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Histological studies on the development of retinotectal projections from nasoventral quarter-eyes in *Xenopus laevis*

N. Degen, K. Brändle* and L. Peter

Zoologisches Institut der Johann Wolfgang Goethe Universität, Fachbereich Biologie, Siesmayerstrasse 70, D-6000 Frankfurt/Main 11, Federal Republic of Germany

*Author for correspondence

SUMMARY

In *Xenopus* larvae, the size and location of the retinotectal projection of nasoventral quarter-eyes was analyzed in early stages (43-47), midlarval stages (50 and 53) and metamorphic stages (56 and 60), by labelling the optic nerve with the cobalt-lysine complex or with horseradish-peroxidase (HRP). For direct comparison, both fragment and normal eye projections were determined simultaneously in the same specimen in brain whole mounts. During early stages (up to stage 47), the projection fields of normal eyes and quarter-eyes are confined to the rostral part of the tectum. The extension of the projection in rostrocaudal direction of eye fragments does not differ from that of normal eyes. During later development up to metamorphosis, normal eyes expand their projection over the newly formed tectal surface in a caudal direction, whereas the fiber terminations of nasoventral quarter-eyes still remain in the rostral part of the tectum. Quantitative studies revealed that there is no difference in the size of both halves of the tectum. At least for quarter-eyes, however, a strict correlation between eye size and extension of the contralateral projection field could be established. According to our results, it is unlikely that during development local tectal markers are involved in determining the location of the projection field and the retinotopic ordering of the optic fibers. Instead we suggest that the optic fibers separate in accordance with their retinal specificity.

Key words: *Xenopus*, visual system, retinotectal, eye fragment, retinotopy, tectal markers, fiber interactions

INTRODUCTION

In lower vertebrates, the retinotectal projection is topographically organized such that the distribution of the optic fiber terminations in the contralateral tectum reflects the spatial arrangement of the corresponding ganglion cells in the retina (Jacobson, 1991): fibers from the temporal retina project to the rostral tectal area, nasal fibers to the caudal part, ventral fibers to the medial part and fibers from the dorsal retina terminate in the lateral part of the tectum. In order to find out the mechanisms responsible for the establishment of the retinotopic projection in regeneration as well as in development, a large number of experiments had been performed in fish and amphibia (reviewed in Cowan and Hunt, 1985; Udin and Fawcett, 1988). The results were interpreted in favour of the involvement of positional cues in the retina and tectum (Sperry, 1963), and/or the self-organization of the optic fibers (Cook and Horder, 1977), and/or a role of correlated impulse activity of retinal axons from neighbouring ganglion cells for the sharpening of the map (Cline and Constantine-Paton, 1989).

Whereas in the eye anlage the positional specification of the retinal ganglion cells is widely accepted (Cooke and Gaze, 1983; Udin and Fawcett, 1988; Fraser and O'Rourke, 1990), the generation of corresponding tectal markers is still doubtful especially in view of several findings pertaining to half-eyes and compound eyes in *Xenopus* (Feldman and Gaze, 1975; Hunt and Berman, 1975; Berman and Hunt, 1975; Feldman, 1978; Straznicky et al., 1980; Straznicky et al., 1981; Straznicky and Gaze, 1982). In these eyes, the optic projection always occupies the entire tectal surface available rather than just those areas of the tectum that correspond to its specificity. Since these eye types eventually develop into eyes of almost normal size, however, the possibility cannot be ruled out that tectal markers are 'overrun' due to the large quantity of ingrowing optic fibers.

In recent years, we have succeeded in creating *Xenopus* quarter-eye fragments that grow to a size substantially smaller than that of normal eyes. At stage 60, the retinotectal projection of these quarter-eyes occurs only in a certain portion of the tectal surface, invariably restricted to the rostral area of the tectum, irrespective of the remaining quadrant (Degen and Brändle, 1986). Despite of the unusual location of the reduced size of the projection field, optic fiber terminations are organized retinotopically (Brändle and Degen, 1988). Furthermore, the direction of field expansion with increasing eye size is quadrant-specific (Brändle and Degen, 1988), indicating that quarter-eyes apparently maintain their original specificity, comparable to half-eyes (Straznicky et al., 1980) and compound eyes (Gaze and

Straznicky, 1980; Straznicky et al., 1981; Straznicky and Tay, 1982).

Since the ingrowing optic fibers of quarter-eyes do not match the corresponding tectal target regions, it is unlikely that in quarter-eyes local tectal markers are involved in determining the location of the retinal projection and the retinotopical ordering of the optic fibers. However, since the retinotectal projection in *Xenopus* tadpoles at metamorphosis reflects almost the mature state of the contralateral visual input (Gaze et al., 1974), we cannot infer from our investigations to the initial innervation of the tectum in early larval stages and the subsequent development of the projection. Recent investigations on the normal development of the retinotectal projection in *Xenopus* have shown that the retinotopic pattern becomes established by dynamic rearrangements of the optic fiber arborizations in the tectum (Fraser and O'Rourke, 1990; O'Rourke and Fraser, 1990). First fibers from the ventral and dorsal retina are roughly separated in the mediolateral direction of the tectum (Holt, 1983, 1984; Holt and Harris, 1983), whereas nasal and temporal axons initially overlap in the rostral part of the tectum. Segregation of nasal and temporal retinal axons in rostrocaudal direction does not occur until stage 45 (O'Rourke and Fraser, 1990).

O'Rourke and Fraser (1990) suggested that nasal fibers expand to the caudal part of the tectum as soon as this area has matured, without any interference of the temporal fibers. It is also conceivable that competitive interactions between the optic fibers are the driving force for the segregation of the optic terminals (Willshaw and van der Malsburg, 1979; Fraser and Perkel, 1990; Wilm and Fritzsch, 1992). Thus, nasal fibers might be displaced by the temporal fibers into the newly formed caudal part of the tectum.

Nasoventral quarter-eyes offer a suitable system to decide between both possibilities, because they only send nasal fibers to the tectum. Therefore, we histologically studied the development of nasoventral quarter-eye projections, starting with stage 43 shortly after the first optic fibers have reached the tectum. In this paper, we report about investigations referring to the location and size of the whole projection field in the contralateral tectum. A preliminary report about this work has previously been published (Degen et al., 1990).

MATERIALS AND METHODS

Animals

Adult specimens of *Xenopuslaevis* were stimulated to reproduce by subcutaneous injection of human chorionic gonadotropin (Primogonyl, Schering). The embryos thus obtained were raised on nettle-powder and kept at room temperature of approximately 20°C. All operations and surgical procedures were carried out while the larvae were anaesthetized with tricaine methane sulphonate (MS 222, Sandoz). Developmental stages were determined according to the normal table of Nieuwkoop and Faber (1956).

Operations of the eye anlage

One-quarter eyes of the nasoventral (NV) type were obtained as follows. Embryos at stage 33/34 were embedded in a cooled mixture of beeswax and paraffin in an operation dish and covered with modified Holtfreter's solution and MS 222. Using fine glass needles to split the eyeball and a glass micropipette to suck out, the temporal half of the right eye anlage was first removed, followed by the dorsal half of the remaining nasal half-eye. The embryos were released into water approximately 30 minutes after termination of the operation.

HRP-staining in stages 43 to 47

In *Xenopus* larvae at early stages (43-47), we determined the optic projection of normal eyes and quarter-eyes by labelling the retinal axons with a solution containing 10% horseradish peroxidase (HRP, Sigma type VI) in 1% nonidet-P40 (Sigma). Following immobilization of the larvae with MS 222, the eyeball was opened by removing the cornea and lens. One drop of the tracer solution was attached to the retina, and subsequently the eyeball was closed and sealed with vaseline and histoacryl blue (Braun-Dexon). After a short survival time (30-60 minutes), the animal was killed and fixed in cold 2% glutaraldehyde in 0.1 M phosphate buffer for 1 hour. Whole larvae heads with the opened brains were reacted for HRP using the cobalt-diaminobenzidine (Co-DAB) method by Adams (1977). Briefly, the specimens were washed in 0.1 M phosphate buffer, rinsed in Tris-HCl buffer and stained in 1% cobalt chloride. The brains were then preincubated in 0.1% DAB (Sigma) for 12 minutes and reacted for HRP in the same solution for 30 minutes by adding 2-4 ml 0.3% hydrogen peroxide. Afterwards, the whole-mount brains were cleared in xylene.

HRP staining from stage 50 to metamorphosis

Horseradish peroxidase (HRP) staining was achieved by applying small pieces of filtration paper soaked with HRP to the optic nerve stump in larvae at stages 50, 53, 56/57 and 60. The animals were anaesthetized and transferred into a solution of full-strength Niu-Twitty. The epidermis that covers the eye as well as the eye-cup were opened with forceps at the animal's dorsal side, immediately behind the cornea. Subsequently, the retina was separated from the pigment epithelium and the optic nerve was severed. HRP filtration paper was applied to the severed optic nerve. The eye-cup was closed with histoacryl (Braun-Dexon). Due to the different levels of development (stages 50 to 60), the survival time of the animals varied from 17 hours at stage 50 up to 45 hours at stage 60. Eventually the larvae were anaesthetized and killed by 0.1 M phosphate buffer perfusion through the heart. Immediately after the perfusion procedure, all the membranes covering the brain were removed, the complete brain was taken out and placed in cold (4°C), buffered fixative (3% glutaraldehyde, 0.3% paraformaldehyde) for one hour.

For HRP-staining reaction, ortho-tolidine (3.3′-dimethyl-(1.1′-biphenyl)-4.4′-diamin, Sigma) was used as a substrate. The HRP reaction product with this substrate was dark blue. The method used was that of Somogyi et al. (1979), in a modified version developed in our laboratory (Holz, unpublished). The extremely sensitive ortho-tolidine was a suitable substrate for labelling of even a small number of neurons, such as those forming the projections of quarter-eyes. A low temperature of 4°C is required in each phase of the staining procedure.

After fixation the brain was washed three times, 15 minutes duration each, in phosphate buffer at pH 7.4, subsequently placed in water for 5 minutes, and finally changed three times, for 15 minutes each, in washing buffer. This buffer is obtained by mixing 50 ml 0.2 M citric acid with 50 ml 0.2 M ammonium acetate, and titrating this solution to pH 4.5-5.0 with concentrated ammonium hydroxide. The brain whole mount was subsequently transferred to the incubation medium (two changes lasting 45 minutes each) prepared as follows. 60 mg ortho-tolidine (Sigma) were dissolved in 50 ml 0.2 M citric acid, and 50 ml 0.2 M ammonium acetate were added to this. The required pH value of 4.5-5.0 was achieved by dropwise addition of ammonium hydroxide. Water was added to increase the volume to 200 ml. Finally, 5% sodium nitroprusside and 1% hydrogen peroxide were added to give the final incubation medium.

After incubation, the brain was washed with washing buffer and subsequently transferred, for a 25-minute period, to a 'stabilizing' solution consisting of the same buffer and 2.5% sodium nitroprusside.

Camera-lucida drawings were made of each preparation and also of both eyes before HRP application. The whole-mount preparations with HRP-filled optic axons were photographed as well.

Cobalt labelling

In some *Xenopus* tadpoles, we labelled the optic projection by filling the optic nerve with the Co^{3+}-lysine complex (Görcs et al., 1979) at stages 56 and 60. The animal was anaesthesized with MS 222 and the optic nerve was dissected from the surrounding tissue. We cut the nerve close to the eyeball and put the distal end into a small plastic tube filled with the cobaltic-lysine complex solution. The open end of the tube was sealed with vaseline and attached to the tadpole's head with histoacrayl. Following a period of 12-15 hours at 5°C, the tadpole was killed with a MS 222 overdose. The brain was dissected and placed in frog's Ringer solution, to which a few drops of ammonium sulphide were added to precipitate the cobalt for 20 minutes and then rinsed in frog's Ringer three times. Fixation and intensification were performed according to the method of Steedman et al. (1979) for brain whole mounts. Briefly, all brain membranes were removed and the brain was fixed in Stieve's fixative for 6 hours and rinsed in 70% ethanol for 12-15 hours (three changes). For intensification of the CoS precipitates, the brains were pre-soaked in the developer solution containing no silver nitrate for 1 hour at 60°C (100 ml 25% gum arabic, 3.5 g citric acid, 0.34 g hydroquinone, 10 g sucrose and 100 ml distilled water) and then transferred to a fresh solution with 0.1% silver nitrate. After intensification was completed, the specimens were rinsed in hot water (three times), dehydrated and cleared in xylene.

Estimation of fiber input from eye size

Histological serial sections of normal, half- and quarter-eyes have revealed that the eye diameter that can be measured from outside is always proportional to the retina surface and that constant proportionality factors can be computed for the different types of fragmentary eyes (Langsdorf and Brändle, 1991). Thus, it appears that the retina surface can be estimated in a fairly reliable manner on the basis of the eye diameter. Furthermore, countings of ganglion cells in normal and half-eyes have shown that the number of cells and the thickness of the ganglion cell layer are also proportional to the size of the respective eye (Degen, unpublished). Tentative counting of cells in quarter-eyes was in good agreement with the above findings. Since the extension of the tectal projection field appears to depend exclusively on the number of ingrowing optic fibers and the overwhelming majority of ganglion cells form only one axon (Sakaguchi et al., 1984; Holt, 1989), it might be expected that eye size and number of outgrowing optic fibers are correlated with each other.

Statistics

For comparison of pairs of mean values, Students *t*-test was applied to establish significance. More than two mean values were compared by a one-way analysis of variance (ANOVA). Individual comparison of mean values was achieved by means of a Scheffé-test (Sachs, 1971). To determine significant differences among the slopes of the straight lines in the regression analyses or if compared with zero, variance-analytic procedures after Riedwyl (1980) were utilized.

RESULTS

Qualitative investigations

(1) Size and morphology of quarter-eyes

At stage 33/34, the eye anlage was fragmented so that only the nasoventral portion remained in the orbit. Within a few days the distal cut edges of the remaining eye quadrant enclosed the lens fragment and fused, displacing the lens, as well as the site of the optic nerve head, to the center of the eye fragment. During further development up to metamorphosis, the quarter-eyes did not show any sign of delayed or decreased growth if compared to growth of normal eyes. In fact the eye fragments seemed to increase their mitotic activity, especially during the first 10-20 days following extirpation (Langsdorf and Brändle, 1991). However, quarter-eyes, despite increased growth rate, remained substantially smaller (between 25% and 70% of the size of contralateral normal eyes) in all the stages studied (Figs 1, 2).

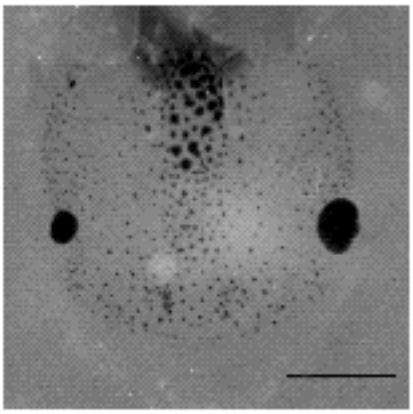

Fig. 1. Head of a *Xenopus* larva at stage 46. Notice the small quarter-eye on the left-hand side. Scale bar, 1 mm.

Histological study of fragmentary eyes revealed that their structure is basically the same as that of normal eyes at comparable stages (Fig. 2). Counting of cells in the ganglion cell layer of the retina yielded a reduced number of ganglion cells, due to the reduced dimensions of the fragment eye. The density of the cells per unit surface of the retina does not differ from that of normal eyes (Table 1).

(2) Location and size of retinotectal projection in normal and fragmentary eyes

In the majority of the experiments, both fragment and normal eye were marked simultaneously in the same specimen, for direct comparison of location and size of the respective retinotectal projection. For control purposes, we occasionally marked just one of the two eyes in order to ascertain that the projection occurred in the contralateral tectum only. We never encountered any instance of ipsilateral projection to the optic tectum (Fig. 4C).

Our investigations of the projection behaviour of nasoventral fragmentary eyes at stages 43-47 (8 specimens), stage 50 (16 specimens), stage 53 (16 specimens), stage 56/57 (16 specimens) and stage 60 (10 specimens) reveal that the surface dimensions of the contralateral retinotectal projection are considerably reduced if compared to those of normal eyes (Figs 3, 4). Furthermore, it is of prime importance that the projection field in all the cases studied is invariably limited to the rostral portion of the tectum surface, a phenomenon previously described for stage 60 (Degen and Brändle, 1986; Brändle and Degen, 1988). Comparing the expansion of the optic projections between

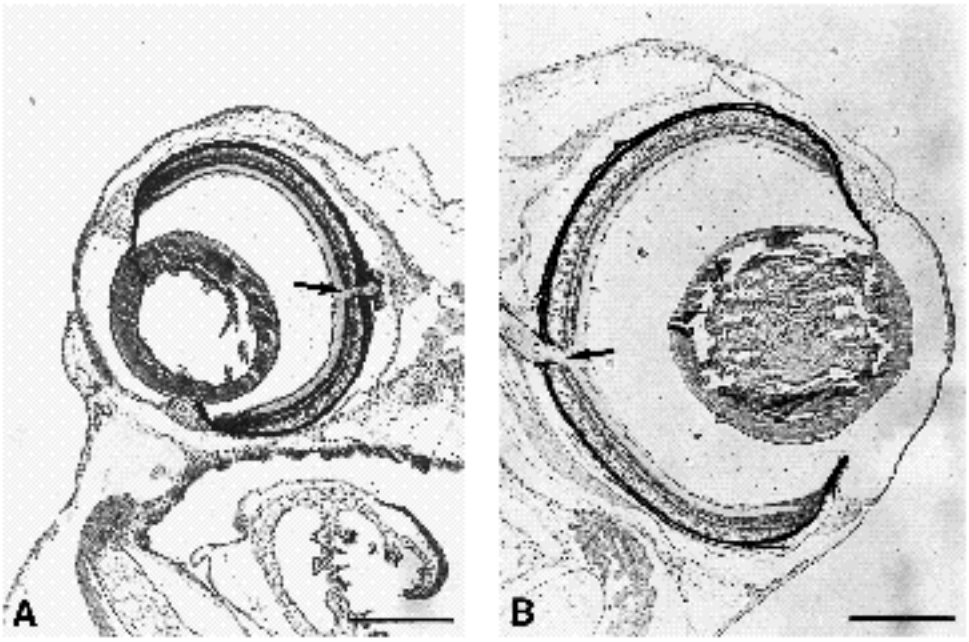

Fig. 2. Histological section of a nasoventral quarter-eye (A) and a normal eye (B) in a *Xenopus* tadpole at stage 60. Haematoxylin-eosin staining. Arrows indicate the optic nerve head. Scale bars, 300 μm. Dorsal is up.

Table 1. Measurements of the relationship between eye size and number of ganglion cells at stage 60 for three normal eyes (n) and three nasoventral quarter-eyes (q)

Type	Eye area (mm^2)	Number of cells	Density (cells/1000 μm^2)
n	2.32	28392	12.24
n	1.93	25887	13.41
n	2.19	25629	11.70
q	1.15	13776	11.98
q	1.28	21327	16.66
q	1.39	15954	11.48

quarter-eyes and normal eyes, two developmental periods can be distinguished:

Early stages in development (43-47)

The projection fields of normal eyes are confined to the rostral part of the tectum extending up to approximately 60% in the rostrocaudal direction (Fig. 3A). In quarter-eyes, the innervation density of the optic fibers in the tectum is considerably smaller due to the reduced number of retinal fibers (Fig. 3B). Nevertheless, the nasoventral eye fragments extend their projection field in the rostrocaudal direction of the tectum nearly as far as the fibers of a normal eye. The width of their projection fields in mediolateral extension, however, is smaller than that of normal eyes.

Midlarval stages up to metamorphosis (50-60)

During this developmental period, the optic fibers of normal eyes increase their projection fields in the tectum rapidly, especially in the caudal direction (Figs 3C,D, 4A,B). At metamorphosis the retinal fibers cover nearly the whole tectal surface.

In contrast to normal eyes, the optic fibers of nasoventral quarter-eyes are still restricted to the rostral part of the tectum and do not show any sign of expansion over the whole tectal surface (Figs 3C,D, 4A,C). Instead, the innervation density of the optic fiber terminations seems to increase especially in the rostral part of the projection field. Only the medial optic tract is occupied by retinal fibers corresponding to their original specificity (Fig. 3C, arrow).

In summary, our histological investigations prove that, irrespective of the developmental stages of the larvae, the projection field of the nasoventral eye fragments is always confined to the rostral portion of the contralateral tectum.

Quantitative investigations

Our quantitative analysis of a possible correlation between projection field and eye size covered the stages 50, 53, 56 and 60. Earlier stages were not included due to the small number of specimens.

(1) Comparison of the surface areas of both tectal halves

The dorsally visible surfaces of both tectal halves were measured at the four stages. The right half of the tectum was occupied by optic fibers of a normal eye and the left tectal half by those of a quarter-eye. In Fig. 5, the two tectal surfaces of each experimental animal are plotted against each other. Linear regression led to equation $Tq=0.03+0.90\times Tn$, where Tq stands for tectum of quarter-eye and Tn for tectum of normal eye. Identical surface dimensions were required to fulfill the function $Tq=0+1.00\times Tn$. The obtained deviation from the expected value 1.00 for the slope is significant ($P<0.01$). Thus, the tectal half innervated by a quarter-eye is somewhat smaller than that of the control side. However, this applies to the regression analysis based on all stages, it is not valid for individual stages. This becomes also apparent in the mean values table (Table 2). One-way analysis of variance (ANOVA) failed to yield any significant size difference between the two tectal halves in any of the stages analyzed.

In summary, it appears that even the strongly reduced

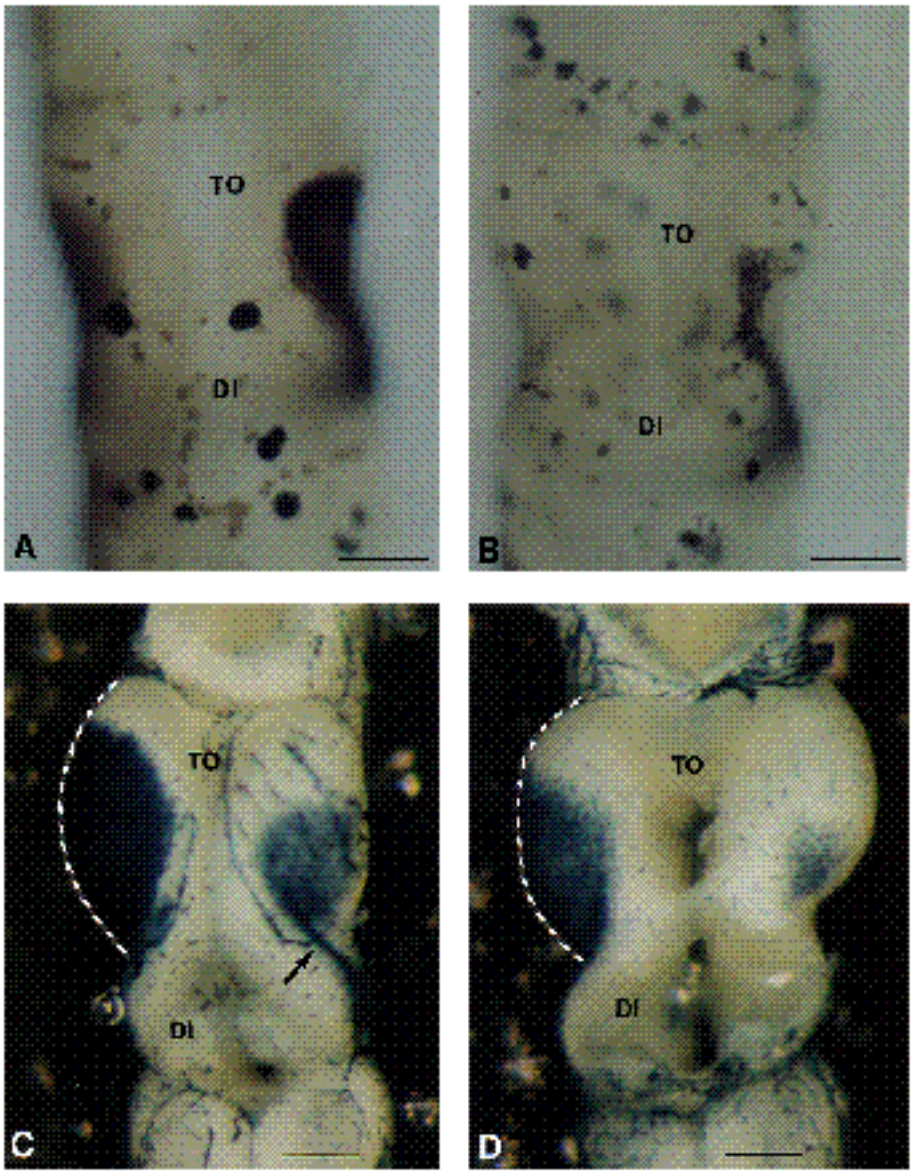

Fig. 3. Dorsal view on brain whole mounts in *Xenopus* at different developmental stages. Optic fibers are labelled with HRP and reacted either by using the Co-DAB method (A,B), or by a modified o-tolidine method (C,D). (A) Projection of a normal eye in a larva at stage 43. (B) Projection of a nasoventral quarter-eye at stage 47. Scale bars, 80 μm. (C,D) Optic projections of both quarter-eye (right side) and contralateral normal eye (left side) in tadpoles at stage 50 (C) and stage 53 (D). Scale bars, 200 μm. TO, optic tectum; DI, diencephalon; arrow in C indicates the medial optic tract.

invasion of optic fibers from nasoventral quarter-eyes has only negligible impact on the surface area growth of the contralateral half of the tectum. Layer thickness and number of neurons in the tectum may, nevertheless, be affected by a reduced input of optic nerve fibers.

(2) Correlation between eye size and contralateral tectum surface

If tectal growth is independent of the number of ingrowing optic fibers, i.e. the eye size, there should be no relationship between topview surface of an eye and contralateral tectum surface.

For normal animals, however, a relation between both parameters appears to exist. Fig. 6A contains the distribution of points and their respective regression line for the four different stages analyzed. Their slopes differ significantly from zero ($P<0.01$). However, the slopes do not differ significantly from one another ($P>0.05$), only their intersections with the ordinate reveals significant differences ($P<0.02$). In other words, the regression lines are shifted parallel towards each other. We assume that the observed correlations between eye and tectal dimensions simply represent variations of body size within an individual stage.

Establishment of a linear regression for normal eyes and their contralateral tecta over all the stages analyzed (Fig. 6A, dotted line) supports this suggestion. With the exception of stage 50, the slope yielded is considerably steeper than the slopes obtained from the regression lines for individual stages, indicating the growth of the larvae between different stages of development.

Apart from being commonly influenced by body size, the overall growth of eye and the tectal dimensions appears to be independent of one other. This becomes particularly clear by analysis of the relation between the surface of quarter-eyes and that of their respective contralateral tectal halves. The extreme values for the tectal halves in the developmental stages of the quarter-eyes differ only slightly from those encountered in normal eyes. In sharp contrast to this,

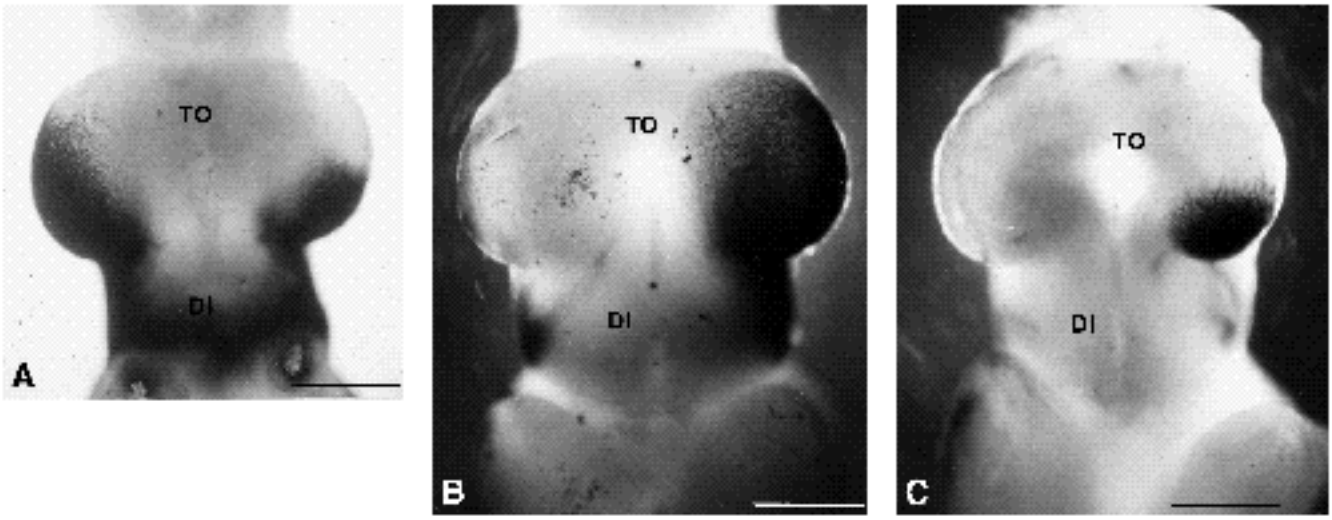

Fig. 4. Dorsal view on brain whole mounts in *Xenopus* tadpoles at stage 56 (A) and stage 60 (B,C). Optic projections are marked by cobalt filling. (A) Projection of a nasoventral quarter-eye (right side) and of the contralateral normal eye (left side). (B) Projection of a normal eye. (C) Projection of a quarter-eye. Scale bars, 500 μm. Abbreviations as in Fig. 3.

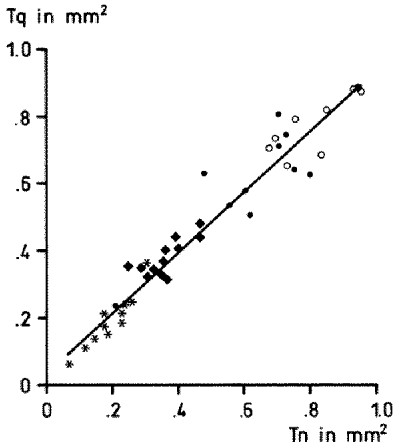

Fig. 5. Correlation between tectal surfaces of all specimens innervated by normal eye (Tn) and by nasoventral quarter-eye (Tq). * stands for stage 50, ◆ for stage 53, ● for stage 56, and ○ indicates stage 60.

the respective values for the eye surfaces scatter to such a degree that they can no longer be correlated with their tectal surfaces (Fig. 6B). The slopes of the regression lines at stages 53, 56 and 60 show no significant deviation from zero. A positive correlation appears to prevail only at stage 50.

(3) Correlation of eye size and tectal projection surface

As has been shown above, the whole tectal surface of quarter-eyes does not differ significantly during the individual stages of development from that found in normal eyes, and the size of the contralateral tectum seems to be independent of eye growth. Therefore, we computed the relative size of the projection surface as percentage of the total tectal surface. This enables us to compare it directly with that of the contralateral eyes. It also allows us to eliminate individual dimensional variation of the larvae.

A comparison of normal-eye size and tectal projection surface in % (Fig. 7A) reveals that there is no correlation whatsoever between eye size and relative fiber distribution on the tectal surface. It has to be taken into consideration, however, that eye size shows little variation in the individual stages of development. With respect to stage 60, the distribution of points for the individual stages in Fig. 7A shows a minor increase in tectal fiber invasion.

In fragmentary eyes, however, a correlation at the level of $P<0.05$ can be established between eye size and percentage of tectal fiber invasion throughout all the stages exceeding stage 50 (Fig. 7B). A linear correlation analysis over the values of all the stages studied (not shown) reveals an unequivocally positive correlation for the percentage of fiber invasion into the tectal surface ($P<0.01$).

At stage 50, the values for both normal and quarter-eyes are found to scatter in an extreme manner. Here, the percentage of tectal surface is particularly high in the presence of small eye diameters.

DISCUSSION

Development of normal projection

The results of our quantitative study can be divided into two developmental periods. Correlation of absolute eye and tectal dimensions in stages 53 through 60 reveals minor linear correlations that are shifted parallel to each other. The linear correlations found within and between the individual stages presumably mirror the fluctuation in body size, which is accompanied by appropriate eye and tectal dimensions. Within each developmental stage, tectum invasion by the retinotectal projection expressed in per cent does not appear to be correlated with eye size. Due to the small differences in eye size, it is not reasonable to expect any unequivocal correlation. Comparison of the mean values of the projection surface expressed in per cent in the four stages analyzed shows that they are a good match with the relative dimen-

Table 2. Mean values and standard deviations of total tectal area and of projection area innervated by normal eyes and quarter-eye fragments

	Total tectal size (mm^2)		Projection field size (mm^2)	
	Normal eyes	NV quarter-eyes	Normal eyes	NV quarter-eyes
Stage 50	0.186 (±0.062)	0.194 (±0.073)	0.096 (±0.033)	0.065 (±0.028)
Stage 53	0.357 (±0.065)	0.403 (±0.075)	0.174 (±0.039)	0.079 (±0.036)
Stage 56/57	0.616 (±0.174)	0.609 (±0.144)	0.313 (±0.128)	0.159 (±0.103)
Stage 60	0.825 (±0.105)	0.784 (±0.085)	0.506 (±0.087)	0.137 (±0.101)

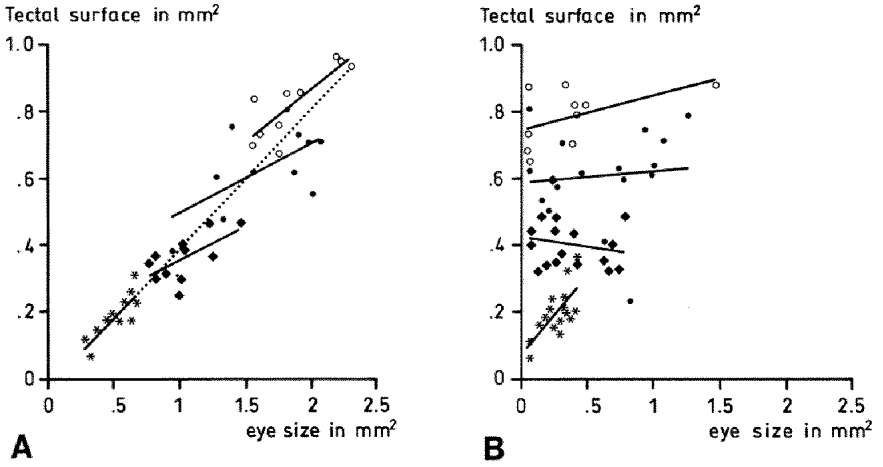

Fig. 6. Relation between top view surface of eyes (abscissa) and contralateral tectum surface (ordinate). (A) Normal eyes; (B) quarter-eyes. Same symbols as those used in Fig. 5. Solid lines, regression lines for the four different stages; dotted line in A, regression line of all measurement values. For further information see text.

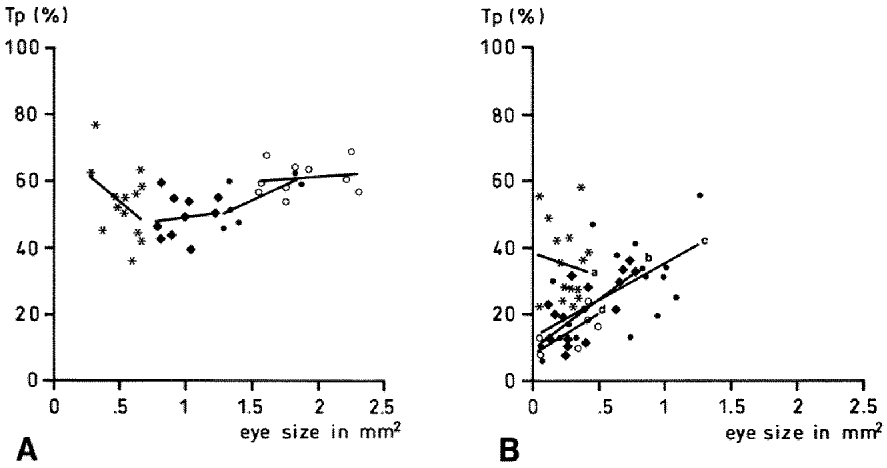

Fig. 7. Relation between top-view surface of eyes (abscissa) and projection surface in the contralateral tectum expressed in percent (TP(%), ordinate). (A) Normal eyes; (B) quarter-eyes. Symbols as in Fig. 5. Solid lines, regression lines for the four different stages.

sions of the maps established during electrophysiological mapping by Gaze et al. (1974).

Stage 50, however, is of particular interest. It is only at this stage that a major increase in tectum size can be observed relative to eye dimensions. Measuring the projection surface in per cent, the values scatter considerably. These observations are in good agreement with the findings of Sakaguchi and Murphey (1985), who found in *Xenopus* a considerable expansion of the tectal neuropil up to stage 50, whereas the rostrocaudal length of the terminal arbors remain more or less unchanged (see Fig. 13B in Sakaguchi and Murphey, 1985). Because the assessment of developmental stages is generally based on fairly rough exterior criteria, it cannot be ruled out at this point that our experiments at stage 50 covered specimens in which neuropil development was at different phases of growth. This might help to explain the strong scattering found in the relative tectum occupation by the projection field.

Development of quarter-eye projection

Development of the eye fragments

Our observations on the development of the quarter-eyes show that at least two kinds of regulatory events are induced by fragmentation. First, a dynamic rounding-up process occurs resulting in the reconstitution of normal eye shape: the lens as well as the optic nerve head are positioned in the center of the small eyeball. This morphological rearrangement seems to be a general reaction of the embryological

eye tissue to a fragmentation, since it could also be found in half-eyes (Feldman and Gaze, 1975) and other types of quarter-eyes (Degen and Brändle, 1986; Brändle and Degen, 1988). Secondly, quarter-eyes as well as half-eyes seem to compensate for the extirpated retinal tissue by increasing their growth rate. Whereas half-eyes can develop by this enhanced cell proliferation into eyes of almost normal size, quarter-eyes never reach the size of normal eyes, probably due to the smaller size of the initial fragment.

The problem of specificity in quarter-eyes

In order to interpret the retinotectal projections of the nasoventral quarter-eyes in relation to tectal markers, it is of prime importance to get information about their state of retinal specificity, since it is conceivable that some kind of positional regulation occurs, altering the original specificity of the remaining fragment. In another series of experiments, not presented in this paper, we found strong indications that the eye fragments retain their original specificity, despite their morphological regulation and the regulation in size. Because this is crucial for this study, the results of these experiments will be briefly considered.

At first we were able to show that different types of quarter-eyes expand their projection fields with increasing eye size in a preferred direction in accordance with their original positional information. For example, we found that nasal fibers preferably expand in caudal direction, while ventral ones prefer a medial expansion, and temporal ones remain in rostral position (Brändle and Degen, 1988).

Further support for the original state of specificity is obtained by investigations about the course and distribution of the retinal fibers in the optic tract. As for normal eyes (Fawcett and Gaze, 1982), the optic fibers of quarter-eyes are distributed according to the different origin of the eye fragments (Degen, 1988): fibers of ventral quarter-eyes pass exclusively through the medial tract; fibers of nasoventral fragments occupy the medial tract (see arrow in Fig. 3C) and, to a minor extent, the lateral tract as well; some fibers of temporoventral fragments follow the medial tract, but the mass of fibers project directly from the rostral pole to the tectum. Comparable results exist for the arrangement of fibers in compound eyes (Straznicky et al., 1979; Fawcett and Gaze, 1982).

All these findings indicate that quarter-eyes keep their positional identity and that a regulation of positional specificities does not take place. Thus, the retinotectal projections of the nasoventral quarter-eyes must be judged on the basis of their original specificity.

Size of the tectum

Experiments involving enucleation during early developmental stages of different anuran species by Currie and Cowan (1974), Kollros (1988), and Kollros and Thiesse (1988) revealed a reduction of the mitosis rate in the contralateral tectum and thus reduced tectal surface dimensions. Enucleation of one eye at stage 33/34 in *Xenopus laevis* embryos yielded similar results (Angert, unpublished). Based on these findings, it would have to be expected that the tectal surface size in quarter-eyes is also influenced by the reduced number of ingrowing optic fibers. Contrary to this expectation, however, the two tectal halves are of almost equal size. This leads us to the assumption that even minor invasions of optic fibers may suffice to guarantee normal tectum growth. This is supported by our observation that even considerable variation in quarter-eye size within one developmental stage does not have an impact on the tectal dimensions (Figs 5, 6B). The schedule of tectal development also seems to be identical for tecta innervated by normal eyes and by quarter-eyes. At stage 50, we found that the extension of the projection field in quarter-eyes reveals a similar degree of strong scattering as in normal eyes. This indicates the same substantial increase of tectal neuropil at that stage, irrespective of the kind of innervation.

This does not imply, however, that innervation does not exert any influence on the structure of the tectum. Preliminary histological analyses of the outer layer of a tectum innervated by a quarter-eye carried out in our labaratory have shown that at least the thickness of layers 8 and 9 is reduced.

Location and extension of quarter-eye projection

Studies of optic fiber innervation of the tectum in normal animals by Holt (1983; 1984), Holt and Harris (1983), Sakaguchi and Murphey (1985), O'Rourke and Fraser (1986; 1990), and Fraser and O'Rourke (1990) have shown for *Xenopus* that the mediolateral and the rostrocaudal polarization of the projection becomes established during different time periods and probably by different mechanisms. Dorsoventral retina specification is fairly early reflected in a tectum occupation in the mediolateral direction. With respect to reduced tectum innervation by quarter-eyes this does no longer apply. Since the fragments are of the nasoventral type it might be predicted that the projection would concentrate only on the medial portions of the tectum surface. However, in all the stages of development analyzed so far, the projection occurs also in the lateral tectum portion. This is also valid for ventral and temporoventral fragment eyes (Degen and Brändle, 1986; Brändle and Degen, 1988).

Similarly, the development of quarter-eye projection in rostrocaudal direction corresponds only in early stages of development to what might have been expected from findings in normal eyes (Sakaguchi and Murphey, 1985; O`Rourke and Fraser, 1986; 1990; Fraser and O'Rourke, 1990). In normal eyes, fiber branchings originating from the nasal and temporal retina do not segregate until stage 45. Then nasal fibers increase the length of their arbors in caudal direction and form arborizations in the caudal tectal area, while rostrally located arborizations are pruned and retracted. For temporal fibers, the total increase of their arbor growth stops, and they remain in the rostral tectal portion. O'Rourke and Fraser (1990) suggest that the initial expansion of the nasal fibers could be restricted by the small area of tectal neuropil, which matured during the first ingrowth of the optic fibers. As tectal growth and maturation proceed at the caudomedial border, nasal fibers can extend their terminal arbors into the newly formed areas in the caudal direction. If this assumption holds true, one should expect the projection of the nasoventral quarter-eyes at later stages to be translocated to the caudal portion of the tectum. In contrast to this prediction, however, the fibers of the nasoventral eye fragments always innervate the rostral

tectum even at later stages leaving the caudal tectum free of fibers. In addition, our quantitative investigations on the development of tectal size as well as Golgi studies on tectal neurons (Angert, unpublished) reveal that tecta innervated by quarter-eyes exhibit almost normal growth and maturation.

Our results suggest that the segregation of nasal and temporal fibers cannot be explained by a selective outgrowth of the nasal arbors merely due to tectal growth and recognition of the proper target region. Instead, we propose that competitive interactions between temporal and nasal fibers are the main force for the translocation of the nasal arbors. Temporal fibers progressively push away the nasal fibers out of the overlapping zone in the rostral tectum. Tectal growth and maturation merely produce free tectal neuropil, to which the nasal fibers can evade.

This concept of self-sorting would presuppose a fairly short-range exchange of information among the fibers concerning their retinal position values. The fibers would have to stop shifting either when a certain distance from each other is exceeded, and/or when nasal and temporal fibers are completely separated. As a consequence, the segregation process stops earlier with a reduced number of ingrowing fibers, the projection field remaining in rostral position.

The observations on the behaviour of growing retinal fibers in in vitro systems corroborate the assumption that nasal fibers are displaced caudally by temporal fibers. In *Xenopus* (Jack et al., 1991; Johnston and Gooday, 1991), as well as in chicken and goldfish (Walter et al., 1987a,b; 1990; Vielmetter and Stuermer, 1989; Cox et al., 1990), temporal retinal fibers avoid caudal glial cells or tectal cell membranes respectively, whereas nasal fibers do not. Provided that the same principles of axonal guidance hold true in vivo, the concept of self-sorting explains, why nasal fibers in normal eyes shift to the caudal part of the tectum instead of covering the whole tectal surface, and why nasal fibers in nasoventral quarter-eye fragments remain restricted to the rostral tectal area.

During later development up to metamorphosis, the correspondence in size between eye and projection field indicates that the dimension of the tectal projection field is determined by the mass of ingrowing optic fibers. In quarter-eyes of at least stage 53 and later, the relation between eye size and tectum occupation expressed in per cent has yielded an unequivocal correlation where position and steepness of the straight lines are almost identical (Fig. 7B). The reason why such correlation has not been detected in normal eyes may reside in the fact that eye size shows little variation at one stage.

Further evidence for a driving capacity of optic fibers comes from the study of Wilm and Fritzsch (1992) in the retinotectal system of the chichlid fish. These authors induced experimentally a permanent ipsilateral retinotectal projection established by regenerated optic axons only from the center of the retina. Fibers of newly formed ganglion cells do not contribute to this projection. In that case, the projection is always confined to the rostral tectum indicating that the shift of axonal terminals from the central retina into more caudal tectal areas (Gaze et al., 1974; Reh and Constantine-Paton, 1984; Fraser, 1983) does not occur due to the absence of newly ingrowing fibers.

Implications for assumption of local tectal markers

Our findings for quarter-eyes are neither in agreement with the hypothesis that local tectal markers should be instrumental in controlling the structure of the retinotopic projection during initial tectum innervation by optic fibers, nor that these markers provide polar information as well as determine a location complementary to the retina specificity. We assume instead a self-sorting mechanism of the optic fibers.

The interpretation above does not per se rule out the existence of locality-specific tectal markers. There is even fairly strong indication for the existence of such markers in the later larval stages and in adult specimens, based on findings from regeneration experiments following partial tectum rotation (reviewed in Udin and Fawcett, 1988) and derived from the totally different behaviour of the terminal arbors during regeneration (Fujisawa et al., 1982) as opposed to development (Sakaguchi and Murphey, 1985). It is still an open question whether the markers are created in conjunction with optic input (Schmidt, 1978; 1983; Willshaw and van der Malsburg, 1979), or whether they develop independent of the whole process of innervation. In this context, an observation made by Stuermer (1988) in *Brachydanio* are interesting. Following extirpation of the temporal retina half prior to optic fiber ingrowth into the tectum, the fibers of the nasal half-eye apparently head directly and exclusively towards the caudal region of the tectum, an observation contradictory to what has been established for *Xenopus* so far. It is conceivable, however, that the concepts of self-sorting and tectal markers constitute parallel orientation mechanisms that are both involved in the formation of retinotopic projection (Fraser and Perkel, 1990), and that the markers in *Brachydanio* - in contrast to *Xenopus* - are already being formed prior to initial innervation of the tectum or have at least a stronger guidance effect on the optic fibers than in *Xenopus*.

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