) are shown. Unless otherwise stated, this colour code is kept for all following pictures. The RGC axons project through the optic nerve and tract to the contralateral tectal lobe. Nasodorsal and temporoventral axons occupy different parts of the optic nerve. The temporoventral axon bundle is located posterior of the nasodorsal bundle. The optic tract is split into a dorsal and a ventral branch. Ventral axons grow into the dorsal branch, dorsal axons into the ventral one. On the tectum, retinal axons map according to their position in the retina. Ventral axons terminate in the dorsal tectum, dorsal axons in the ventral tectum. Likewise, temporal axons terminate in the anterior tectum, nasal axons in the posterior tectum. On the tectum, anterior is also referred to as rostral, posterior as caudal, dorsal as medial and ventral as lateral. (B) Terminology for the retinal and tectal positions. Retinal positions and the corresponding positions on the contralateral tectal lobe have been labeled with identical colours. a, anterior; ad, anterodorsal; av, anteroventral; c, caudal; d, dorsal; dbr, dorsal branch of optic tract; l, lateral; m, medial; md, middorsal; mv, midventral; n, nasal; nd, nasodorsal; nv, nasoventral; p, posterior; pd, posterodorsal; pv, posteroventral; r, rostral; t, temporal; td, temporodorsal; tv, temporoventral; v, ventral; vbr, ventral branch of optic tract.

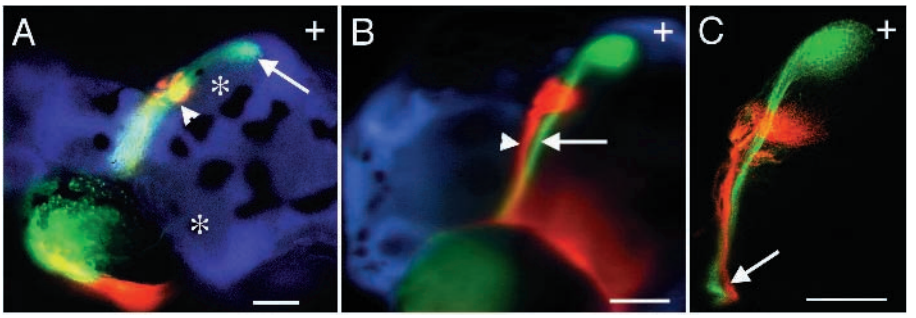

Fig. 2. Retinotectal projection of wild-type zebrafish, dorsal view. Retinal ganglion cells were labeled by injections of DiO (green fluorescence) and DiI (red or yellow fluorescence) into the nasodorsal or temporoventral quadrant of the retina, respectively. (A) Injected fish, counterstained with DAPI. The neuropils of the two tectal lobes (*) are visible. The two tectal lobes together form the tectum. Temporoventral axons (yellow) terminate in the anterodorsal tectum (arrowhead), while nasodorsal axons (green) terminate in the posteroventral tectum (arrow). (B) A more ventral focal plane of another wild-type fish. The two branches of the optic tract are visible. Temporoventral axons (red) grow in the dorsal branch (arrowhead), while nasodorsal axons grow in the ventral branch (arrow). (C) Confocal image of the RGC axons of a wild-type fish. Nasodorsal and temporoventral axons grow as separate bundles through the optic nerve (arrow). Temporoventral axons occupy a more posterior portion of the nerve than the nasodorsal axons. Compare with Fig. 1. Scale bars, 0.1 mm.

Transverse sections through the brain of *box* mutants at 6 days postfertilization show that the dorsal diencephalon and the dorsal mesencephalon are compressed along the superficial-deep axis (Fig. 5 and data not shown). The cartilage that forms the jaw is more massive than usual, due to irregular shaped cartilage cells (data not shown) (for a further description of jaw defects, see Schilling et al., 1996). The pectoral fins are reduced in size. This fin abnormality is the first visible deviation from normal development in *box*, and is apparent at 2 days postfertilization.

In *dak* mutants, the forebrain, especially the telencephalon, is smaller than in wild-type fish and the olfactory placodes are reduced in size or missing. The jaw cartilage is severely affected by this mutation. Gill arches two to five are reduced, as is the hyoid. The pectoral fins are missing. For a further description of these defects, see Schilling et al. (1996) and also van Eeden et al. (1996). Obviously, *box* and *dak* not only share a similar retinotectal phenotype, but also a similar combination of other defects. This suggests that the genes affected in *box* and *dak* are involved in the same pathway(s).

pinscher (*pic*)

As in *box* and *dak*, nasodorsal axons in *pic* mutant embryos grow through both branches of the optic tract. However, in *pic*, the ectopic nasodorsal axons grow onto the tectum in only about 75% of cases (n=16). In more than 50% of cases, a fraction or all of the ectopic nasodorsal axons are deflected medially before reaching it (Fig. 4F, arrowhead). The deflected axons sometimes enter the ipsilateral tectal lobe. On the ipsilateral lobe, they can navigate to their retinotopic position (data not shown). Ventral axons appear not to be affected in their choice of the correct branch of the optic tract by the mutation. But frequently, ventral axons leave the optic tract and grow medially as well (Fig. 4F). The gill arches in this mutant are massive and abut each other (Schilling et al., 1996). The pectoral fins are normal. The telencephalon is smaller than normal and does not protrude anterior to the eyes.

Mutations affecting the mapping of retinal axons

Five mutations in four genes, *nevermind* (*nev*), *who-cares* (*woe*), *gnarled* (*gna*) and *macho* (*mao*), affect the mapping of retinal axons. The first two affect mapping primarily along the dorsoventral dimension of the tectum. Dorsal axons in *nev* and *woe* grow and terminate on the ventral and on the dorsal side of the tectum. The remaining two mutants affect mapping along the anteroposterior dimension. In *gna* and to a lesser extend in *mao*, nasodorsal axons defasciculate and terminate

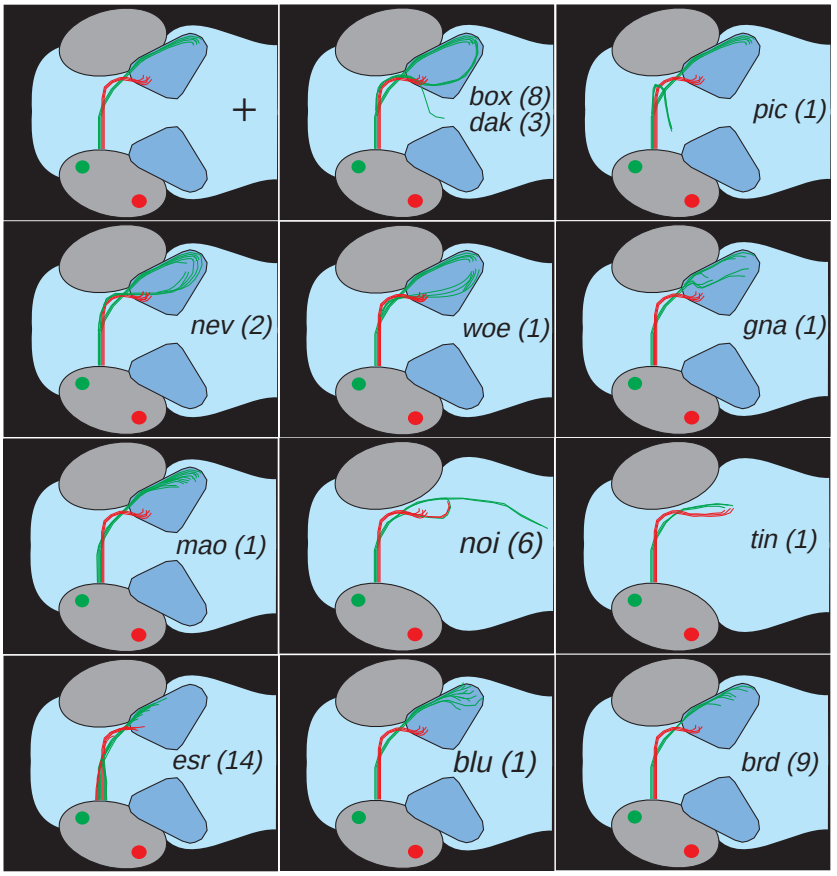

Fig. 3. Schematic drawings of the mutants discussed in this paper. The phenotypes of all the mutants are described in detail in the text. The tectal neuropils are outlined. When the tecta are much smaller than in wild-type fish, the outlines are missing (*noi*, *tin*). The number of alleles is in parentheses. Compare with Fig. 1.

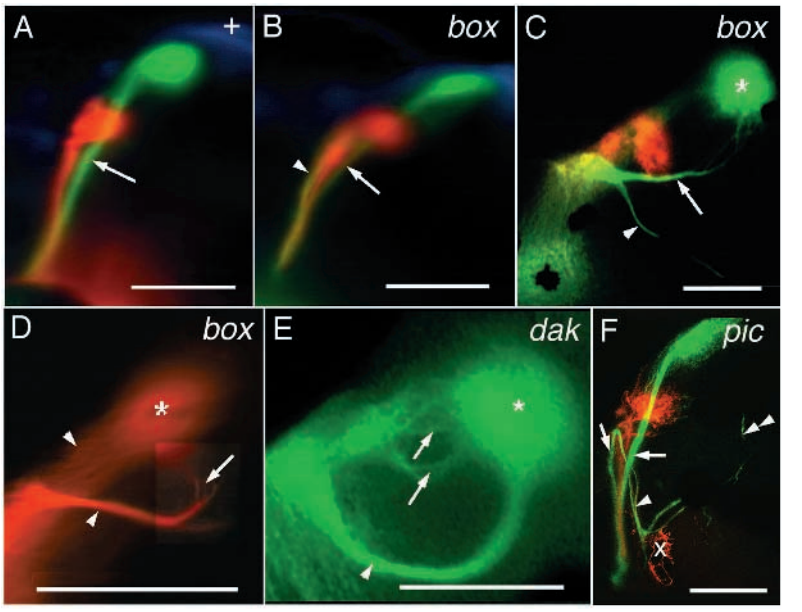

Fig. 4. Mutations affecting the sorting of axons into the two branches of the optic tract. (A) In wild-type animals, nasodorsal axons (green) grow in the ventral branch of the optic tract (arrow). (B) In *box* mutant fish, nasodorsal axons grow in the ventral (arrow) and in the dorsal branch (arrowhead) of the optic tract. (C) Another *box* mutant fish. Nasodorsal axons that enter the tectum through the dorsal branch continue their dorsal trajectory on the tectal lobe (arrow). Near the posterior margin of the lobe, they turn and terminate retinotopically, posteroventrally (*). Some of the ectopic nasodorsal axons leave the contralateral tectal lobe and grow to the ipsilateral one (arrowhead). (D) Trajectory of middorsal RGC axons of a *box* mutant. Dorsal view of the contralateral tectal lobe. Middorsal axons grow in both branches of the optic tract and on the dorsal and the ventral side of the tectal lobe (arrowheads). Ectopic middorsal axons make a sharp turn middorsally (arrow) and grow to their retinotopic midventral target area (*). (E) Nasodorsal axons in a *dak* mutant. As in *box*, these axons grow in both branches of the optic tract. Ectopic nasodorsal axons grow around the dorsal side of the tectal lobe (arrowhead), but terminate retinotopically, posteroventrally (*). The nasodorsal axons on the dorsal side of the lobe are much more strongly fasciculated than the ones on the ventral side (arrows). (F) Confocal image of the retinotectal projection of a *pic* mutant. Nasodorsal axons grow in both branches of the optic tract (arrows). The nasodorsal axons in the dorsal branch and some temporoventral axons turn and grow to the ipsilateral tectal lobe (arrowhead). Here, the temporoventral axons terminate retinotopically (×), while the nasodorsal axons first grow around the dorsal margin of the lobe and then leave it to grow towards the contralateral lobe again (double arrowhead). Scale bars, 0.1 mm.

too soon in the anterior tectum. *Woe* and *mao* mutants have expanded melanophores. *nev*, *woe* and *mao* show abnormal motility.

never mind (*nev*)

As shown in Fig. 6C,D, nasodorsal axons in *nev* terminate in a large zone that extends from the posteroventral to the posterodorsal side of the tectum. The termination field of the nasodorsal axons usually does not extend as far dorsally as it does ventrally, and judged by the intensity of labeling, fewer axons terminate dorsally than ventrally. On transverse sections, the intensity of labeling can be seen to decrease smoothly from dorsal to ventral (Fig. 5C). As in *box*, *dak* and *pic*, dorsal axons grow through both branches of the optic tract (Fig. 6D), although a majority of the labeled axons usually use the proper ventral branch. Dorsally growing nasodorsal axons in *nev* do not leave the tectum, as they do in *box*. They defasciculate normally upon approaching their target area.

Ventral axons terminate properly in the dorsal tectum, but unlike axons in wild-type animals, they do not grow in a smooth curved line toward their target area, but appear to meander instead (Figs 6E, 7B). The order of axons in the optic nerve is changed in a characteristic fashion (Fig. 6C).

Sections through 5-day-old embryos homozygous for *nev* show that as in *box*, the dorsal di- and mesencephalon are smaller than in wild-type animals (Fig. 5 and data not shown). *nev* fish fail to develop a swim bladder and die, usually around day 8. The mutant embryos show abnormal motility. They rotate around their long body axis when swimming.

who-cares (*woe*)

In *woe* mutants, nasodorsal axons terminate posteroventrally and posterodorsally in the tectum (Fig. 6F,G). Judged by the

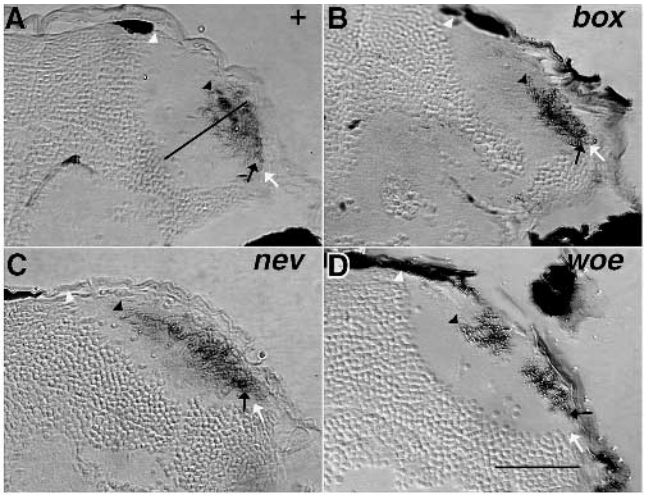

Fig. 5. Transverse sections through the tectal lobes of a wild-type fish (A) and three mutants (B,C,D). Before sectioning, nasodorsal RGCs were labeled with DiI and the dye was photoconverted. Medial is to the left, dorsal is up. (A) A wild-type fish. The termination field of the nasodorsal axons occupies the ventral half of the tectum. Black arrows mark the ventral margin of the termination fields in all four pictures, black arrowheads the dorsal margin. The dorsal margin of the tectal lobes is labeled by white arrowheads, the ventral margin by white arrows. The black bar marks the superficial-deep axis of the tectum. (B) In *box* mutants, the termination field of the nasodorsal axons occupies the ventral half of the tecum, as in wild-type fish. The tectum is compressed along the superficial-deep axis. (C) In *nev* fish, the termination field of nasodorsal axons reaches into the dorsal side of the tecum. It is compressed along its superficial-deep axis. (D) In *woe* mutants, nasodorsal axons terminate in two separate fields posteroventrally and posterodorsally in the tectum. The dorsal field is oriented more towards the center of the neuropil than towards the dorsal margin. Scale bars, 50 μm.

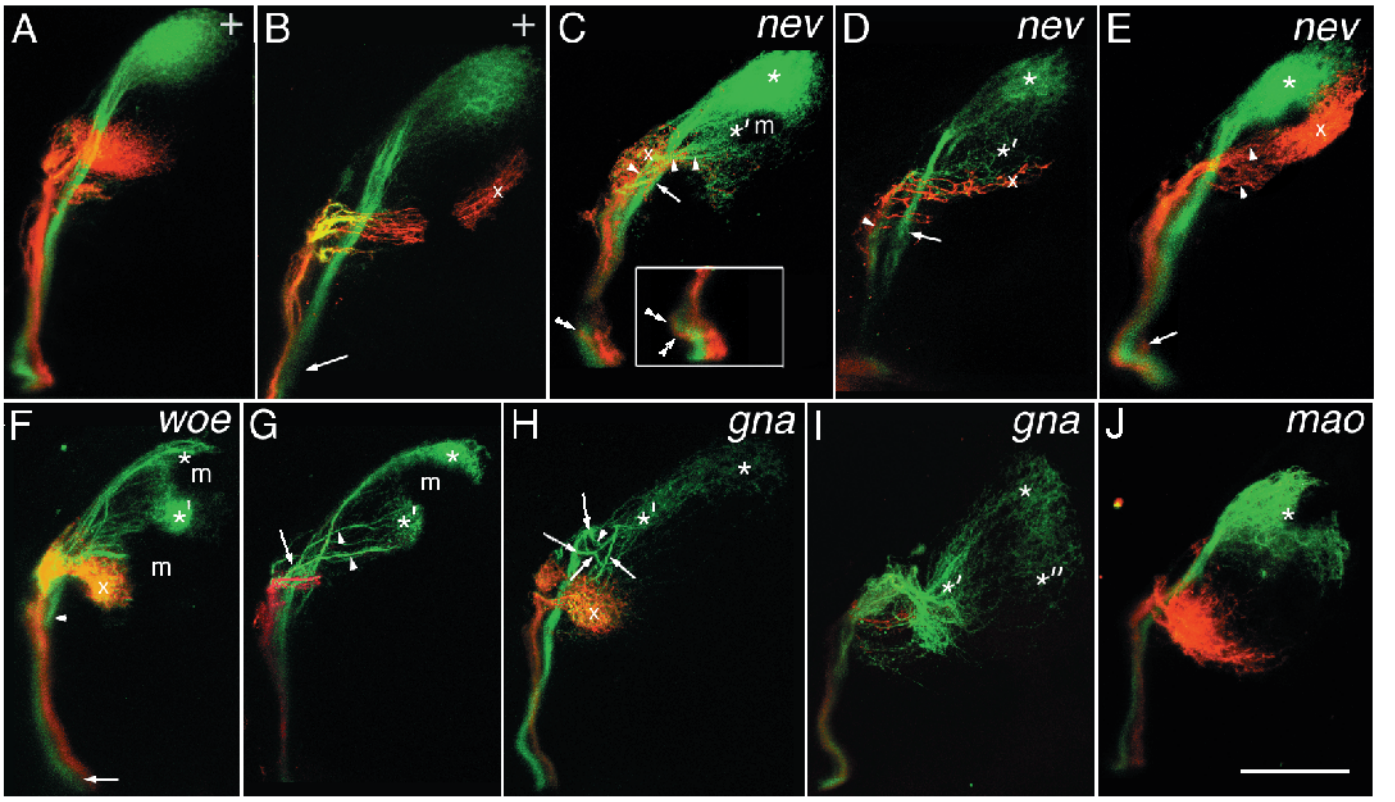

Fig. 6. Mutations affecting the mapping of RGC axons. All images shown in this figure are projections of optical sections obtained with a confocal microscope. (A) Retinotectal projection of a wild-type fish. The absolute position of the termination areas on the tectum cannot be seen, but the temporoventral axons (red) terminate anterior and dorsal of the nasodorsal axons (green), as is typical for a wild-type projection. For all the confocal images shown, the absolute positions of the termination fields has been ascertained using conventional fluorescence microscopy. (B) Retinotectal projection of another wild-type fish. In this animal, red axons are of nasoventral origin and terminate in the posterodorsal tectum (×). Note that the dorsal and ventral axons, although both originating in the nasal retina, still occupy different parts of the optic nerve (arrow). (C) In *nev* mutants, nasodorsal axons terminate posteroventrally (*) and posterodorsally (*′) in the tectum. Temporoventral axons terminate retinotopically in the anterodorsal tectum (×). The nasodorsal axons grow through both the ventral (arrow) and the dorsal (arrowheads) branch of the optic tract. RGC axons are already missorted in the optic nerve. Some of the temporoventral axons grow ectopically in the anterior portion of the optic nerve (double arrowheads). The inlet shows the optic nerve of another *nev* mutant individual showing a similar phenotype. (D) Another *nev* mutant fish. This picture shows more clearly that the nasodorsal axons grow in the ventral (arrow) and the dorsal (arrowhead) branches of the optic tract. The nasodorsal axons terminate again in the posteroventral (*) and posterodorsal (*′) tectum. Nasoventral axons (red) terminate retinotopically in the posteroventral tectum (×). (E) Nasoventral axons (red) in *nev* mutants always terminate correctly in the dorsal tectum (×), but reach their target area on meandering paths (arrowheads). A subpopulation of the nasoventral axons grows ectopically in the posterior portion of the optic nerve (arrow). Compare with B. In this individual, the nasodorsal axons terminate correctly in the ventral tectum, but the termination area is larger and fuzzier than in wild-type fish. (F) A *woe* mutant fish. Nasodorsal axons terminate on the ventral (*) and the dorsal (*′) side of the tectum. Note that the majority of the nasodorsal axons appear to terminate at the ectopic dorsal side. Temporoventral axons terminate retinotopically in the anterodorsal tectum (×). The RGC axons are normally sorted in the optic nerve (arrow) and tract (arrowhead), but the nasodorsal and the temporoventral fascicles cross each other further away from the eye than usual. The two branches of the tract itself are less widely searated than in wild-type animals. G: Another *woe* mutant fish. Nasodorsal axons (green) terminate again in the posteroventral (*) and the posterodorsal (*′) tectum. Nasodorsal axons split only upon reaching the tectum (arrow). Ectopic nasodorsal axons grow in several fascicles along the dorsal margin of the tectum (arrowheads). (H) Retinotectal projection of a *gna* mutant fish. The termination area of the nasodorsal axons is enlarged and encompasses posteroventral (*) and anteroventral (*′) parts of the tectum. The nasodorsal fascicle splits abruptly into several smaller fascicles upon reaching the tectum (arrows), some of which turn sharply dorsally before continuing to the posterior tectum (arrowhead). Temporoventral axons terminate correctly in the anterodorsal tectum (×). The sorting of the axons along the optic nerve and tract is normal. (I) In some *gna* mutant fish, nasodorsal axons terminate not only anteroventral (*′) and posteroventral (*), but also posterodorsal (*″). (J) A *mao* mutant fish. The termination area, especially of the nasodorsal axons (*), is larger and fuzzier than in wild type. m, melanophore. Scale bar, 0.1 mm.

intensity of labeling, the axons distribute themselves about evenly between the two termination fields. Analysis of transverse araldite sections shows two distinct termination fields, one in the ventral and one in the dorsal tectum (Fig. 5D). The actual position of the dorsal termination area in the dorsal tectal half still has to be accurately determined. Due to the shape of the tectal lobe, sections might give a wrong impression of the position of an termination field. Unlike in *nev* mutants, nasodorsal axons grow appropriately in the ventral branch of the optic tract, and it is only on entering the tectum that some of them grow across the tectal lobe to its dorsal side (Fig. 6F,G). These axons grow in several widely spaced fascicles

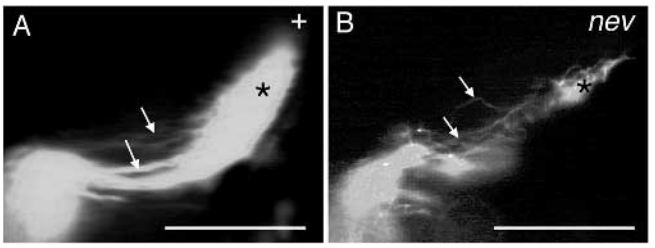

Fig. 7. Nasoventral axons of a wild-type fish and of a *nev* mutant. Dorsal views of the tectal lobes, anterior is to the left. (A) In wild-type fish, nasoventral axons grow in straight, smoothly curved trajectories (arrows) on the dorsal side of the tectal lobe and terminate posterodorsally. (B) A *nev* mutant fish. Nasoventral axons terminate retinotopically (*), but they grow on tortous paths (arrows). m, melanophore. Scale bars, 0.1 mm.

toward their posterodorsal termination zone (Fig. 6G). The two branches of the optic tract itself are not separated as widely in the mutant as in a wild-type fish.

The sorting of axons in the optic nerve appears to be normal, although the nasodorsal and the temporoventral fascicle cross further away from the eye than usual. Ventral axons terminate retinotopically in the dorsal tectum.

Mutant individuals have a pigment phenotype at 6 days postfertilization. Their melanophores show no background adaptation when exposed to bright light. About 50% of embryos homozygous for *woe* develop a swim bladder. These embryos show very little spontaneous movement, but react normally to touch. They die around 12 days postfertilization.

gnarled (*gna*)

In *gna* mutant fish, nasodorsal axons split abruptly into several diverging fascicles in the anterior tectum (Fig. 6H). Some of the fascicles turn dorsally before continuing their posterior course. A fraction of the nasodorsal axons terminates prematurely anteroventrally, shortly after arriving on the tectum (Fig. 6H,I). The area covered by the termination field(s) of the nasodorsal axons varies between individuals. In extreme cases, it encompasses almost the entire tectum (Fig. 6I). Ventral axons project retinotopically to the dorsal tectum. In particular, the nasoventral axons also terminate normally in the posterodorsal tectum (data not shown). No further abnormalities have so far been identified in this mutant.

macho (*mao*)

In *mao* mutant fish, the nasodorsal axons defasciculate and terminate prematurely in a more anterior position on the tectum than usual. The termination field of the nasodorsal axons is enlarged compared to a wild-type, but the enlargement is less pronounced than in *gna* (Fig. 6J). The termination zone of temporoventral axons appears to be enlarged as well.

mao mutants do not have a touch response at day 2 (Granato et al., 1996) and at day 4 their melanophores are expanded.

Retinotopic maps on small tecta

We have kept 17 mutants in which mapping of retinal axons onto the tectum is little or not at all disturbed but in which the size of the tectum itself is greatly reduced (Table 1). Often, eye size is diminished as well (Table 1). Two examples of such mutants are shown in Fig. 8.

no isthmus (*noi*)

In the two alleles of *noi* mutants we found, *tb21* and *ty31z*, temporoventral and nasodorsal axons project near the posterior part of a small tectum (Fig. 8B). The tectal remnant in *noi* probably has anterior identity, since it does not express posterior markers (Brand et al., 1996). Nasodorsal axons often continue to grow beyond the posterior margin of the tectum (Fig. 8C). Part of the labeled axons project frequently to the ipsilateral tectal lobe (data not shown).

The earliest visible defect in *noi* mutant embryos is the absence of the midbrain/hindbrain boundary (Brand et al., 1996).

tiny neuropil (*tin*)

In *tin* mutant embryos, the tectal neuropil is tiny. Nasodorsal and temporoventral axons are consequently barely separated on the tectum (Fig. 8D). But even in this tiny tectum, nasodorsal axons terminate posterior of the temporoventral axons. Clearly, tectal development rather than mapping of retinal axons is affected. We have kept another ten mutants with small tectal neuropils (Table 1). For several reasons it is not yet clear to what extent mapping is affected in these mutants. Small eyes make the localization of the dye injections less precise, thus introducing a great variance into the location of labeled termination fields on the tectum. The small tecta further increase the difficulty of judging the retinotopy of the projections. However, during the screen we found many mutants with small tecta displaying a clearly retinotopic map, showing that a reduction of tectal size does not necessarily disturb retinotopy (data not shown).

Mutations affecting the arborization pattern of retinal axons

In this group we placed mutants whose RGC axons project to

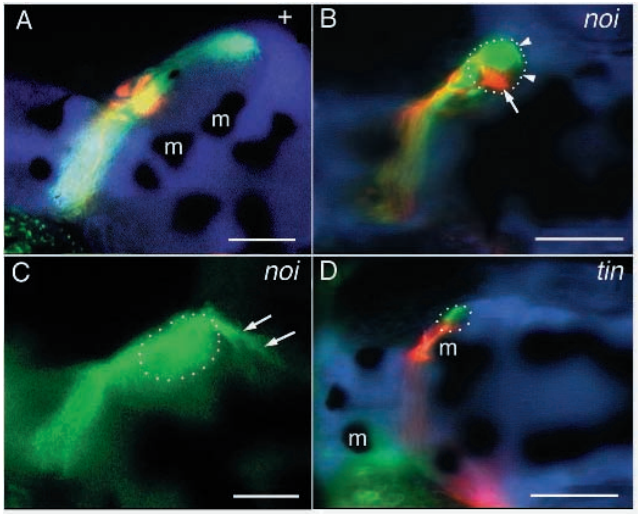

Fig. 8. Mutations affecting tectal size. (A) Retinotectal projection of a wild-type fish. (B) Retinotectal projection of a *noi* mutant. The tectum (dotted line) is smaller than in wild-type fish. Temporoventral axons (arrow) project to the posterior margin of the tectal remnant (arrowheads). (C) Nasodorsal axons of a noi mutant. They pass the posterior end of the tectal remnant (dotted line) and grow caudally (arrows). (D) Retinotectal projection of a *tin* mutant. The tectum (dotted line) is tiny. Even in this tiny tectum, nasodorsal axons (green) project posterior and ventral of the temporoventral axons (red). m, melanophore. Scale bars, 0.1 mm.

Table 1. Overview of all genes whose phenotypes are described in this paper

Gene	Alleles (*n*)	Retinotectal phenotype	Other phenotypes	Other references
Sorting mutants				
boxer (box)	*tm4, tm70g, tg308c, te242, tm317c, tw24, tp67z, to232* (8)	Optic tract	Jaw, fins	a,b,c
dackel (dak)	*to273b, tw25e, tf205* (3)	Optic tract	Jaw, fins, brain	a,b,c
pinscher (pic)	*to216z* (1)	Optic tract	Jaw	c
Mapping mutants				
nevermind (nev)	*tr230b, ta229f* (2)	Topography	Behavior	d
who-cares (woe)	*tr221z* (1)	Topography	Pigmentation	
gnarled (gna)	*tc236z* (1)	Topography	–	
macho (mao)	*tt261a* (1)	Topography	Behavior	d
Termination mutants				
tilsitt (til)	*tz130B* (1)	Termination	Pathfinding, pigmentation	c, e
esrom (esr)	*th222, tg5, th36b, tb241a, te250, te279, tg265a, tj236, ts208, tp203, tn207b, te376, te275, tf4z* (14)	Termination	Pathfinding, pigmentation	c, e
tofu (tof)	*tq213c* (1)	Termination	Pathfinding, pigmentation	c,e
blumenkohl (blu)	*tc257z* (1)	Termination	–	–
delayed fade	*AJ41a* (1)	Termination	Degenerating tectum	f,g
braindead (brd)	*tl21, ty91, tl41, tp41z, tv59y, tm46y, ty103z, tm42z, tc265z* (9)	Termination	Degenerating tectum	h
Tectum mutants				
no isthmus (noi)	*tu29a, tm343a, th44, tb21, ty22b, ty31z* (6)	Small tectum	Brain	i
–	*tc265y*	Small tectum	–	
–	*tc38z*	Small tectum	–	
tiny-neuropil (tin)	*tm101z* (1)	Small tectum	Small eyes	
–	*tm147z*	Small tectum	–	
–	*tm46b*	Small tectum	Small brain, degenerating tectum jaw defects	
–	*tp32z*	Small tectum	Small eyes and brain	
–	*tv56z*	Small tectum	Small eyes	
–	*tv59z*	Small tectum	–	
–	*ty6z*	Small tectum	Small eyes	
eisspalte (ele)	*ty77z* (1)	Small tectum	Abnormal hindbrain, small eyes	j
miro (mio)	*tm88z*	Small tectum	Small eyes	

Complementation analysis is not yet complete. In particular, complementation analysis has not been performed among *gna* and *mao*, and among many of the mutants with a small tectum.

References: a, van Eeden et al. (1996); b, Schilling et al. (1996); c, Karlstrom et al. (1996); d, Granato et al. (1996); e, Odenthal et al. (1996); f, Heisenberg et al. (1996); g, Kelsh et al. (1996); h, Furutani-Seiki et al. (1996); i, Brand et al. (1996); j, Jiang et al. (1996).

their normal target area, but fail to develop a normal termination field. 27 mutants in six complementation groups fall into this class. Mutants in two complementation groups, *braindead* (*brd*) (Fig. 9E) and *delayed fade* (*dfd*), show a degenerating tectum around day 3 (Furutani-Seiki et al., 1996), and the retinotectal phenotype in these cases is likely to be a consequence of this degeneration.

esrom (*esr*), *tilsit* (*til*) and *tofu* (*tof*)

In *esr*, *til* and *tof* mutants, which show an identical projection phenotype, RGC axons form a thickening right behind the optic papilla, and fewer axons than normal project to the tectum (Fig. 9B). Usually, most of the nasodorsal axons stop in the anterior tectum, and only a few axons project posteriorly. However, this seems not to reflect a topographic but an outgrowth defect, since, judged by the intensity of labeling, the axon bundles thin out already before reaching the tectum. In some mutant individuals, an apparently normal number of axons arrives at the tectum. In these cases, the nasodorsal axons spread out to cover a big part of the tectal neuropil evenly (Fig. 9C).

All three mutations also result in a reduced xanthophore pigmentation (Odenthal et al., 1996).

Blumenkohl (*blu*)

Nasodorsal and nasoventral axons in *blu* mutants defasciculate strongly upon reaching the posterior tectum. The axons appear to turn away from each other. They arborize in a cauliflower-like fashion and end up covering a much larger area than usual (Fig. 9D). The melanophores of *blu* fish show no background adaptation when exposed to bright light.

DISCUSSION

In a large-scale screen in zebrafish, 45 mutants were identified that affect either the order of retinal axons en route to the tectum, the establishment of a retinotopic map on the tectum or the formation of proper termination fields. The analysis of the mutant phenotypes allows a dissection of the process of map formation.

boxer, *dackel* and *pinscher* affect the sorting of RGC axons into the two branches of the optic tract but do not affect their mapping

We found 11 mutations in three loci that affect the sorting of retinal axons into the branches of the optic tract in an identical manner: dorsal axons, instead of using only the ventral branch, split and grow into both the ventral and the dorsal branch. Nasodorsal axons that grow in the inappropriate dorsal branch continue their dorsal trajectory on the tectum. These misrouted axons can locate their appropriate target area, demonstrating that the sorting of axons onto the two branches of the optic tract is not required for proper mapping.

This finding is consistent with earlier observations, which indicated that the point of entry of retinal axons into the tectum is not crucial for the establishment of a normal map. If, for example, retinal quadrants are directly explanted onto the tectum, retinal axons will grow toward their target area from a variety of positions (DeLong and Coulombre, 1967; Fujisawa, 1981; Thanos and Dütting, 1987). Axons from eye transplants that grow to the tectum on abnormal paths also map normally (Constantine-Paton and Law, 1978; Harris, 1980, 1982; Sharma, 1972).

Analysis of *box* mutants supports the hypothesis that gradients of guidance molecules are used to establish the retinotectal map

In *box* mutants ectopic middorsal axons continue their dorsal trajectory after entering the tectum. After reaching the middorsal tectum, a retinotopic position with respect to the anteroposterior tectal axis, they make a sharp turn and grow across the tectum directly towards their midventral target area, thus correcting their dorsoventral displacement. This observation is in agreement with experiments in chick showing that retinal axons that have been displaced by antibody perturbations first establish their position along the anteroposterior axis, and only then proceed to seek out their proper dorsoventral position (Thanos et al., 1984). The course correction of ectopic axons, as shown for example by dorsal axons in *box* mutants, is easily explained by the operation of graded positional cues (Gierer, 1983, 1987).

Two of the genes identified are important for dorsoventral mapping

Two of the genes identified in this screen, *nev* and *woe*, are important for mapping in the dorsoventral dimension. If either of these genes is mutated, dorsal axons terminate on the ventral and on the dorsal side of the tectum. In *nev*, the RGC axons are also missorted in the optic nerve and tract, indicating that *nev* affects the positional identities of ganglion cells in the retina. In contrast, retinal axons in *woe* mutant fish are sorted normally in the optic nerve and tract.

Gradient models can account for an extension or duplication of termination fields in several ways (Fig. 10). For example, if one assumes that linear gradients specify dorsoventral positions in the retina or the tectum (Fig. 10A), a decrease in slope (Fig. 10B) or a duplication of gradients (Fig. 10C) can in principle explain the observed dorsoventral extension or duplication of termination fields, respectively. On the other hand, radial gradients (Fig. 10D) may be used to specify the positions in the retina or the tectum. In this case, one additional cue is required to define dorsal and ventral (and/or nasal and temporal) sides (Fig. 10E). The functional disruption of such a symmetry-breaking cue could also explain the phenotypes of *nev* and *woe*. This cue might be a dorsoventral gradient, a circumferential gradient, or simply a step, that is, a cue expressed in either the dorsal or the ventral retinal/tectal half (Fig. 10E). Steps between the nasal and the temporal and between the dorsal and ventral retina have been reported (e.g. Macdonald et al., 1994; McLoon, 1991; Rissi et al., 1995; Walter et al., 1987), and it has been suggested already that in the retina, a step function along the nasotemporal axis together with a radial (i.e. a centroperipheral) and a dorsoventral gradient could be used to label position (Holt and Harris, 1993).

In *nev* and *woe* mutants, ventral axons map retinotopically on the dorsal side of the tectum. Theoretical gradient models designed to account for topographic projections with a minimal number of cues use the same gradients for guiding both the dorsal and the ventral axons. Hence we take the result, that mapping of dorsal axons is affected while that of ventral axons is normal, as an indication that more gradients are involved than are minimally required in theory. Furthermore, ventral axons in *nev* mutants, although projecting retinotopically to the dorsal tectum, reach their target area by tortuous paths, unlike axons in wild-type animals. Although the ventral axons still possess sufficient information to reach their target area, the precision of their tracking has been affected. It is possible that

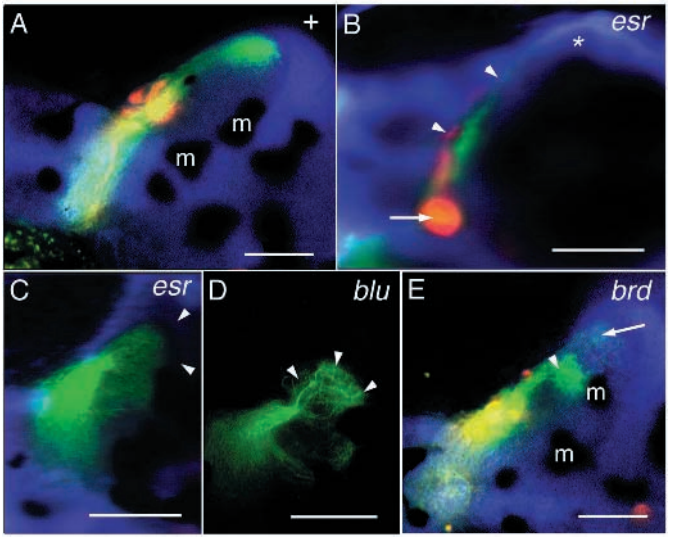

Fig. 9. Mutations affecting the formation of termination fields. (A) Retinotectal projection of a wild-type fish. (B) Retinotectal projection of an *esr* mutant. Retinal axons form a thickening right behind the optic papilla (arrow). Although many axons are still seen in the optic tract, the termination fields of the RGC axons on the tectum are tiny (arrowheads). Nasodorsal axons are not seen in the posterior tectum (*). (C) Nasodorsal axons of *esr*. In this case, the nasodorsal axons reach the posterior margin of the tectum (approximate position marked by arrowheads). Their termination field is spread out and covers a larger area than usual. (D) Nasodorsal axons of *blu*. Upon reaching their target area in the posteroventral tectum, they arborize in a cauliflower-like fashion (arrowheads). (E) Retinotectal projection in a *brd* mutant. Many nasodorsal axons terminate prematurely already in the midventral tectum (arrowhead). Nasodorsal axons in the posteroventral tectum are marked by an arrow. m, melanophore. Scale bars, 0.1 mm.

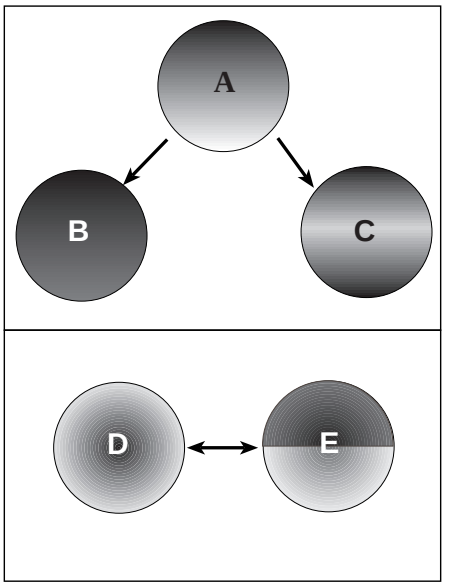

Fig. 10. Concentration distributions discussed in context with the formation of a topographic projection. (A) A linear gradient can label positions along one axis unequivocally. In the example shown, each point along the vertical axis is defined by a different shade of grey. (B) The slope of the gradient is decreased. (C) In a gradient duplicated as shown, positions along the vertical axis are not unequivocally defined by the concentration. Positions along the vertical axis at an equal distance from the center have the same shade of grey. (D) A radial gradient on its own cannot be used to define positions along the vertical axis, because positions along the vertical axis at equal distances from the center have the same shade of grey. (E) A radial gradient in combination with a step function can define each positions along the vertical axis of the circle by a specific shade of grey.

the ventral axons use multiple cues to orient with respect to the dorsoventral axis.

Two genes affect anteroposterior mapping

In *gna* and *mao*, nasodorsal retinal axons terminate in a more anterior position on the tectum than in wild-type animals. This defect is much more pronounced in *gna* than in *mao*. In *gna*, the nasodorsal but not the nasoventral axons split abruptly into several diverging fascicles upon arriving on the tectum. Some fascicles frequently enter the dorsal tectum. The ventral axons map normally.

In *gna* and other mutants, for example *nev*, changes in the mapping of RGC axons always go together with changes in their degree of fasciculation. This argues that both processes are connected. Defasciculation could be a direct consequence of the increasing permissiveness of the tectal environment once axons approach their target. This permissiveness is perhaps mediated by the same graded cues that direct the formation of a topographic map. The degree of fasciculation of ectopic nasodorsal axons in *box* and *nev* mutants correlates with their site of termination, supporting this hypothesis. In *box* mutants, ectopic nasodorsal axons terminate in the ventral tectum. They are highly fasciculated while growing through the dorsal tectum. In contrast, ectopic nasodorsal axons in *nev* mutants terminate in the dorsal tectum. In this mutant, the ectopic axons defasciculate gradually while approaching their dorsal target.

Most mutations affecting the mapping of retinal axons also affect the motility of the mutant fish

Three of the four genes that affect the mapping of retinal axons onto the tectum also affect the motility of the embryos. *nev* mutants rotate around their long axis when swimming, which might be a direct consequence of their retinotectal phenotype. However, the relative position of the dorsoventral axis of fish to the gravitational axis is affected by vision as well as gravitation (Parzefall, 1993). The relationship between the behavioural and the retinotectal phenotype is probably less direct. The behavioural defects of the other mutants are not explicable by their retinotectal phenotypes in an obvious way. In this context it is important to note that the tectum is a multimodal structure that also receives sensory inputs other than vision (Udin and Fawcett, 1988). Mutations affecting tectal polarity can probably influence the behaviour of fish, not only by changing the formation of visual, but also of other sensory maps.

noi confirms ideas about the role of *engrailed (en)* in determining tectal polarity

Transplantation experiments in the chick have shown that the polarity of the anteroposterior axis of the tectum anlage, the mesencephalic alar plate, correlates with the expression of *en*, which is expressed in an increasing anterior to posterior gradient on the tectal primordium (Itasaki et al., 1991; Itasaki and Nakamura, 1992; Martinez and Alvarado-Mallart, 1990; Patel et al., 1989). The tectum of *noi* mutants does not express *en* (Brand et al., 1996), consequently, one might expect it to have lost anteroposterior polarity. The tectum of *noi* mutants is very small and its innervation pattern (see Results) is indeed consistent with the idea that this remnant has anterior identity.

Conclusion

By screening a large number of mutagenized zebrafish lines, we obtained a set of mutants with defects in retinotectal mapping. We expect that the molecular and functional analyses of these mutants will help us to understand the principles underlying the formation of neuronal projections.

It is a pleasure to acknowledge the help of many individuals who made this study possible. Christian Bayertz, Eckard Dominik, Dr Uwe Drescher, Carmen Gitter, Claudia Handwerker, Ingrid Horschke, Julita Huf, Dr Hirojuki Ichijo, Kurt Knauf, Dr Sigrun Korsching, Ulrike Nell, Thomas Stroh, Dr Thomas Voigt and Franko Weth helped with the processing of the fish. Under the supervision of Franz Endress, our machine shop patiently developed the gadgets needed to mount and inject the fish. Jürgen Jung and Jürgen Löschinger helped with the computers. We would also like to thank Drs D. Dütting, S. S. Easter, A. Gierer, S. Jesuthasan, S. Thanos and two anonymous referees for helpful comments on the manuscript.

REFERENCES

Baier, H., Klostermann, S., Trowe, T., Karlstrom, R., Nüsslein-Volhard, C. and Bonhoeffer, F. (1996). Genetic dissection of the retinotectal projection. *Development*, **this issue.**

Brand, M., Heisenberg, C.-P., Jiang, Y.-J., Beuchle, D., Lun, K., Furutani-Seiki, M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D., Kelsh, R., Mullins, M., Odenthal, J., van Eeden, F. J. M. and Nüsslein-Volhard, C. (1996). Mutations in zebrafish genes affecting the formation of the boundary between midbrain and hindbrain. *Development* **123**, 179-190.

Burrill, J. D. and Easter, S. S., Jr. (1995). The first retinal axons and their microenvironment in zebrafish: cryptic pioneers and the pretract. *J. Neurosci.* **15,** 2935-47.

Cheng, H.-J., Nakamoto, M., Bergemann, A. D. and Flanagan, J. G. (1995). Complementary gradients in expression and binding of ELF-1 and MEK4 in development of the topographic retinotectal projection map. *Cell* **82,** 371-381.

Constantine-Paton, M. and Law, M. I. (1978). Eye-specific termination bands in tecta of three-eyed frogs. *Science* **202,** 639-641.

DeLong, G. R. and Coulombre, A. J. (1967). The specificity of retinotectal connections studied by retinal grafts onto the tectum in chick embryos. *Dev. Biol.* **16,** 513-531.

Drescher, U., Kremoser, C., Handwerker, C., Löschinger, J., Noda, M. and Bonhoeffer, F. (1995). In vitro guidance of retinal ganglion cell axons by RAGS, a 25 kDa tectal protein related to ligands for Eph receptor tyrosine kinases. *Cell* **82,** 359-370.

Fraser, S. E. (1980). A differential adhesion approach to the patterning of nerve connections. *Dev. Biol.* **79,** 453-464.

Fraser, S. E. and Perkel, D. H. (1990). Competitive and positional cues in the patterning of nerve connections. *J. Neurobiol.* **21,** 51-72.

Fujisawa, H. (1981). Retinotopic analysis of fiber pathways in the regenerating retinotectal system of the adult newt *Cynops pyrrhogaster*. *Brain Res.* **206**, 27-37.

Furutani-Seiki, M., Jiang, Y.-J., Brand, M., Heisenberg, C.-P., Houart, C., Beuchle, D., van Eeden, F. J. M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D. A., Kelsh, R. N., Mullins, M. C., Odenthal, J. and Nüsslein-Volhard, C. (1996). Neural degeneration mutants in the zebrafish, *Danio rerio*. *Development* **123**, 229-239.

Gaze, R. M. and Sharma, S. C. (1970). Axial differences in the reinnervation of the goldfish optic tectum by regenerating optic nerve fibres. *Exp. Brain Res.* **10,** 171-181.

Gierer, A. (1983). Model for the retino-tectal projection. *Proc. R. Soc. Lond. B* **218,** 77-93.

Gierer, A. (1987). Directional cues for growing axons forming the retinotectal projection. *Development* **101,** 479-489.

Granato, M., van Eeden, F. J. M., Schach, U., Trowe, T., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D. A., Kelsh, R. N., Mullins, M. C., Odenthal, J. and Nüsslein-Volhard, C. (1996). Genes controlling and mediating locomotion behavior of the zebrafish embryo and larva. *Development* **123**, 399-413.

Haffter, P., Granato, M., Brand, M., Mullins, M. C., Hammerschmidt, M., Kane, D. A., Odenthal, J., van Eeden, F. J. M., Jiang, Y.-J., Heisenberg, C.-P., Kelsh, R. N., Furutani-Seiki, M., Vogelsang, E., Beuchle, D., Schach, U., Fabian, C. and Nüsslein-Volhard, C. (1996). The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*. *Development* **123**, 1-36.

Harris, W. A. (1980). Regions of the brain influencing the projection of developing optic tracts in the salamander. *J. Comp. Neurol.* **194,** 319-333.

Harris, W. A. (1982). The transplantation of eyes to genetically eyeless salamanders: visual projections and somatosensory interactions. *J. Neurosci.* **2,** 339-353.

Heisenberg, C.-P., Brand, M., Jiang, Y.-J., Warga, R. M., Beuchle, D., van Eeden, F. J. M., Furutani-Seiki, M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D. A., Kelsh, R. N., Mullins, M. C., Odenthal, J. and Nüsslein-Volhard, C. (1996). Genes involved in forebrain development in the zebrafish, *Danio rerio*. *Development* **123**, 191-203.

Holt, C. E. and Harris, W. A. (1993). Position, guidance and mapping in the developing visual system. *J. Neurobiol.* **24,** 1400-1422.

Hope, R. A., Hammond, B. J. and Gaze, R. M. (1976). The arrow model: retinotectal specificity and map formation in the goldfish visual system. *Proc. R. Soc. Lond. B* **194,** 447-466.

Horder, W. T. J. and Martin, K. A. C. (1978). Morphogenetics as an alternative to chemospecificity in the formation of nerve connections. *Symp. Soc. Exp. Biol.* **32,** 275-358.

Itasaki, N., Ichijo, H., Hama, C., Matsuno, T. and Nakamura, H. (1991). Establishment of rostrocaudal polarity in tectal primordium: *engrailed* expression and subsequent tectal polarity. *Development* **113,** 1133-1144.

Itasaki, N. and Nakamura, H. (1992). Rostrocaudal polarity of the tectum in birds: Correlation of *en* gradient and topographic order in retinotectal projection. *Neuron* **8,** 787-798.

Jiang, Y.-J., Brand, M., Heisenberg, C.-P., Beuchle, D., Furutani-Seiki, M., Kelsh, R. N., Warga, R. M., Granato, M., Haffter, P., Hammerschmidt, M., Kane, D. A., Mullins, M. C., Odenthal, J., van Eeden, F. J. M. and Nüsslein-Volhard, C. (1996). Mutations affecting neurogenesis and brain morphology in the zebrafish, *Danio rerio*. *Development* **123**, 205-216.

Kaethner, R. J. and Stuermer, C. A. (1992). Dynamics of terminal arbor formation and target approach of retinotectal axons in living zebrafish embryos: a time-lapse study of single axons. *J. Neurosci.* **12,** 3257-71.

Karlstrom, R. O., Trowe, T., Klostermann, S., Baier, H., Brand, M., Crawford, A. D., Grunewald, B., Haffter, P., Hoffmann, H., Meyer, S. U., Müller, B. K., Richter, S., van Eeden, F. J. M., Nüsslein-Volhard, C. and Bonhoeffer, F. (1996). Zebrafish mutations affecting retinotectal axon pathfinding. *Development* **123**, 415-426.

Kelsh, R. N., Brand, M., Jiang, Y.-J., Heisenberg, C.-P., Lin, S., Haffter, P., Odenthal, J., Mullins, M. C., van Eeden, F. J. M., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Kane, D. A., Warga, R. M., Beuchle, D., Vogelsang, L. and Nüsslein-Volhard, C. (1996). Zebrafish pigmentation mutations and the processes of neural crest development. *Development* **123**, 369-389.

Levine, R. L. and Jacobson, M. (1975). Discontinous mapping of retina onto tectum innervated by both eyes. *Brain Res.* **98,** 172-176.

Macdonald, R., Xu, Q., Barth, K. A., Mikkola, I., Holder, N., Fjose, A., Krauss, S. and Wilson, S. W. (1994). Regulatory gene expression boundaries demarcate sites of neuronal differentiation in the embryonic zebrafish forebrain. *Neuron* **13,** 1039-1053.

Martinez, S. and Alvarado-Mallart, R.-M. (1990). Expression of the Homeobox *Chick-en* gene in chick/quail chimeras with inverted mesmetencephalic grafts. *Dev. Biol.* **139,** 432-436.

McLoon, S. C. (1991). A monoclonal antibody that distinguishes between temporal and nasal retinal axons. *J. Neurosci.* **11,** 1470-1477.

Meyer, R. L. (1982). Tetrodotoxin blocks the formation of ocular dominance columns in goldfish. *Science* **218,** 589-591.

Mullins, M. C., Hammerschmidt, M., Haffter, P. and Nüsslein-Volhard, C. (1994). Large-scale mutagenesis in the zebrafish: in search of genes controlling development in a vertebrate. *Curr. Biol.* **4,** 189-202.

Odenthal, J., Haffter, P., Vogelsang, E., Brand, M., van Eeden, F. J. M., Furutani-Seiki, M., Granato, M., Hammershmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D. A., Kelsh, R. N., Mullins, M. C., Warga, R. M., Allende, M. L., Weinberg, E. S. and Nüsslein-Volhard, C. (1996). Mutations affecting the formation of the notochord in the zebrafish, *Danio rerio*. *Development* **123**, 103-115.

Orike, N. and Pini, A. (1996). Axon guidance: following the EPH-plan. *Curr. Biol.* **6,** 108-110.

Parzefall, J. (1993). Behavioural ecology of cave-dwelling fishes. In *Behaviour of Teleost Fishes* (ed. T. J. Pitscher), pp. 573-606. London: Chapman & Hall.

Patel, N. H., Martin-Blanco, E., Coleman, K. G., Poole, S. J., Ellis, M. C., Kornberg, T. B. and Goodman, C. S. (1989). Expression of engrailed proteins in arthropods, annelids, and chordates. *Cell* **58,** 955-968.

Rissi, M., Wittbrodt, J., Délot, E., Naegli, M. and Rosa, F. M. (1995). Zebrafish *Radar*: A new member of the TGF-β superfamily defines dorsal regions of the neural plate and the embryonic retina. *Mech. Dev.* **49,** 223-234.

Sanes, J. R. (1993). Topographic maps and molecular gradients. *Curr. Opin. Neurobiol.* **3,** 67-74.

Schilling, T. F., Piotrowski, T., Grandel, H., Brand, M., Heisenberg, C.-P., Jiang, Y.-J., Beuchle, D., Hammerschmidt, M., Kane, D. A., Mullins, M. C., van Eeden, F. J. M., Kelsh, R. N., Furutani-Seiki, M., Granato, M., Haffter, P., Odenthal, J., Warga, R. M., Trowe, T. and Nüsslein-Volhard, C. (1996). Jaw and branchial arch mutants in zebrafish I: branchial arches. *Development* **123**, 329-344.

Schmidt, J. T. (1978). Retinal fibers alter tectal positional markers during the expansion of the half retianl projection in goldfish. *J. Comp. Neurol.* **177,** 279-300.

Schmidt, J. T., Cicerone, C. M. and Easter, S. S. (1978). Expansion of the half retinal projection to the tectum in goldfish: an electrophysiological and anatomical study. *J. Comp. Neurol.* **177,** 257-278.

Sharma, S. C. (1972). Retinotectal connexions of a heterotopic eye. *Nature New Biology* **238,** 286-287.

Sperry, R. W. (1963). Chemoaffinity in the orderly growth of nerve fiber patterns and connections. *Proc. Natl. Acad. Sci. USA* **50,** 703-710.

Stuermer, C. A. O. (1988). Retinotopic organization of the developing retinotectal projection in the zebrafish embryo. *J. Neurosci.* **8,** 4513-4530.

Stuermer, C. A. O., Rohrer, B. and Münz, H. (1990). Development of the retinotectal projection in zebrafish embryos under TTX-induced neural-impulse blockade. *J. Neurosci.* **10,** 3615-3626.

Thanos, S., Bonhoeffer, F. and Rutishauser, U. (1984). Fiber-fiber

interaction and tectal cues influence the development of the chicken retinotectal projection. *Proc. Natl. Acad. Sci. USA* **81,** 1906-1910.

Thanos, S. and Dütting, D. (1987). Outgrowth and directional specificity of fibers from embryonic retinal transplants in the chick optic tectum. *Dev. Brain Res.* **32,** 161-179.

Udin, S. B. and Fawcett, J. W. (1988). Formation of topographic maps. *Ann. Rev. Neurosci.* **11,** 289-327.

van Eeden, F. J. M., Granato, M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.-P., Jiang, Y.-J., Kane, D. A., Kelsh, R. N., Mullins, M. C., Odenthal, J., Warga, R. M. and Nüsslein-Volhard, C. (1996). Genetic analysis of fin formation in the zebrafish, *Danio rerio*. *Development* **123**, 255-262.

Walter, J., Kern-Veits, B., Huf, J., Stolze, B. and Bonhoeffer, F. (1987). Recognition of position-specific properties of tectal cell membranes by retinal axons *in vitro*. *Development* **101,** 685-696.

Westerfield, M. (1993). *The Zebrafish Book.* Eugene: University of Oregon Press.

Whitelaw, V. A. and Cowan, J. D. (1981). Specificity and plasticity of retinotectal connections: A computational model. *J. Neurosci.* **1,** 1369-1387.

Willshaw, D. J. and von der Malsburg, C. (1976). How patterned neural connections can be set up by self-organization. *Proc. R. Soc. Lond. B* **194,** 431-445.

Yoon, M. G. (1972). Transposition of the visual projection from the nasal hemiretina onto the foreign rostral zone of the optic tectum in goldfish. *Exp. Neurol.* **37,** 451-462.

Yoon, M. G. (1973). Retention of the original topographic polarity by the 180° rotatet tectal reimplantant in young adult goldfish. *J. Physiol. (London)* **233,** 575-588.

Yoon, M. G. (1975). Readjustment of retinotectal projection following reimplantation of a rotated or innervated tectal tissue in adult goldfish. *J. Physiol. (London)* **252,** 137-158.

(Accepted 12 March 1996)