First publ. in: Journal of Comparative Neurology 284 (1989), pp. 148-168

Pathfinding and Target Selection of Goldfish Retinal Axons Regenerating Under TTX-Induced Impulse Blockade

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ABSTRACT

To define the extent to which impulse blockade interferes with the morphological changes of regenerating retinal axons during their growth through the tectum, axons were deprived of activity by repeated intraocular injections of TTX. At intervals between 24 and 189 days after optic nerve section (ONS), a defined group of TTX-silenced axons and of axons with normal activity (controls) were labeled by applications of HRP to the ventro- or dorsotemporal retina. The trajectories of these labeled axons were traced in DAB processed tectal wholemounts.

As in controls, TTX-blocked axons went through a phase of exploratory growth at early regeneration stages (24 to 80 days after ONS). Coursing in abnormal routes, the axons initially distributed their growing endings widely over the tectum. Axons with and without activity extended side branches with growth cones and filopodia over all regions of the tectum. These ramifications were of similar dimensions for the TTX-blocked and control axons. Despite abnormal routes and branching over inappropriate territories, axons showed a preference for the rostral tectum. At late regeneration stages (120–189 days after ONS), axons had lost their side branches and their growth cones. Their preterminal segments exhibited striking bends, suggesting that they had undergone course corrections to achieve access to the retinotopic target. Axonal processes had disappeared from the caudal tectum, and the preferential accumulation of axons over the rostral tectum had increased. The majority of the TTX-blocked and control axons ended in terminal arbors at retinotopic regions. The labeled arbors of the TTX-group were no larger than those of the control group. The arbors of each group lay close together in a continuous cluster in the TTX-group as well as in two-thirds of the control group. In the other one-third of the control group, however, terminal arbors were aggregated into separate patches. The clusters of the TTX-blocked axons covered between 2.2 and 3.9% (mean 2.95%) of the tectal surface and the clusters and/or patches of active axons between 1.9 and 3.4% (mean 2.7%). Thus the terminal arbor clusters of the TTX-silenced axons were not significantly larger than those of the active axons.

These data show that retinal ganglion cell impulse activity is required for neither the extension of side branches in the early exploratory phase of regeneration nor for the withdrawal of these branches nor for the establishment of target-directed routes and the deployment of normal-size terminal arbors at retinotopic loci. Our data further suggest that the retinotopic map is refined considerably with time even in the absence of activity, a finding that is consistent with an abstract by Olsson and Meyer ('87).

Key words: Regenerating retinal axons, extension and loss of exploratory branches, terminal arbor at retinotopic regions

How growing nerve fibers manage to form selective connections during development or regeneration is not well understood. Many studies have addressed this question by

Accepted February 24, 1989.

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using the retinotectal system of nonmammalian vertebrates, especially goldfish, taking advantage of the accessibility of the system and its capacity to regenerate (for reviews see Gaze, '70; Purves and Lichtman, '85). In the visual system of fish, the retinal axons deploy their terminal arbors in a geometrically simple retinotopic order, in which the neighborhood relationship of their parent ganglion cells is reproduced at the level of the axon terminals in the tectum. This retinotopic "map" is reestablished to near-normal precision by regenerating axons following optic nerve transsection (Jacobson and Gaze, '65; Schmidt et al., '83). In their classic work, Attardi and Sperry ('63) conveyed the view that regenerating axons follow their normal pathways and terminate at predesignated targets promptly after their arrival in tectum. They proposed that axons are guided to their target by cytochemical positional cues or markers that are associated with axons and the tectal cells (Attardi and Sperry, '63; Sperry, '63).

Subsequent studies, however, using more sensitive methods, revealed that regenerating axons travel in abnormal routes (Cook, '83; Stuermer and Easter, '84a; Stuermer, '86, '88a,b). Furthermore the terminal endings of axons from small and well-defined sites in the retina were found to be initially distributed widely over the tectum and to accumulate at retinotopic sites only after a period of several months (Meyer et al., '85; Rankin and Cook, '86; Stuermer, '88a,b). These findings were taken to indicate that axon growth is not goal-directed (Cook and Rankin, '86). Further experiments suggested that axon-axon and axon-tectum interactions mediated by activity play a decisive role for the assembly of the axons at their appropriate target sites (Schmidt and Edwards, '83; Schmidt, '85; Cook and Rankin, '86). When fish, during optic nerve regeneration, were reared in stroboscopic light, which causes excessive correlation in the firing pattern of neighbouring ganglion cells and their axons, the condensation of the terminal endings failed to occur (Cook and Rankin, '86). Likewise, when regenerating axons were silenced by repeated intraocular injections of TTX, the multiunit receptive fields, recorded electrophysiologically from the axon terminals in the tectum, were approximately three times larger than normal (Schmidt and Edwards, '83). These results could mean that activity-impaired axons maintained endings in incorrect positions at times when axons with normal impulse patterns had settled at their predesignated targets (Schmidt, '85). In contrast to these reports is a recent study in which TTX-silenced retinal axons were deflected into an ipsilateral tectum. Here they ultimately terminated in retinotopically appropriate areas very much like axons with normal activity (Meyer, '87).

The results mentioned above were obtained with electrophysiological or anatomical mapping techniques or with autoradiography to label the axons. These experiments relate the positions of the ganglion cells to their axon terminals in the tectum. However, they do not illuminate the morphology or pathways of individual axons. Directly visualizing the pathways and axonal arborizations could reveal structural correlates of the topographic targeting errors. In earlier reports, we have traced the trajectories of regenerating axons labeled with HRP (Stuermer, '88a,b; Busse and Stuermer, '87; Humphrey and Stuermer, '88). We have shown that the morphology of and pathways taken by regenerating axons change considerably between early and late regeneration stages.

Regenerating axons follow abnormal routes through the tectum. However, their course is not random, since most axons cross through or course into their retinotopically more appropriate tectal halves. On their growth through tectum, at early regeneration stages the axons place their endings, the growth cones, transiently at various sites. The number of these growth cones is high, since a substantial population of axons extends several branches into various regions of the tectum, and these branches are equipped with multiple growth cones and filopodia (Stuermer, '88b). These growth-related appendages are numerous at times when anatomical mapping experiments show a diffuse distribution of axon endings (Meyer et al., '85; Rankin and Cook, '86). At later regeneration stages when axonal endings were found with anatomical mapping techniques only at retinotopically appropriate sites in the tectum (Meyer et al., '85; Rankin and Cook, '86), the HRP-labeled axons were found to have lost most of their side branches and growth cones and to have formed terminal arbors at their target sites (Stuermer, '88a). Most of the earlier branches and growth-related appendages disappeared from ectopic tectal territories. The trajectories of the axons were then strikingly goal-directed in that the axons ran in target-directed routes towards their destination (Stuermer, '88a; Busse and Stuermer, '87). We provided evidence that misrouted axons can use two strategies to connect with their target. They appeared either to alter their course directly and continue to grow into the direction of the target or to select branches directed toward the target and loose the remaining branches (Fujisawa et al., '82; Stuermer, '88a,b).

Anatomical studies can thus clarify whether and to what extent the manoeuvers of the regenerating axons may depend on the maintenance of their normal impulse activity. We blocked impulse activity in axons with TTX and asked

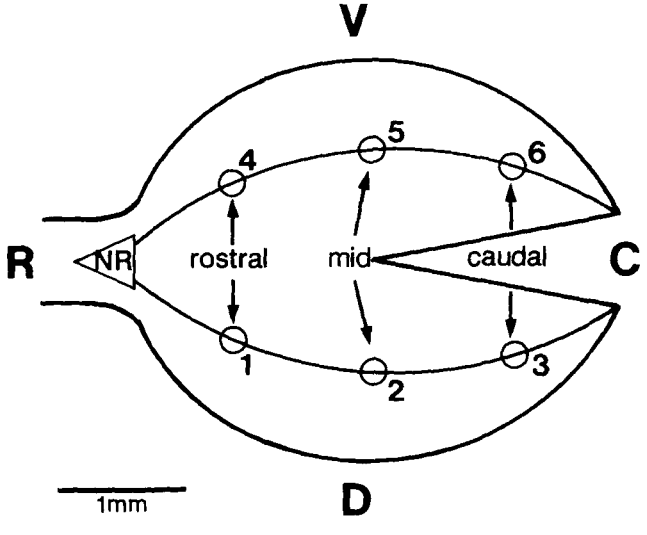


Fig. 1. Diagrammatic illustration of a tectum. The circles, numbered 1 to 6, give the areas over which the labeled axons were counted; 1, 2, 3 are the sites on the correct hemitectum (ventral for dorsotemporal axons, dorsal for ventrotemporal axons); 4, 5, 6 mark the sites on the incorrect hemitectum (dorsal for dorsotemporal, ventral for ventrotemporal axons). Abbreviations: R = rostral, V = ventral, D = dorsal, C = caudal, NR = nucleus rotundus.

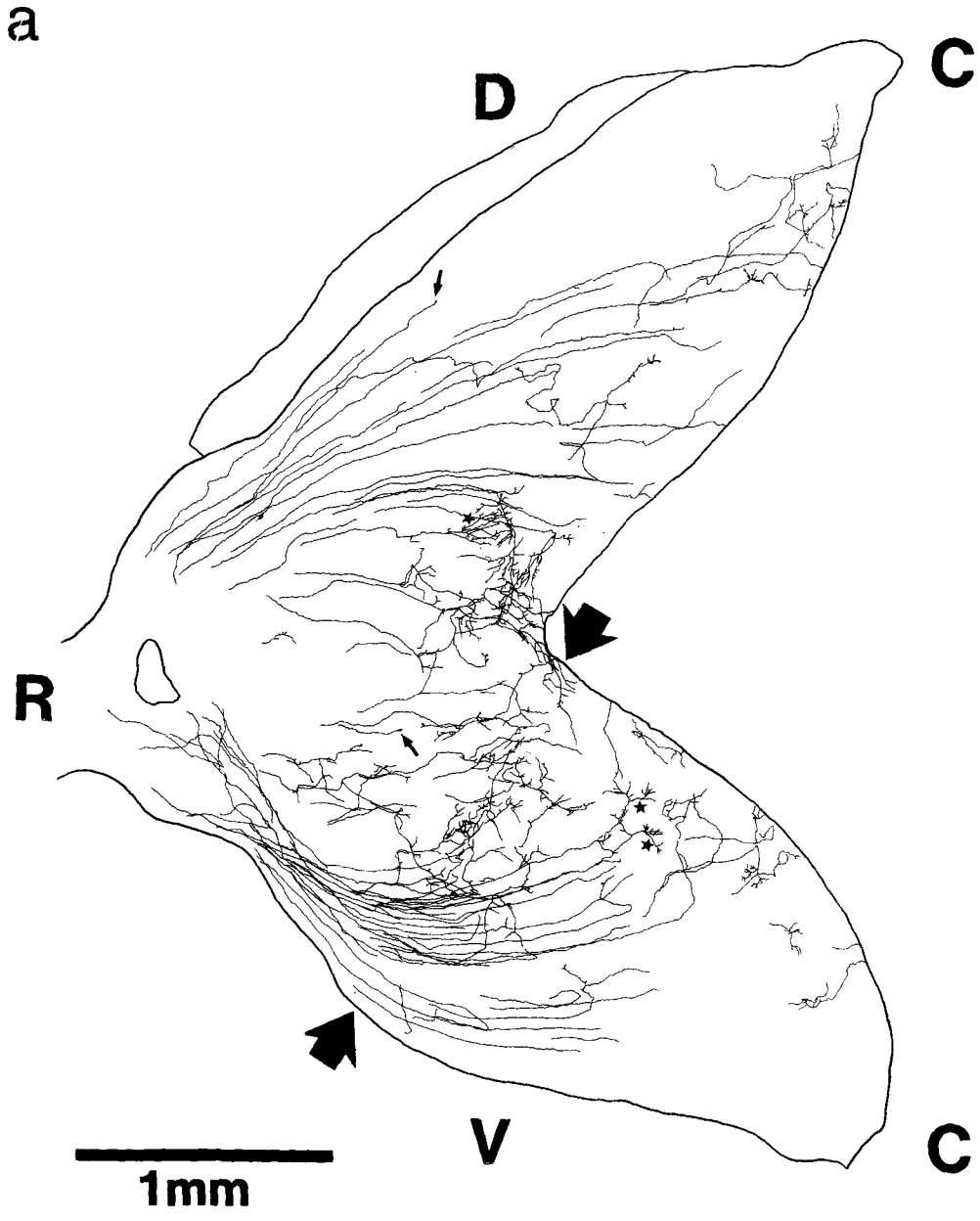


Fig. 2. (a) Camera lucida tracing of HRP-labeled regenerating axons at 24 days after optic nerve section originating from a dorsotemporal sector of the TTX-injected eye. From their rostral points of entrance the axons grew caudally along the path of the previous fascicles. Some exhibit growth cones at their tips (small arrows). Axons that have entered into the synaptic layers stratum fibrosum et griseum superficiale (SFGS, here and in subsequent figures) extend side branches with growth cones and filopodia (examples are marked by asterisks). The large arrows indicate the prospective termination region of the labeled axons. (b) Examples of branching axons depicted from (a). The branching pattern is highly variable, from moderate to highly branched. The highly branched axon marked by a small arrow is shown enlarged in Figure 4a. Large arrows as in (a). Abbreviations R, V, D, C as in Figure 1.

whether they fail to correct their course, whether they maintain their extra branches that normally would disappear with progress in regeneration time, and whether they deploy their terminal arbors at ectopic sites. We pursued these questions with an experimental procedure similar to that in our earlier investigations, labelling small groups of regenerating axons with HRP and tracing their path in tectal wholemounts. These tracings were performed both at early stages of regeneration, when active axons would still be on their way through the tectum, and at late regeneration stages, when axons normally connect to their retinotopic target sites and have formed their terminal arbors.

Part of these results were published in abstracts (Hartlieb and Stuermer, '87, '88).

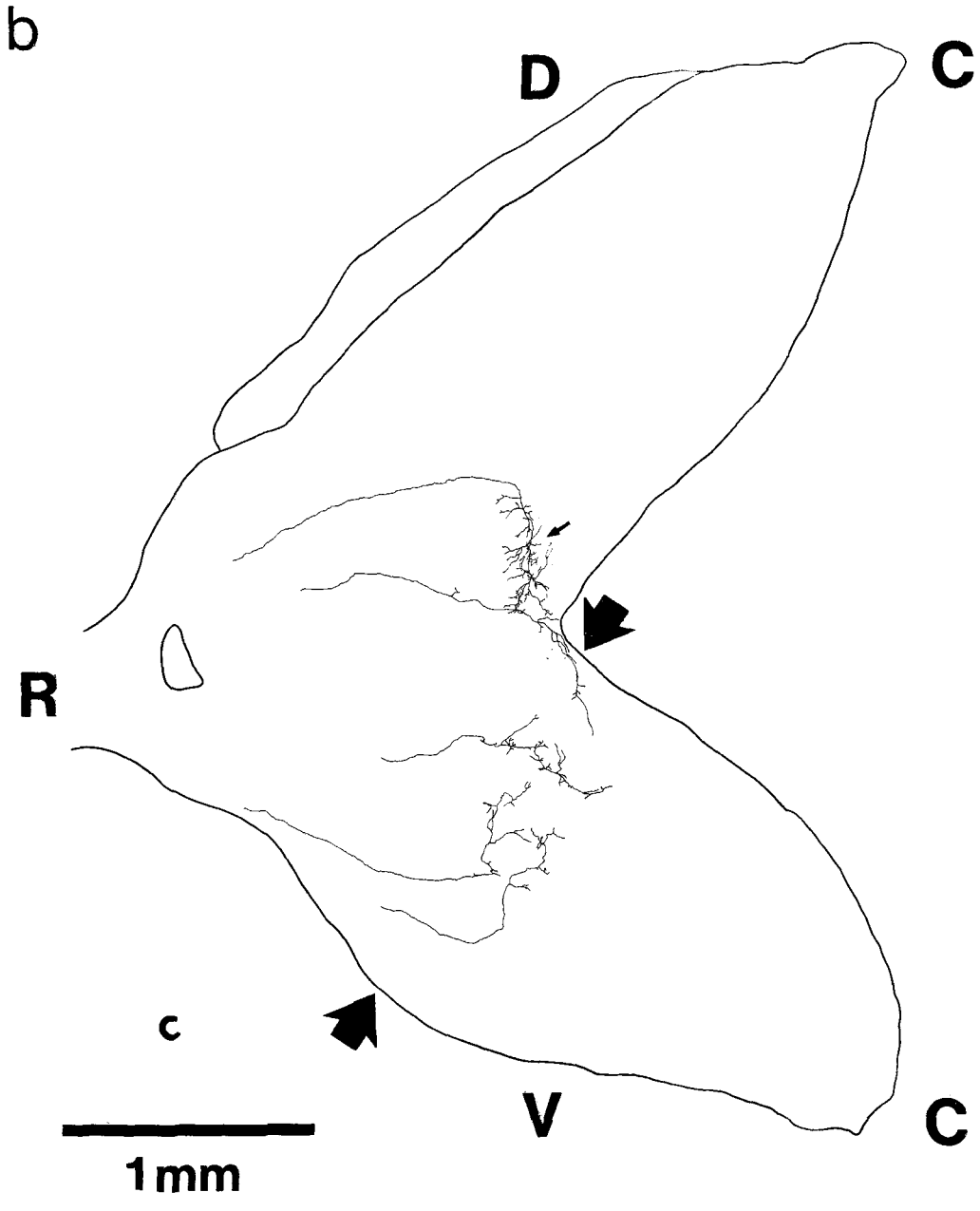


Figure 2

MATERIAL AND METHODS

The two optic nerves of goldfish (4–6 cm length) were cut intraorbitally with an iridectomy scissor under MS 222 (Sigma) anesthesia. Fish survived for various periods from 18 to 189 days after optic nerve section. At 2-day intervals beginning at the second day after optic nerve section, the right eye was injected with TTX (0.5 μl of 0.12 mM TTX in Ringer's solution), whereas the left (control) eye received injections of 0.5 μl Ringer. Seven μl of 0.1 mM TTX-solution is reported to block the impulse activity of normal and regenerating axons for at least 2–2.5 days in fish of 10–13 cm body length (Edwards and Grafstein, '83; Schmidt et al., '83). The blockade of impulse activity after intraocular TTX-injections was confirmed in our experiments by electrophysiological recordings from tecta of normal fish that had received TTX at regular 2-day intervals for 150 days. Whereas visually evoked potentials were reliably recorded from the visuorecipient layers on the right tectum, connected to the left (control) eye, no such potentials were elicited on the left tectum, connected to the right TTX-injected eye. Thus repeated TTX-injections over several months can still effectively block the axonal impulse activity, consistent with other reports (Schmidt and Edwards, '83; Boss and

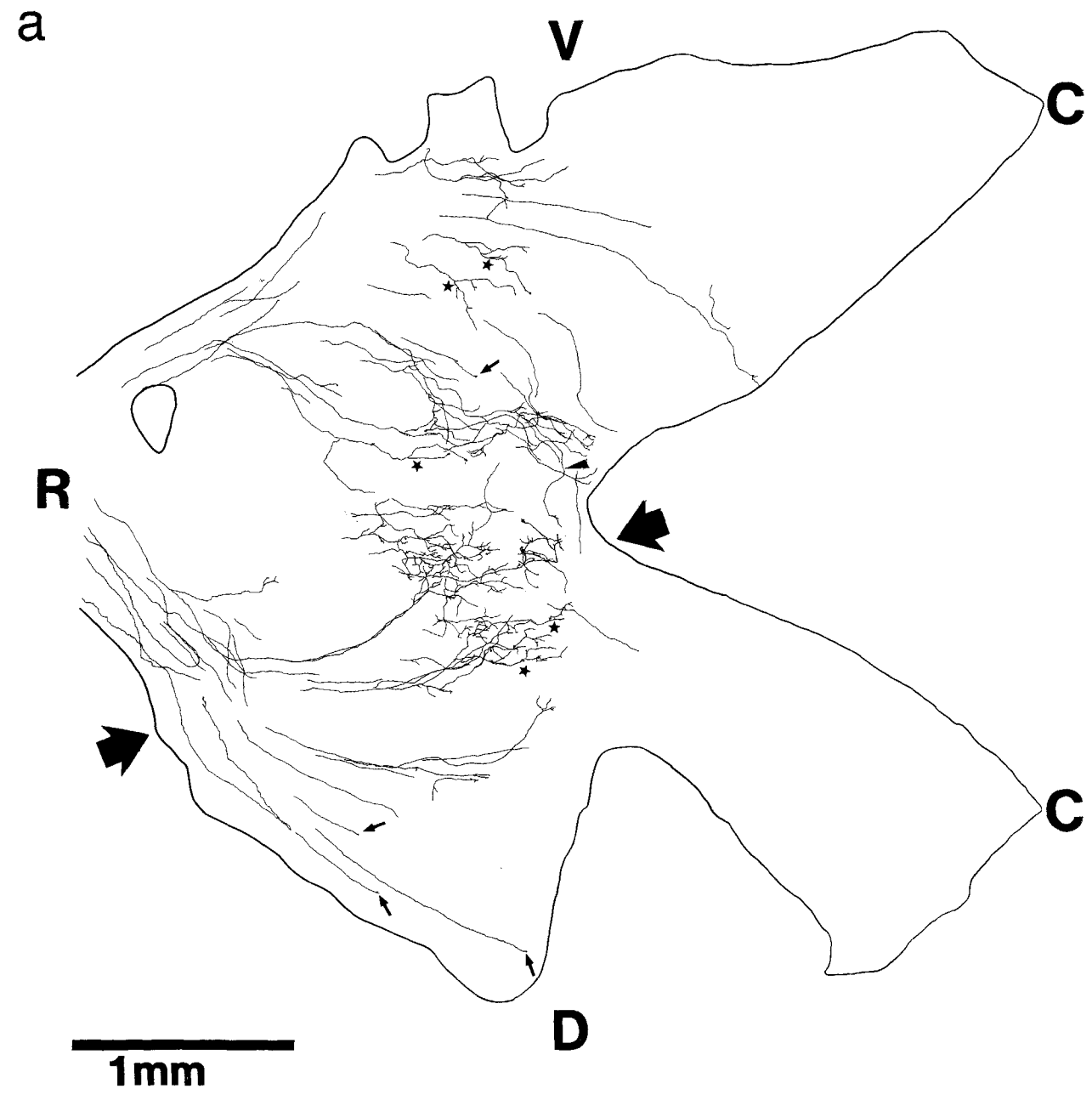


Fig. 3. (**a**) Tectal wholemount showing the path of axons, originating from a dorsotemporal sector of the Ringer-injected eye, at 24 days after optic nerve section. Similar to the axons from the TTX-injected eye axons travel in abnormal routes. They are tipped with a leading growth cone (small arrows) as long as they follow the path of the previous fascicles or they give rise to side branches (examples with asterisks) with growth cones and filopodia in SFGS. Four of the branching axons are illustrated separately in (b). (**b**) Examples of branching axons. Some produced large ramifications (asterisks) and branches carried growth cones (arrowheads), others bifurcated or were moderately branched. Large arrows in (a) and (b) indicate the prospective termination region of the labeled axons. Abbreviations R, V, D, C as in Figure 1.

Schmidt, '84; Meyer and Wolcott, '87; Olsson and Meyer, '87).

To label small groups of retinal axons with HRP and to visualize these axons in the tectum, we followed a protocol described earlier (Stuermer, '88a,b). In brief, HRP was applied intraretinally to severed axons in the dorso- or ventrotemporal retina of the TTX-injected and control eyes. Fish were perfused 4 to 5 days later with 0.75% saline through the heart. The left and right optic tecta were isolated and processed unfixed in diamino-benzidine (DAB) (5 mg DAB in 5 ml phosphate buffer with 130 μl of 10% H2O2) for 40 minutes, and then fixed in 4% glutaraldehyde. The tecta were then slit from the caudal pole to allow us to flatten them between a slide and a coverslip. Retinae were isolated from dark adapted fish, fixed for 20 minutes in 4% glutaraldehyde and incubated in 0'dianisidine (10 mg in 10

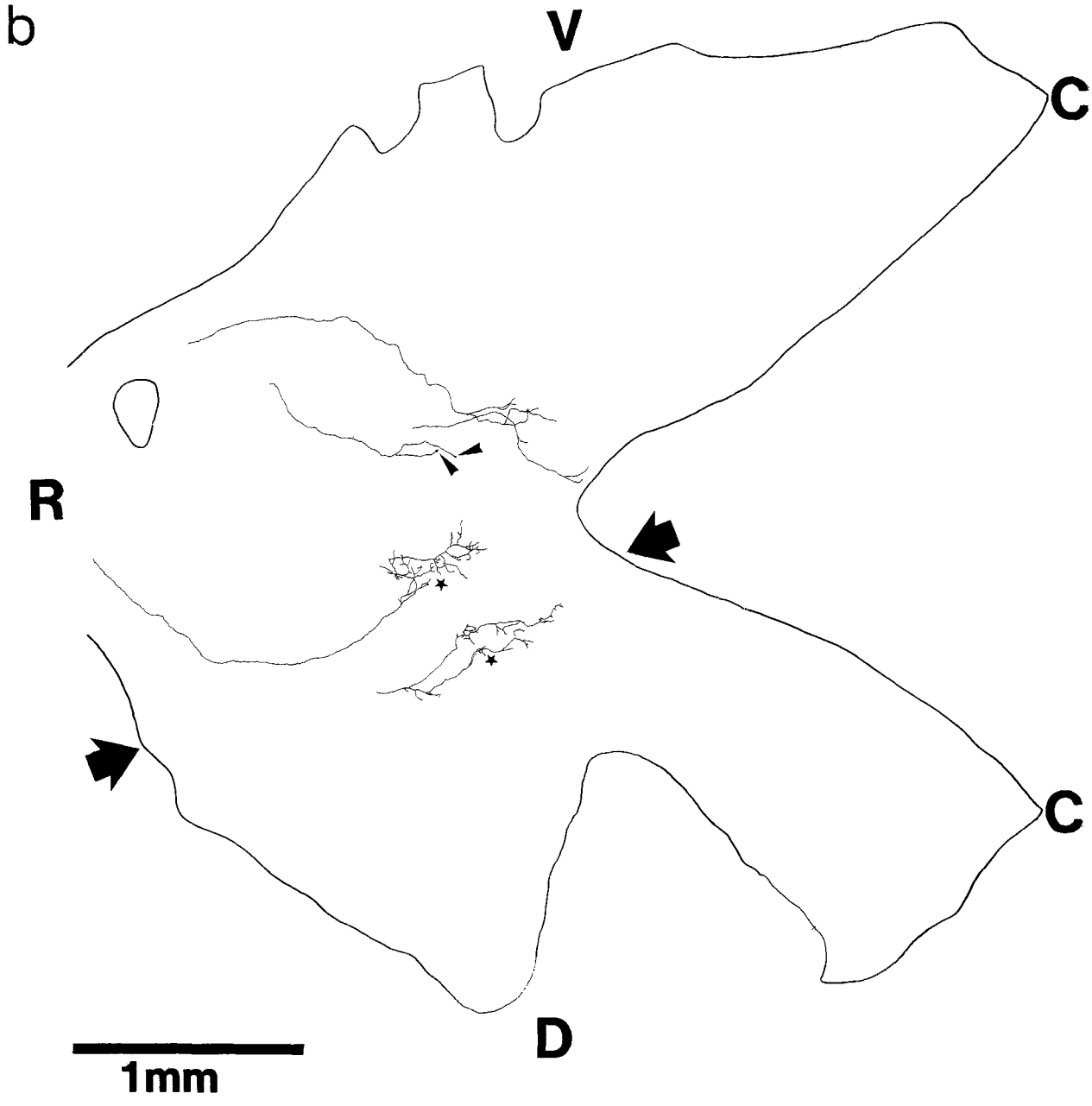


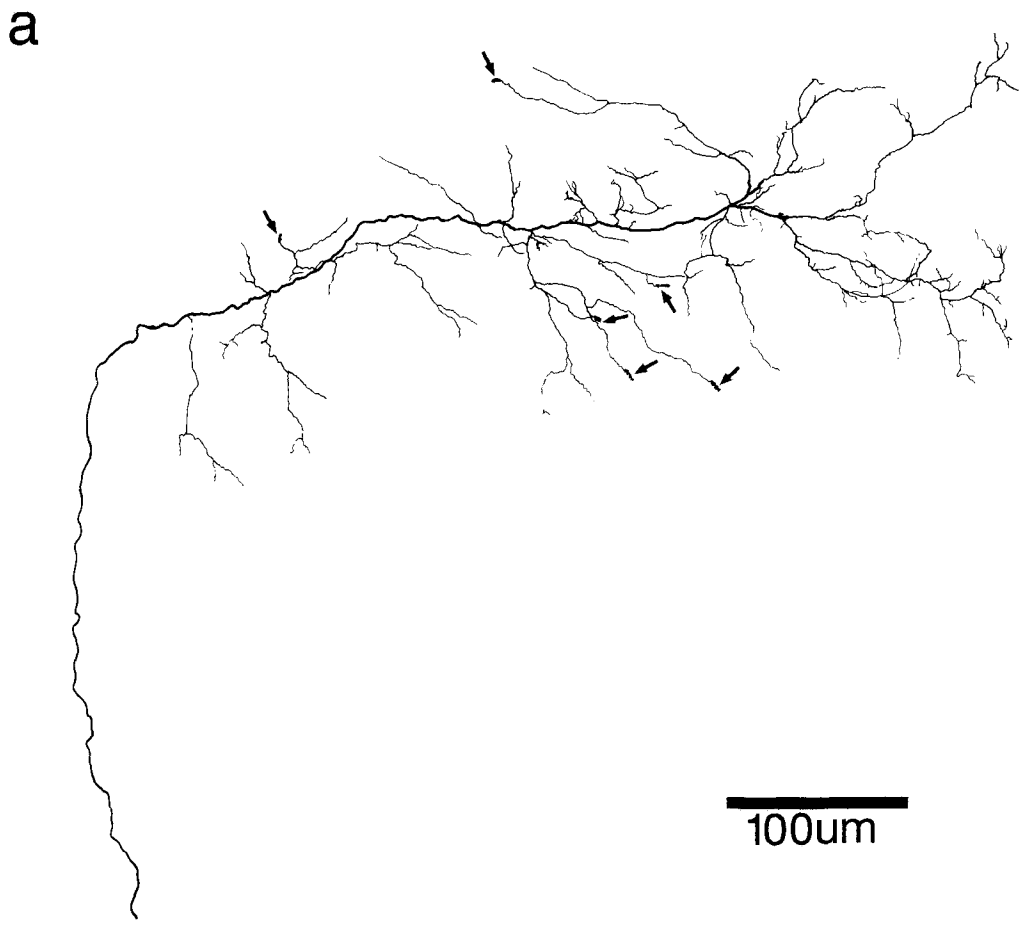
Figure 3

ml with 3 drops of 10% hydrogen peroxide added) for 20 minutes. Retinae and tecta were then placed on coated slides as wholemounts, dehydrated through a graded series of ethanol, cleared in Xylene, and embedded in Permount.

Microscopic inspections of the wholemounted retinae showed no apparent difference between the TTX-injected and control eyes with respect to the size, morphology, and number of the labeled ganglion cells, an observation that is consistent with previous reports (Edwards and Grafstein, '83; Meyer, '83; Boss and Schmidt, '84).

The axons anterogradely labeled with HRP were clearly visible under microscopic observation. In earlier studies (Humphrey and Stuermer, '88; Stuermer, '88a,b) we analysed the pathways and morphologies of retinal axons regenerating from eyes that had not been subjected to any injections prior to the HRP-application. Compared to those axons, the axons originating from the Ringer- or TTX-injected eye were no different in morphology and staining properties. These observations rule out the possibilities that TTX, or Ringer, or the repeated fluid injections caused damage to the retinal axons, altered their morphological characteristics (specified in Results), or impaired the transport (Edwards and Grafstein, '83; Antonian et al., '87) and/or diffusion of the HRP (Reh and Constantine-Paton, '85; Shatz and Stryker, '86).

Since the blood vessels in the tectum were stained after the DAB reaction, making photographic documentation difficult, the labeled axons were traced by means of a drawing



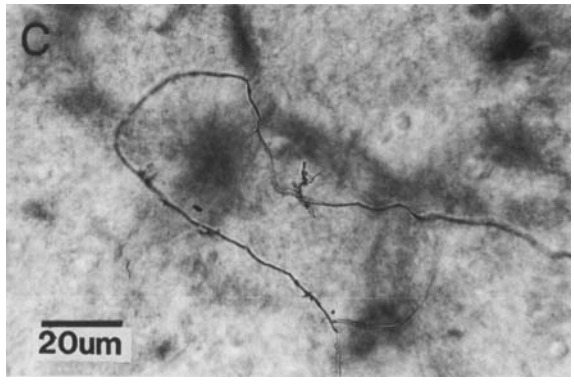


Fig. 4. (**a,b**) Two typical examples of the large ramifications that are abundant in the TTX (a) and control group (b) at early regeneration periods. Branches carry growth cones (arrows) and filopodia. The boxed region in (b) is shown in (c). (**c**) Photomicrograph of part of the ramification and one of its growth cones.

tube throughout most of their intratectal path. For documentation of the axonal trajectories, camera lucida tracings were made from observations with a ×25 oil immersion lens resulting in drawings of 2 × 2 m. For more detailed inspections of axons, ×40 oil, ×63 oil, and ×100 oil immersion lenses were used. In all camera lucida tracings the axons coursing at various levels of depth of the tectum were drawn in one plane.

The camera lucida tracings show all labeled axons and axonal segments that were visible under inspection with the ×25 oil immersion lens. Not all axons could be traced throughout their course through the tectum, even after further inspections with the ×63 and ×100 oil immersion lenses, since they often traveled in fascicles or crossed each other repeatedly or were in portions too weakly stained. Processes that were not readily assigned to one or another axon were drawn separately and omitted from drawings that depict isolated axon trajectories or arbors.

To determine the extent of the axonal ramifications and arbors, they were drawn and encircled by a line connecting the tips of the branches. As described in Stuermer ('84), the long and short axes were measured and plotted.

The regional distribution of the HRP-labeled axons over the tectum was quantified according to a procedure in Stuermer ('88a,b). The numbers of labeled axons were determined in 6 areas of 180 μm diameter (visual field pro-

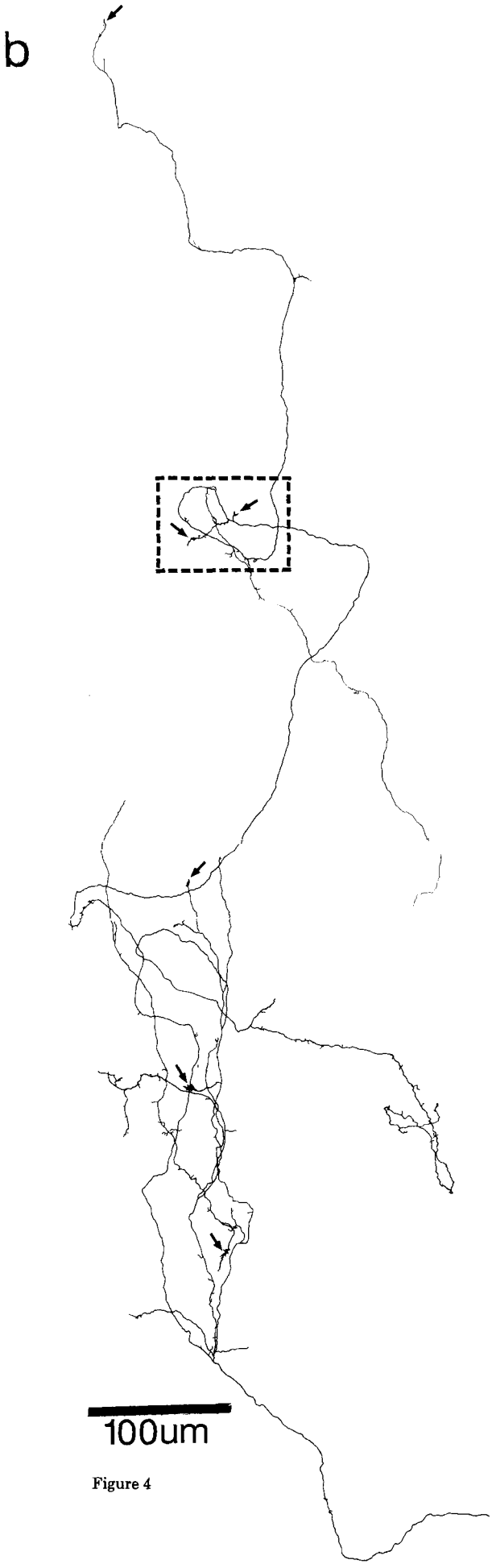


Figure 4

vided by the ×63 oil immersion lens) in the rostral, middle, and caudal tectum (Fig. 1). These areas lie on a line running rostrocaudally from the nucleus rotundus through the middle of the dorsal and ventral hemitectum to the caudal tectal ends. The numbers of axons at comparable sites in different tecta were pooled. The relative numbers of axons over these sample areas were plotted in a diagram.

Measurements of areas (the sector of labeled ganglion cells in retina, the cluster sizes, and the surfaces of retinae and tecta) were made from camera lucida tracings with the aid of a digitizing tablet (Kontron).

RESULTS

When HRP was applied to the radially oriented axons near the optic disk in the retina, it retrogradely labeled the severed axons and their parent ganglion cells in a sector extending from the site of application to the retinal periphery. The sector of labeled ganglion cells comprised an area of 2,2 to 6% of the retinal surface. The axons from this sector were anterogradely labeled in the optic nerve, tract, and optic tectum. In both TTX-injected and Ringer-injected control eyes, HRP-application sites were in the ventro- or dorsotemporal retina. In normal fish the retinotopic termination site of the labeled axons from these sectors is a wedge-shaped region in the dorsorostral or ventrorostral tectum, respectively (Stuermer, '88a). Regenerating axons ultimately return to their retinotopic region, but at early regeneration stages their growing endings are widely distributed over tectum (Stuermer, '88a,b). However, even then the prospective termination sites of the labeled axons can be predicted, since we know their retinal origin.

Tecta prior to day 18 after optic nerve section contained either a very few axons, which had just entered into tectum, or none. In tecta at 24 days, however, labeled axons were more numerous, and they had progressed into the caudal tectum and had developed their typical morphological characteristics (Stuermer, '88b). To determine whether TTX-induced impulse blockade would affect the morphology of the regenerating axons and their pathfinding strategies, we compared the trajectories of the regenerating axons from TTX-injected eyes to those from Ringer-injected (control) eyes and to those of uninjected eyes (preparations of our earlier study, Stuermer, '88a,b). The trajectories of regenerating TTX-silenced axons were traced in 40 tecta at survival periods ranging from 24 to 189 days, and those from the Ringer-injected eye in 40 tecta of similar survival periods.

Pathways and morphologies of TTX-silenced axons at regeneration periods of 24 to 80 days

As typical for regenerating axons (Stuermer, '88a,b), TTX-silenced as well as control axons from dorsotemporal (or ventrotemporal) retina entered into tectum through both the appropriate ventral (dorsal) and the inappropriate dorsal (ventral) brachia of the optic tract (Hartlieb and Stuermer, '87). Once in the tectum they took various abnormal routes, which are exemplified on two typical whole-mounts in Figure 2a, 3a. The camera lucida tracing in Figure 2a illustrates the trajectories of TTX-silenced dorsotemporal axons and Figure 3a shows ventrotemporal axons from a Ringer-injected eye, both at 24 days after optic nerve section. The axons coursed through all regions of tectum. They traveled through the appropriate ventral (Fig. 2), or dorsal

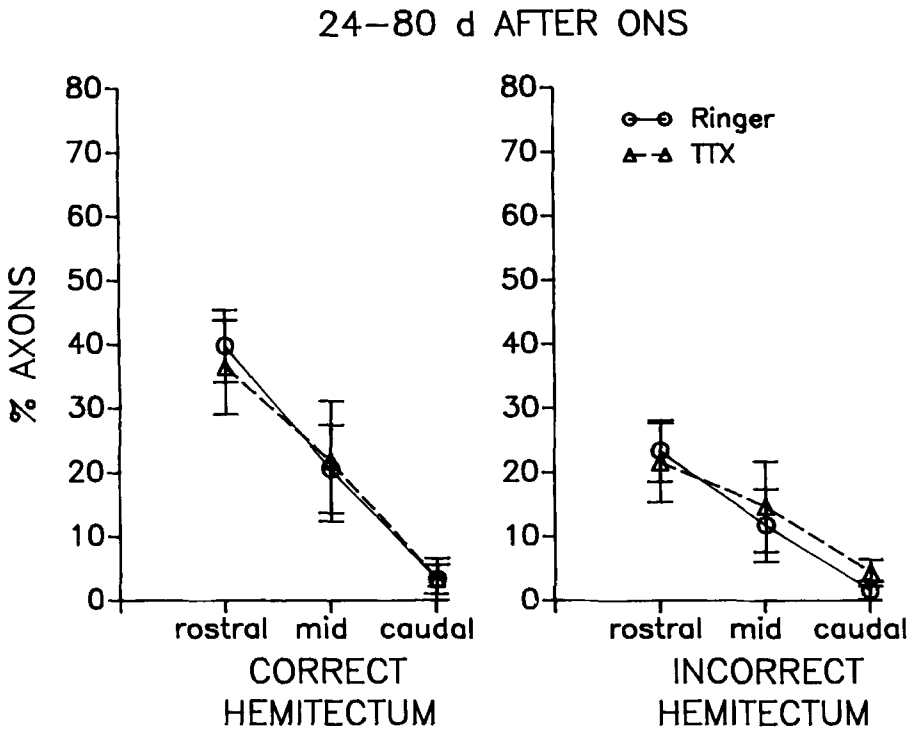


Fig. 5. These graphs illustrate the distribution of labeled dorso- and ventrotemporal axons over the 6 sample sites (defined in Fig. 1) in the correct and incorrect hemitectum at 24–80 days after optic nerve section (ONS). The vertical bars give the values of the standard deviation. Note that the distribution of the TTX-injected eye resembles closely to that of the axons from the Ringer-injected eye. Both show a decline of the relative axon distribution from rostral over midtectal to caudal tectal regions.

(Fig. 3), and inappropriate dorsal, or ventral, hemitectum, and erred into caudal tectal territories. The TTX-silenced axons and the axons from the control eye exhibited morphological characteristics typical of regenerating axons at this early regeneration period (Stuermer, '88b). From their rostral points of entrance, the axons traveled caudally for various distances following the curved paths of the previous fascicles in Stratum opticum (SO) (Stuermer and Easter, '84). Here they were always unbranched and carried a leading growth cone at their tip (Fig. 2). Axons that had entered into the synaptic layers Stratum fibrosum et griseum superficiale (SFGS) were either tipped with one or several, usually large, growth cones and exhibited numerous filopodial sprouts or gave rise to several side branches (Figs. 2a,b, 3a,b) oriented in various directions. These side branches were studded with an abundance of growth cones and filopodia (Fig. 4a,b,c). These ramifications formed by the branching axons resided in all regions of tectum and subtended over retinotopically appropriate and inappropriate tectal territories. Most, however, were seen in the rostral tectum. Five of such ramifications of axons from the TTX-injected eyes and four of axons from the Ringer-injected eye were depicted from Figures 2a, 3a, and are shown separately in Figures 2b, 3b, respectively.

To determine whether these early axonal ramifications may be larger or smaller when the regenerating axons were silenced, the extreme tips of the branches were circumscribed and the long and short axis was measured (Stuermer, '84, '88c). Thirty-nine of such ramifications of TTX-silenced axons from 8 tecta that were stained into their finest processes were drawn. They had dimensions ranging from 67 × 89 to 536 × 1084 μm with a mean and its standard deviation of 219 (±127) × 456 (±234) μm. The ramifications of the control axons (N = 35 from 10 tecta) had long and short axes ranging from 95 × 96 to 642 × 1327 μm, with a mean of 179 (±118) × 403 (±240) μm. The mean of the ramifications of the TTX-blocked axons was larger than that of the control axons, but this difference was statistically insignificant (Kolmogoroff-Smirnoff-test). These results suggest that TTX does not inhibit the extension of side branches nor does it influence the size of the ramifications.

Particularly at rostral and midtectal levels, individual misrouted axons from TTX- and Ringer-injected eyes exhibited striking bends or turns (Figs. 2, 3) that occurred when axons had left the fascicle path and entered into the synaptic layers. Thereupon most of the axons or the main axis of the ramification appeared oriented in the direction of the retinotopic target region (Figs. 2, 3, 4a).

Our earlier investigation demonstrated that temporal axons course and ramify preferentially over the rostral half tectum, whereas nasal axons exhibit a preference for caudal tectum (Stuermer, '88a,b). Whether TTX-silenced temporal axons possess a similar preference for rostral tectum was assessed by quantifying the relative number of axons over 6 representative areas in tectum. The location of the areas was defined in Methods (Fig. 1) and in Stuermer, '88a,b. Labeled TTX-silenced axons crossing through these areas were counted in 12 tecta between 24 and 80 days after optic nerve section (24–30d (N = 3), 31–40d (N = 3), 55–65d (N = 2), 70–75d (N = 2), 80d (N = 2)). The counts at each

TABLE 1. Distribution (in %) of Regenerating TTX-Blocked and Control Axons from Temporal Retina over Tectum: Early Regeneration Stages

	Correct hemitectum			Incorrect hemitectum		
Region	1	2	3	4	5	6
TTX	36.4 (7.4)[1]	21.7 (9.4)	3.3 (2.3)	21.5 (6.2)	14.5 (7.1)	4.3 (2.1)
Ringer	39.7 (5.7)	20.5 (6.9)	3.9 (3.3)	23.3 (4.8)	11.7 (5.7)	1.5 (1.5)

[1]Standard deviation in brackets.

area were pooled. The same quantification procedure was performed for axons from the control eye in 10 tecta of regeneration periods of 27 to 80 days (27d (N = 1), 31–40d (N = 4), 55–65d (N = 2), 70–75d (N = 2), 80d (N = 1)). The relative distribution of the TTX-silenced axons and the axons from Ringer-injected eyes are listed in Table 1 and are illustrated in the graphs in Figure 5. On the appropriate hemitectum (ventral for dorsotemporal and dorsal for ventrotemporal axons) TTX-silenced axons were most numerous in area 1, rostral to the retinotopic termination region. The axon number decreased slightly in the midtectal region 2 and dropped in the caudal region 3 (Table 1). On the inappropriate dorsal (or ventral) hemitectum axons declined at rostral region 4 over midtectal region 5 to caudal region 6. The relative distribution of temporal axons of Ringer-injected eyes showed a similar decline from rostral to caudal sites of tectum (compare Table 1). Thus axons from temporal retina whether originating from TTX- or Ringer-injected eyes were more numerous over their retinotopically appropriate rostral half of tectum. This differential distribution of the regenerating TTX-blocked and control axons is comparable to that shown in our earlier report (Stuermer, '88a,b) and suggests that activity is not required for its expression.

We have shown earlier that the morphology of the regenerating axons and their pathways change with progress in regeneration time (Stuermer, '88a,b). Here we asked whether axons need their impulse activity to undergo these changes. The analysis of tecta at late regeneration stages—described in the following section—suggests that they do not. The axonal morphology and pathways that were manifested at the late regeneration stages appear to evolve gradually over time. This was apparent from inspection of tecta between 24 and 80 days after optic nerve section. The following description applies to both TTX-silenced and control axons. Between 24 and 40 days after optic nerve section, the number of labeled axons in the tectum increased. With progress in regeneration time, many more misrouted axons exhibited bends in their path and had come closer to or had arrived at their retinotopic target region. But even in tecta at 80 days, we found axons at ectopic sites that were lead by growth cones or that maintained branches with growth cones and filopodia. However, their number appeared to be smaller than at 24–40 days. In 3 tecta at 80 days (1 with TTX-silenced axons, 2 with control axons), a few terminal arbors of mature appearance and confined to retinotopic regions were apparent. These terminal arbors, as well as those at 120–189 days, differed from the large ramifications in that all branches of the terminal arbors emerged over the distal end of the axons. The terminal arbor branches of these mature arbors never reached dimensions like those of the ramifying axons (see below) and they lacked growth cones and filopodia. The preterminal parts of the axons were bare of side branches except for an occasional short side sprout on some axons. These sprouts may be remnants of the earlier long side branches.

The pathways of TTX-silenced axons between 120 and 189 days after optic nerve section

Figures 6 and 8 illustrate dorsotemporal axons from a TTX- and a Ringer-injected eye, at 150 and 181 days after optic nerve section, respectively. The vast majority of the axons in both tecta ran towards their retinotopic target region and ended in terminal arbors (Fig. 7). The terminal arbors lay clustered together and their processes formed a dense meshwork.

A few terminal arbors, particularly those in the deeper strata of the synaptic layer SFGS and at the margin of the clusters, were distinct (Figs. 6a,b, 8a,b). This allowed us to draw them and to determine their extent. We examined whether terminal arbors of TTX-silenced axons were larger than those from the control axons, since this was found in the frog (Reh and Constantine-Paton, '85) and was proposed to apply to fish (Schmidt and Edwards, '83). The long and short axes of 69 arbors of TTX-injected eyes and of 66 arbors of Ringer-injected eyes were measured. The arbors' long and short axes from the TTX-treated axons ranged from 41 × 65 μm to 269 × 389 μm, mean and standard deviation: 106 (±49) × 162 (±64) μm. Those of control axons had values from 37 × 66 to 315 × 428 μm, mean and standard deviation: 105 (±49) × 171 (±82) μm. Thus arbors in both groups fell into similar size ranges and were of near normal dimensions (Stuermer, '84). The morphology of most arbors was closely related to that of arbors of normal axons (Stuermer, '84) (Fig. 9a,b,d,e). However, a few axons of Ringer- and TTX-injected eyes gave rise to arbors that were sparsely branched and of unusual shape (Fig. 9c,f). We did not find arbors of small sizes, which typically had dimensions of 34 × 52 μm in normal fish (Stuermer, '84) either in the control or in the tectum with TTX-blocked regenerating axons. This may not be surprising, since such small arbors are less distinct, and they might have easily escaped our observation in the dense meshwork of arbor processes. These data show that the terminal arbors of TTX-silenced axons are no larger than those of axons with normal activity. This finding is consistent with recent results of J.T. Schmidt (personal communication).

The labeled arbors from TTX-injected eyes always formed a continuous cluster, such as the one in Figure 6 extending from rostral peripheral regions to the tectal center. The arbors of axons from Ringer-injected eyes were arranged in a continuous cluster in two-thirds of all tecta. In the other third, however, the arbors were condensed into separate patches, as shown in Figure 8. Such patch formation by axons with normal activity but not by TTX-blocked axons was observed in earlier studies (Meyer et al., '85; Rankin and Cook, '86; Stuermer, '88a). Since they were never observed in tecta innervated by TTX-blocked axons, their formation must require neural activity (Olsson and Meyer, '87).

Anatomical mapping experiments have shown that the tectal projection of axons that regenerated under TTX-blockade remained diffuse (Meyer, '83b). Electrophysiological recordings from the tectum have shown that the multiunit receptive fields (MURFs) of axons that regenerated under TTX-blockade were roughly 2.5 to 3 times larger than the MURFs of axons without impairment of their activity (Schmidt and Edwards, '83). We therefore expected that the terminal arbors would be distributed over larger tectal territories than those of active axons. However, the terminal

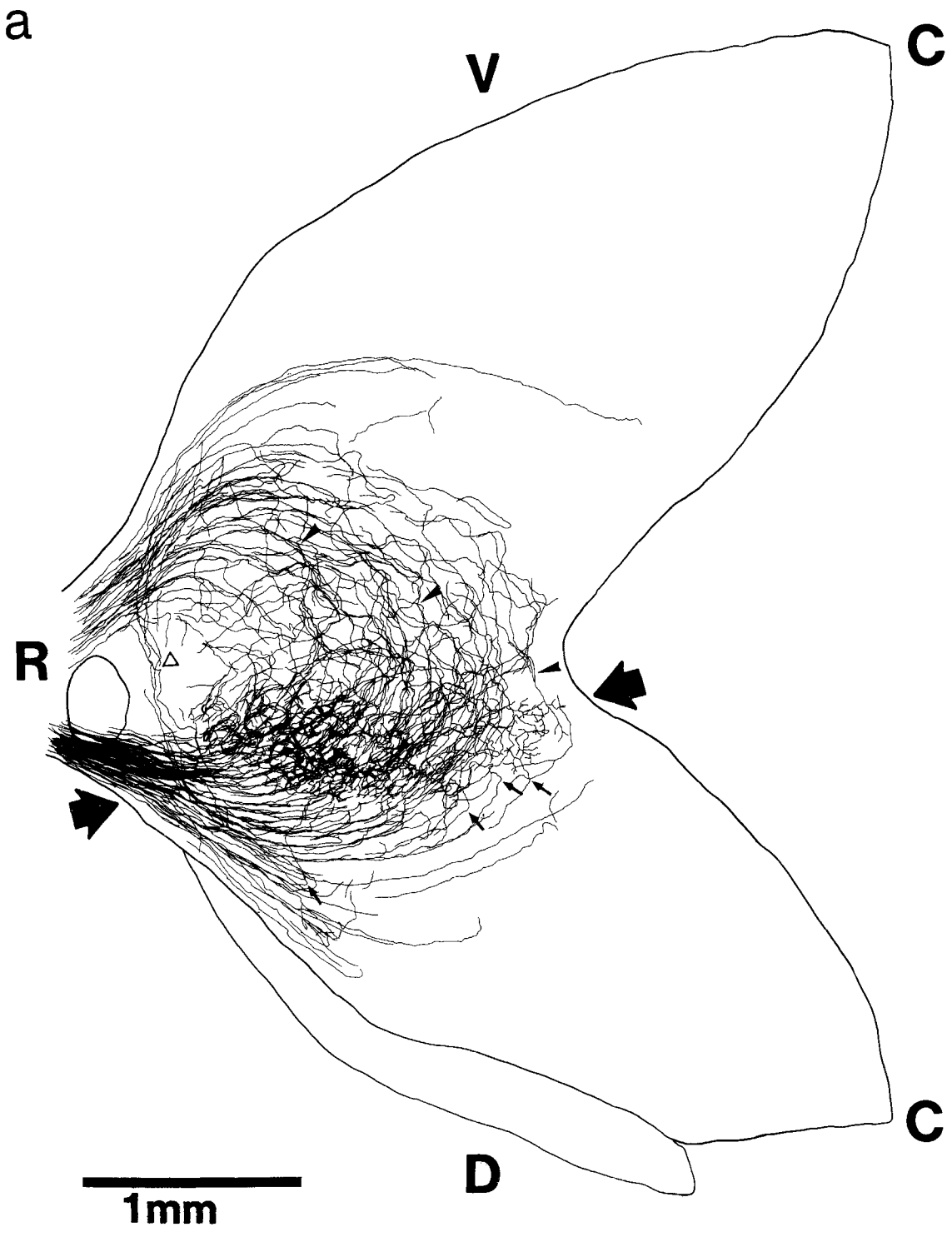


Fig. 6. (**a**) Regenerating axons from a ventrotemporal sector in the TTX-injected eye at 152 days after optic nerve section. The axons end in terminal arbors that are clustered at retinotopic regions (large arrows). Misrouted axons from the ventral hemitectum travel toward their retinotopic target region in curved path across the tectal equator (examples with arrowheads). The triangle points to axons that bend shortly after their entrance into the inappropriate ventral hemitectum to course into the dorsal tectal half. Axons that have grown past the target region exhibit right angle turns or gradual bends (small arrows) to return to the target. Similar to the control axons (Fig. 8a), the TTX-blocked axons had disappeared from caudal tectum. (**b**) Examples of axons from (a) that grow over abnormal but target-directed routes and merge into the terminal arbor cluster (dotted line). Some arbors were clearly visible when they lay in isolation (arrowhead). Abbreviations: R, V, D, C in a,b) as in Figure 1.

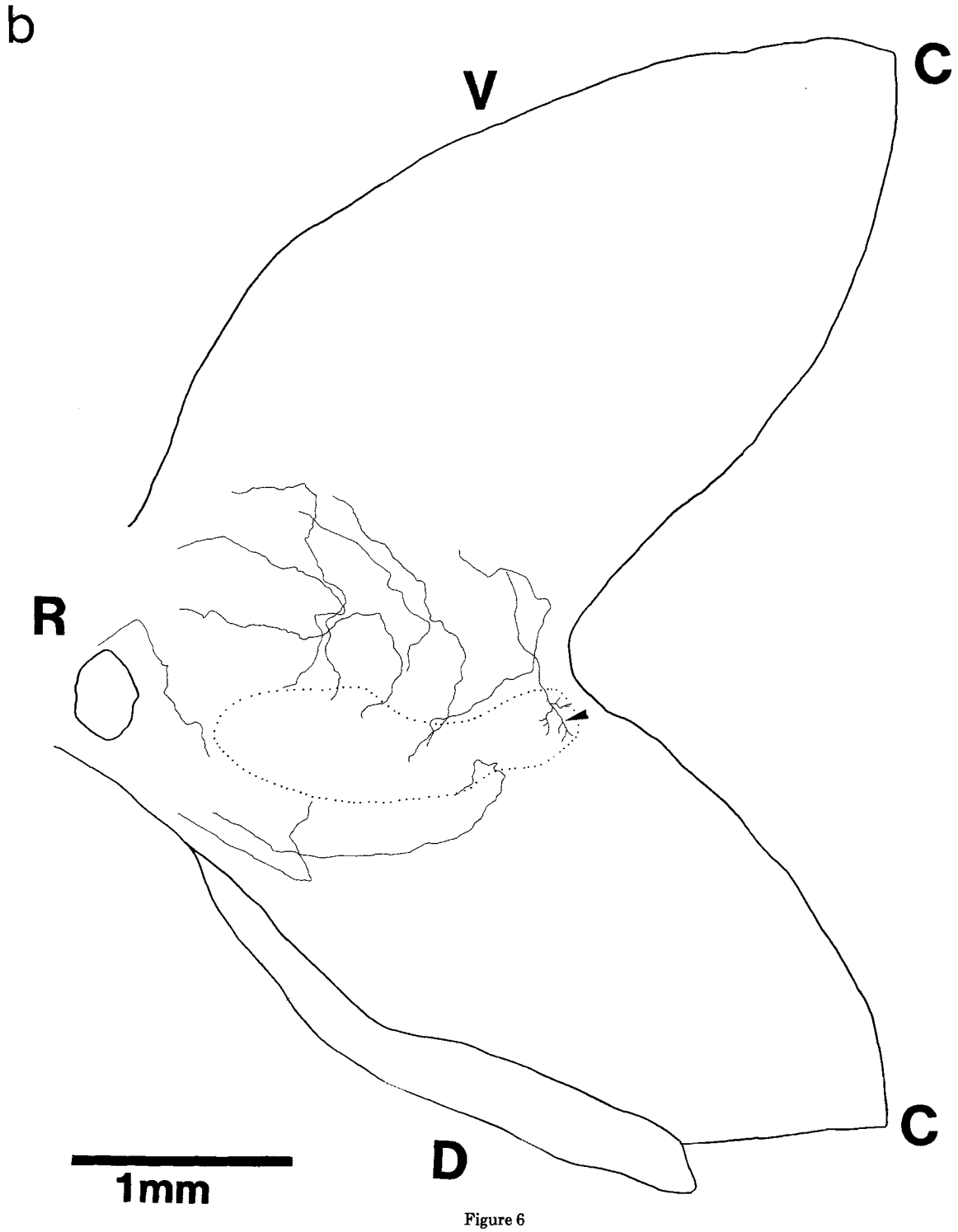


Figure 6

arbors of the TTX-blocked axons were confined to regions that were only slightly larger than the terminal arbor areas of the active axons. To achieve an estimate of the percentage of the tectal surface occupied by the terminal arbors, the cluster and patches, respectively, were encircled and the area determined. The meshwork of the terminal arbors was quite distinct and could readily be distinguished from its surround (Fig. 10). The area of the individual clusters of the TTX-group and the sum of the areas of the patches or the clusters of the control group were determined for each group in 6 tecta at 150–184 days and set in relation to the sector of the labeled ganglion cells in the retina. In the TTX-group, the retinal sectors ranged from 2.4 to 5.9% of the retinal surface with a mean of 3.5%. The tectal clusters covered 2.2 to

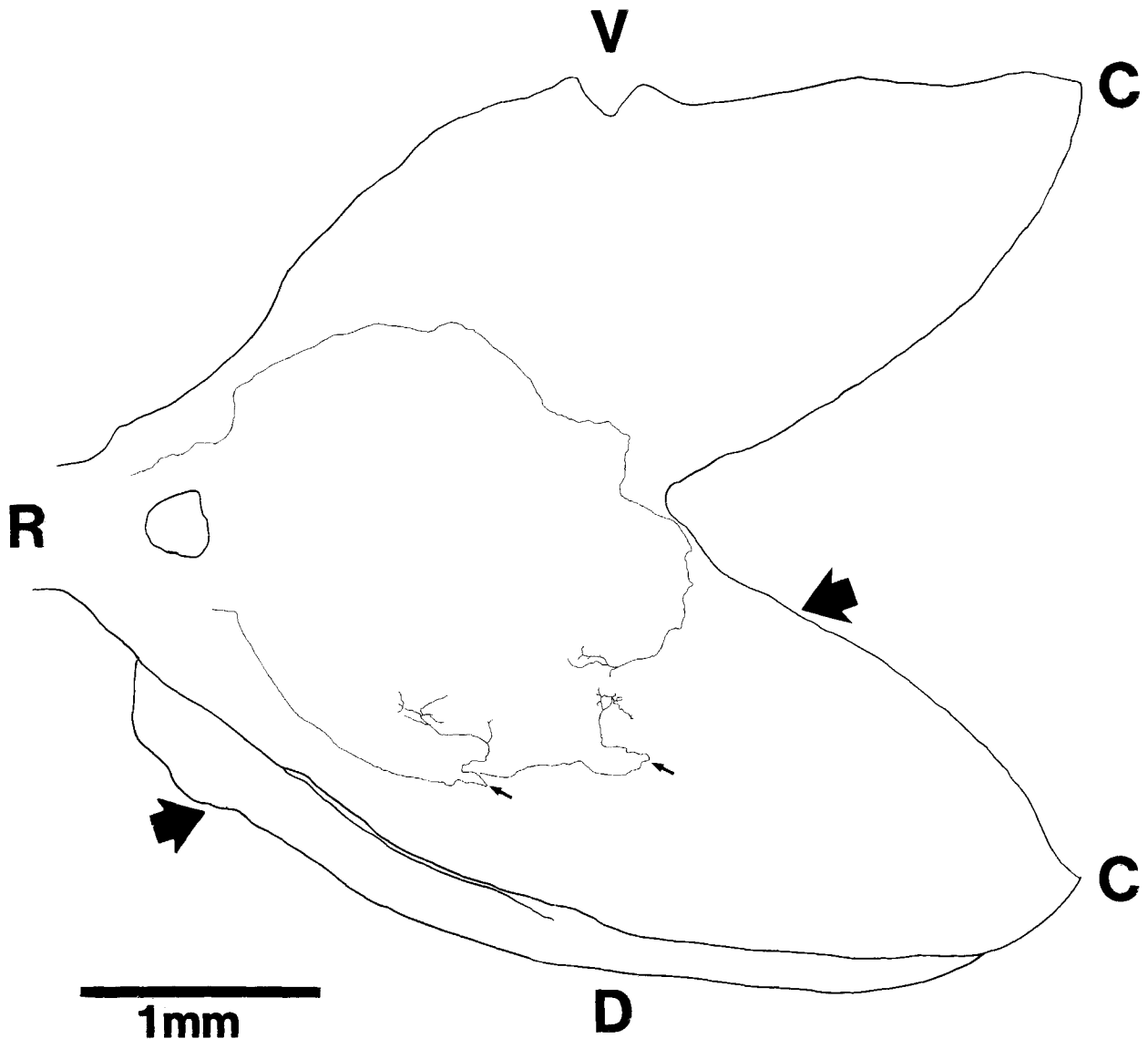


Fig. 7. Individual TTX-blocked ventrotemporal axons at 122 days after optic nerve section that have reached their retinotopic target region (large arrows) in abnormal routes. The axon that has entered through the incorrect ventral brachium of the optic tract and coursed over the ventral hemitectum, bent to cross the tectal equator and to approach the retinotopic target region (between large arrows) where it arborized. The axons traveling in abnormal routes over the correct dorsal hemitectum exhibit abrupt course alterations (small arrows) turn and arborize at their target region. Abbreviations R, C, D, V as in Figure 1.

3.9% of the tectal surface with a mean of 2.95%. The retinal sectors of the Ringer-injected eyes ranged from 2.2 to 6% (mean: 3.7%) and the tectal clusters from 1.9 to 3.4% (mean: 2.7%). Thus the means of both retinal and tectal sectors in the TTX-group was within 10% of those means in the control group. This difference is small when compared to the 2.5 to 3 times-enlargement of the terminal fields found with electrophysiological recordings (Schmidt and Edwards, '83).

The axons, whether from TTX- or Ringer-injected eyes, that ended in terminal arbors were bare of growth cones and filopodia and did not generally show branches proximal to the terminal arbor (Figs. 6, 7, 8). The large ramifications typical of early regeneration stages were absent. Activity therefore is obviously not needed for the removal of the side branches and the formation of terminal arbors over retinotopically correct regions. The axons exhibited strikingly target-directed orientations. Among the axons that coursed over the inappropriate dorsal hemitectum, a few were seen to bend in sharp right angles medially shortly after their entrance into the tectum (Figs. 6a,b, 8a,b). Most axons first grew caudally for various distances, then curved medially and crossed the tectal equator, the boundary between the ventral and dorsal hemitectum (Stuermer and Easter, '84)

and then merged into the terminal arbor cluster (Figs. 6a,b, 7, 8a,b). Axons that had grown past the retinotopic target region were observed to turn, abruptly or more gradually, and to approach the retinotopic region in rostrally directed routes (Figs. 6a,b, 7, 8a,b). These axonal routes suggest that the misrouted axons had undergone course corrections that could either have happened directly or after the exploration of the tectal territories with side branches and the selection of the appropriately oriented branches (Fujisawa et al., '82; Stuermer, '88a,b).

The comparison of Figures 6 and 8 shows that the pathways established by TTX-silenced axons (Fig. 6) were qualitatively indistinguishable from axonal routes of axons regenerating with normal impulse activity (Fig. 8; Stuermer '88a,b). This was substantiated by an analysis of an additional 17 tecta connected to Ringer-injected eyes and 17 tecta connected to TTX-injected eyes. These findings, therefore, suggest that impulse activity is not needed for regenerating axons to undergo morphological changes during their path through tectum, to approach their target and ultimately to terminate in a terminal arbor cluster of near normal dimensions at retinotopically appropriate sites.

The formation of target-directed routes and the deployment of the terminal arbors was paralled by a disappearance of ectopic axonal ramifications and by a reduction of axons in the caudal tectum. This is reflected in the quantification of the axonal processes over rostral, midtectal, and caudal tectal regions that was performed as described above (Table 2). The proportion of the axons over the 6 tectal sample regions is illustrated in the graph in Figure 11. The number of axons from TTX-injected eyes over regions 1 to 6 were pooled from 8 tecta between 120 and 184 days after optic nerve section, (120–125d (N = 3), 150–155d (N = 2), 180–185d (N = 3)) (Table 2). The retinotopically appropriate hemitectum, area 1, rostral to the retinotopic target site, contained by far the most axons. The number of axons fell in the midtectal region 2 and further in the caudal region 3. On the inappropriate tectal half, the number of axons in region 4 was less than half of that in area 1, and declined over midtectal region 5 to 0 in caudal region 6. The regional distribution of temporal axons from Ringer-injected eyes was determined from 6 tecta of 120–184 days after optic nerve section (122d (N = 1), 150–155d (N = 2), 180–184d (N = 3)). The decline of axonal numbers from rostral to caudal regions of tectum was nearly identical to that of TTX-silenced axons (compare Table 2). These data show that the preferential accumulation of the regenerating temporal axons over the rostral tectum and the disappearance of these axons from ectopic caudal tectal regions becomes more pronounced with progress in regeneration time. This applies to both TTX-silenced and active axons and suggests that the dynamical changes of the axonal trajectories and morphology that underlie this reorganization are activity independent.

DISCUSSION

The foregoing experiments showed that TTX-induced axonal impulse blockade has only a minor effect on the way in which regenerating retinal axons in goldfish gain access to their retinotopic target regions in the tectum. During their growth through the tectum the regenerating retinal axons undergo a variety of morphological changes. The axons course in abnormal routes and extend branches with growth cones into various directions of the tectum. With progress in time the ectopic side branches are lost. The axons establish target-oriented routes and deploy their terminal arbors over retinotopic regions. The same morphological changes, target-oriented routes, and the assembly of terminal arbors over well-defined retinotopic regions are exhibited by axons that regenerate under TTX-induced impulse blockade. The sole difference between TTX-treated and control axons is that regenerating control axons form patches in many cases, whereas only continuous clusters were seen in TTX-treated axons. These observations, therefore, suggest that Na+-channel-dependent activity is not required for axonal path- and home-finding.

Technical considerations

The validity of this conclusion rests on the notion that intraocular injections of TTX successfully abolish the Na+-channel-dependent impulse activity of the retinal axons. The volume and the concentration of the TTX-solution that we used to silence retinal axons was adapted from earlier publications, which had successfully demonstrated the effects of the TTX-induced impulse blockade on the retinotectal projection in goldfish (Meyer, '83b; Boss and Schmidt, '84). The effectiveness of the TTX-solution in blocking the spike activity of the retinal axons was verified by electrophysiological recordings in normal fish at 1 and 2 days after the injection and in fish that had received repeated injections over 150 days. Together with evidences of earlier studies that showed that TTX blocked the activity of the axons even when the injections were repeated over 6 months (Boss and Schmidt, '84; Meyer and Wolcott, '87; Olsson and Meyer, '87), we can rule out the possibility that the ganglion cells and their axons might have lost their sensitivity to the toxin. It was technically not possible to test whether each of our repeated TTX-injections had always blocked all Na+-channel-dependent activity or whether low levels of activity might occasionally have arisen. This criticism, however, applies not only to this but to all other studies in fish. The observation that the axons from the TTX-injected eyes never formed the patches of terminal arbors that axons from the Ringer-injected eye did strongly suggests that the TTX-injections were effective. Further support comes from two other studies, which used periodic injections of TTX over long time periods and which showed that the segregation of axons in dually innervated tecta failed to develop (Meyer, 1982; Boss and Schmidt, '84). Injections of subthreshold doses of TTX, however, did not prevent the segregation (Meyer, '82).

Since we aimed at solving whether axons regenerating under TTX-induced impulse blockade differ in their morphology or in their mode of approaching the retinotopic target region from axons with normal impulse activity we labeled as in previous studies (Humphrey and Stuermer, '88; Stuermer, '88a,b) the axons of defined sectors in the retina. This procedure gives axons that are sufficiently labeled to trace them throughout most of the path, and axons that can only be seen as fragments. Both were included in the Camera lucida drawings. For illustrations of individual axons or terminal arbors, however, only those were drawn that appeared labeled into their finest processes and that were distinct from other axons. Still, unless HRP is injected into individual cells or axons, the possibility always remains that two tightly joined axons are mistaken as one

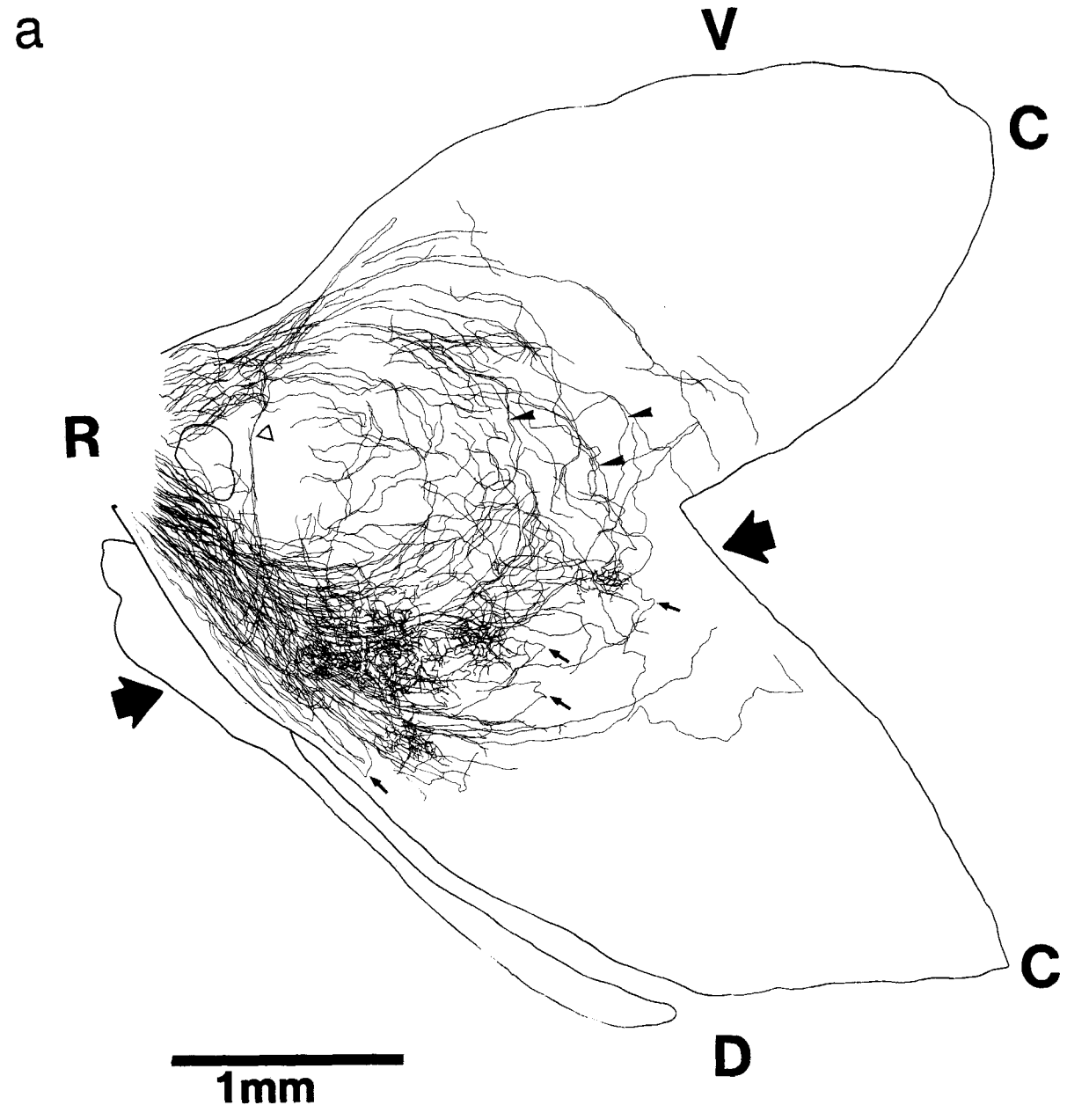
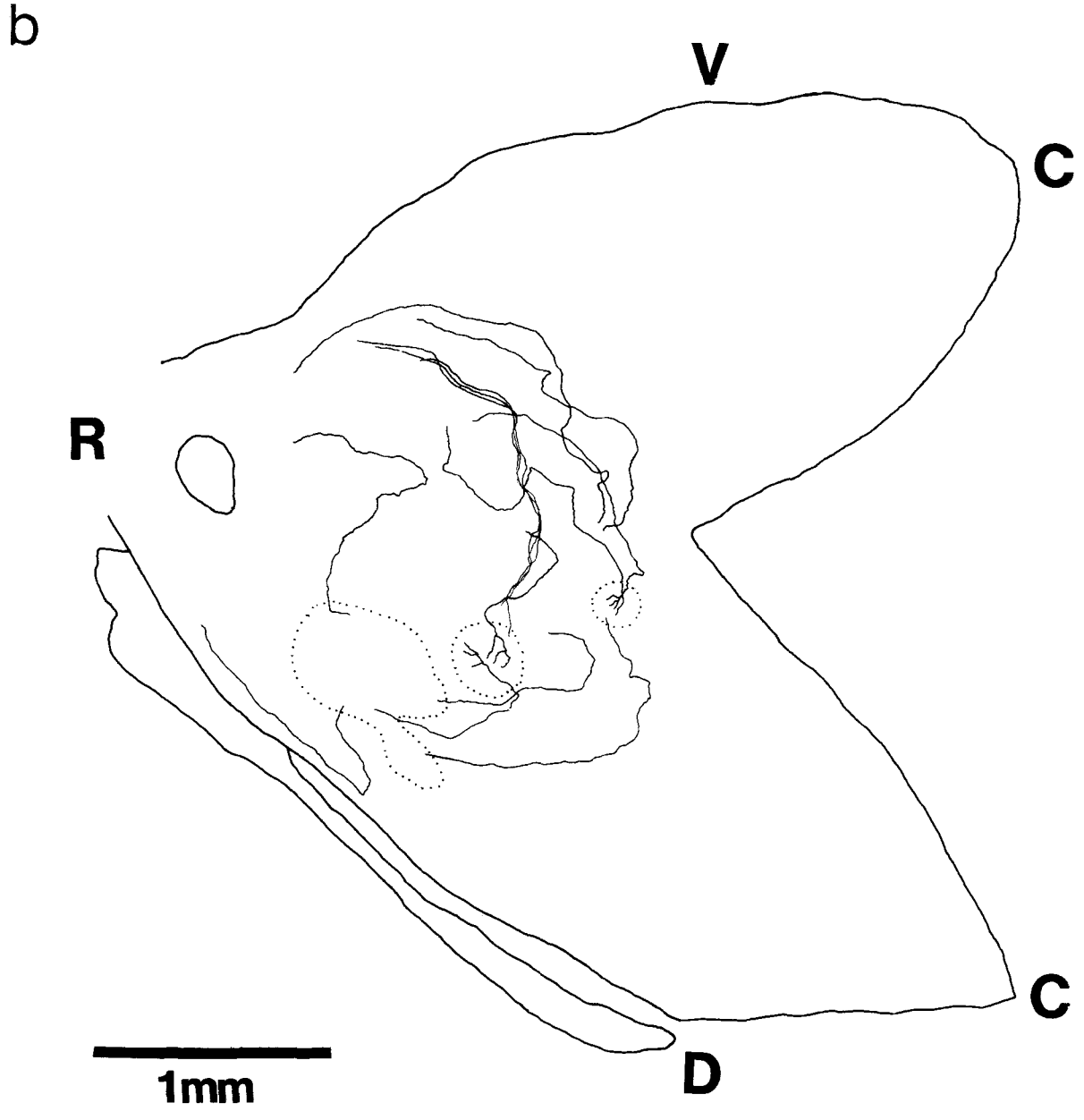


Fig. 8. (**a**) Tectal wholemount with regenerating axons originating from a ventrotemporal sector of the Ringer-injected eye at 181 days after optic nerve section. The axons terminate at or close to their retinotopic target region (large arrows) in 3 separate patches. The axons coursing over the inappropriate ventral hemitectum exhibit right angle turns (triangle) or strikingly curved paths (arrowheads). They cross the tectal equator at various levels to travel toward and to terminate at their retinotopic target region. Some axons on the dorsal hemitectum that had passed the level of their target are seen to return into the direction of the target (small arrows). Examples of the course correcting axons are shown separately in (b). (**b**) Examples of course correcting axons depicted from (a), which travel toward or merge into the terminal arbor patches (dotted lines). In some instances the terminal arbors are clearly visible as the ones in the smaller patches. Abbreviations R, V, D, C in (a,b) as in Figure 1.

axon, or that certain processes are falsely assigned to an axon or that processes are not recognized to belong to an axon. These mistakes, however, affect both groups of regenerating axons considered here to the same extent. These technical limitations are not unique to this study but apply to studies that used similar techniques as well. The axonal trajectories observed here were strikingly similar (1) to those seen in earlier studies (Humphrey and Stuermer, '88; Stuermer, '88a,b) and (2) to those illustrated in a recent publication by Schmidt et al. ('88) and to those in an investigation (Busse and Stuermer, '87), in which only a very small number of axons were labeled and traced individually.



Comparison with results and implications of related studies

The conclusion that regenerating retinal axons manage to seek out their targets despite TTX-induced impulse blockade is in agreement with earlier studies (Meyer, '83b; Schmidt and Edwards, '83). These studies showed that TTX-silenced axons restore a gross retinotopic order and therefore implied that TTX does not interfere with the navigation of the regenerating axons to their target. Our documentation of the axonal pathways has added new information. They reveal the complexity of the path- and target-finding strategies and show that the deprivation of Na+-channel-dependent activity does not interfere with axonal manoeuvering. Moreover, the visualization of the axonal pathways also enabled us to evaluate the possibilities offered to account for the lack of map refinement under TTX-blockade. It has been shown with anatomical mapping techniques that the tectal projection formed by regenerating axons is initially diffuse and only matures to near-normal precision after several months (Meyer et al., '85; Rankin and Cook, '86). The structural basis of this diffuseness is most likely the axonal branches and growth cones, which are spread widely over the tectum at early regenerating stages (Stuermer, '88b) and which disappear with time (Stuermer, '88a,b). Electrophysiological and anatomical mapping experiments showed that the projection established by activity-deprived axons appeared less precisely organized and

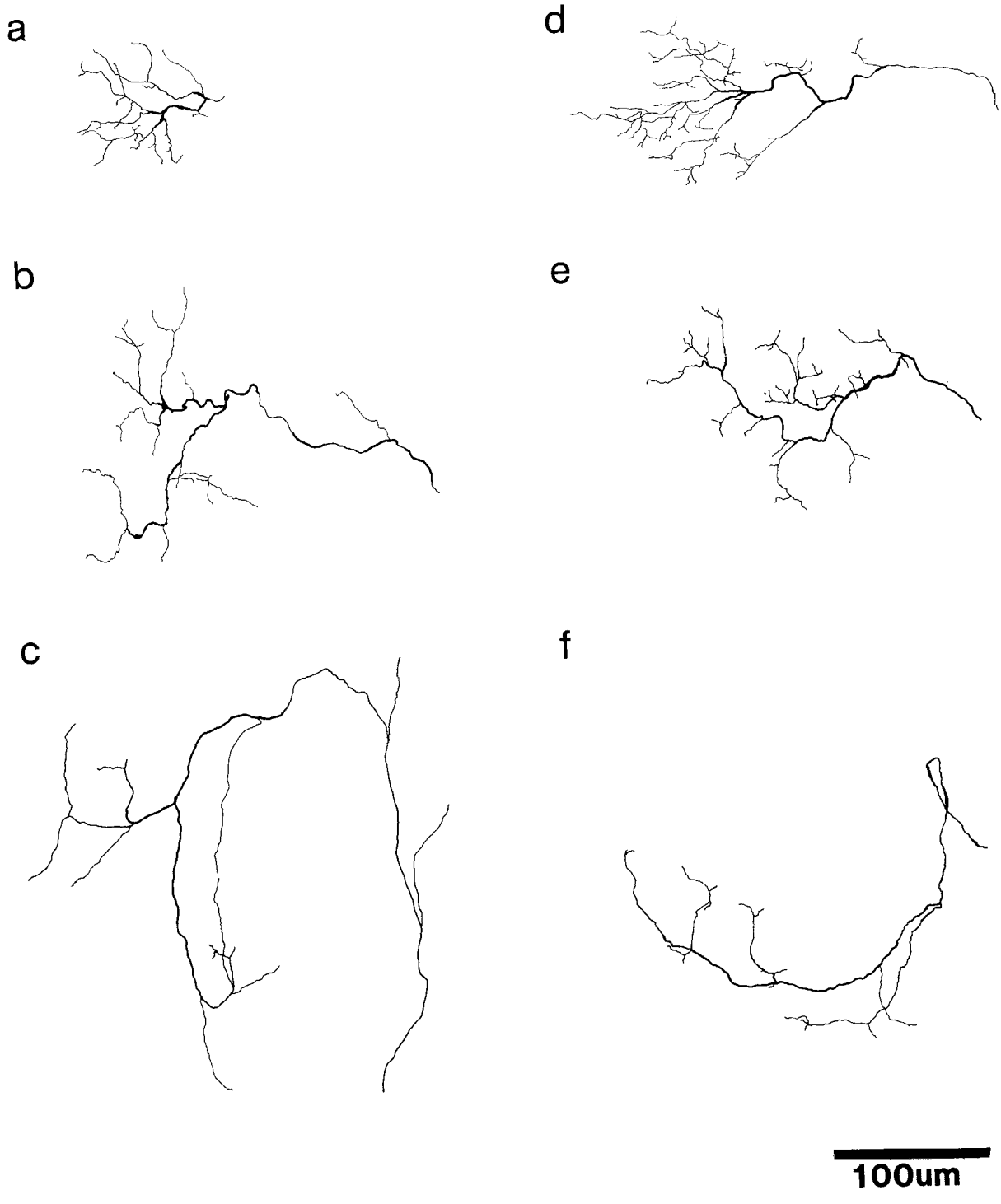


Fig. 9. Examples of terminal arbors from retinotopic regions at 80 to 181 days after optic nerve section. (**a,b,c**) Arbors of TTX-silenced axons: (a) 80 days, (b) 94 days, (c) 181 days after optic nerve section. (**d,e,f**) Arbors of control axons: (d) 94 days, (e) 184 days, (f) 181 days after optic nerve section; (c and f) show arbors that are abnormal in morphology and sparsely branched.

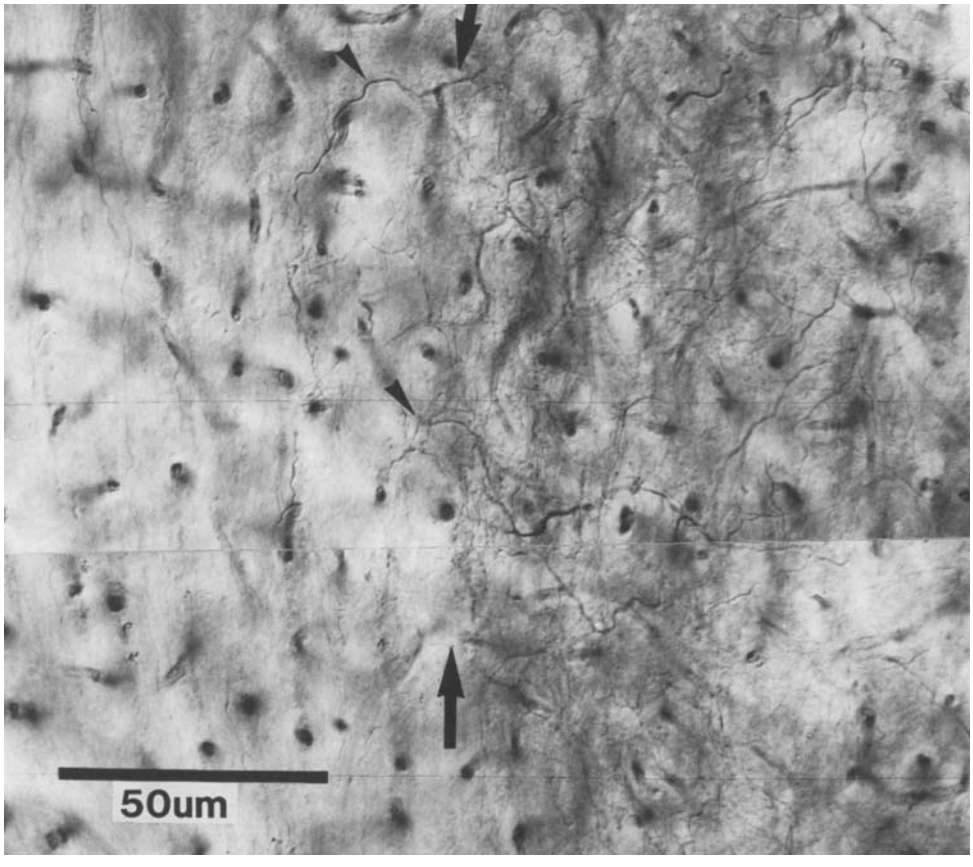


Fig. 10. Photomicrograph of the border (arrows) of a terminal arbor cluster (to the right). Two axons merging into the cluster are to the left (arrowheads).

does not achieve the precision that is reached when the tectum is innervated by normally active axons (Meyer, '83b; Schmidt and Edwards, '83). The explanations that have been proposed to account for the failure of map refinement under TTX were: first, that TTX-silenced axons might maintain ectopic branches, which are lost from normal axons; second, that terminal arbors may be larger although appropriately positioned; or third, that terminal arbors are normal-size but inappropriately positioned (Meyer, '83b; Schmidt and Edwards, '86).

Maintenance of ectopic branches?

The idea that activity may be important for the abolition of ectopic branches was supported by findings in other systems. In the kitten lateral geniculate nucleus, the embryonic retinal axons maintain or even increase their side branches over inappropriate layers when the axons are silenced by TTX (Sretavan et al., '87). Outside the CNS, such as in neuromuscular connections, the elimination of polyneural innervation requires pre- and postsynaptic activity (Thompson, '85). The current results on the fish retinal axons, however, revealed that ectopic branches are extended and then retracted by TTX-silenced axons just as they are by normally active axons. This rules out the possibility that the imprecision of the map originates from a persistence of branches that would normally disappear. The side branches on the fish regenerating axons may subserve a special function. As proposed for Xenopus (Fujisawa et al., '82) they may be extended randomly and repeatedly to explore the tectal territories widely. Their exploratory role is suggested by their multitudes of growth cones and filopodia. Branches that encounter more favourable environments than others (possibly those oriented towards the target) may be elongated, whereas the other branches are lost (Stuermer, '88a,b). Thus it appears most likely that these transient branches are involved in and needed for axonal pathfinding during regeneration. Our present results indicate that their production and elimination is not regulated by Na+-channel-mediated activity of the axons.

Larger arbors?

That terminal arbors of TTX-silenced axons were larger than those of active axons was supported by such observations in the frog (Reh and Constantine-Paton, '85). However, terminal arbors in fish, whether originating from activity-deprived or active axons were of normal sizes, as demonstrated by our measurements of the terminal arbors. Similar results were obtained by Schmidt (personal communication) in his recent anatomical studies. In none of the tecta—whether connected to TTX- or Ringer-injected eyes—did we see the very small arbors that have been found in the upper strata of SFGS in normal fish (Stuermer, '84). In his recent report on terminal arbors developing after optic nerve section, Schmidt et al. ('88) were also unable to

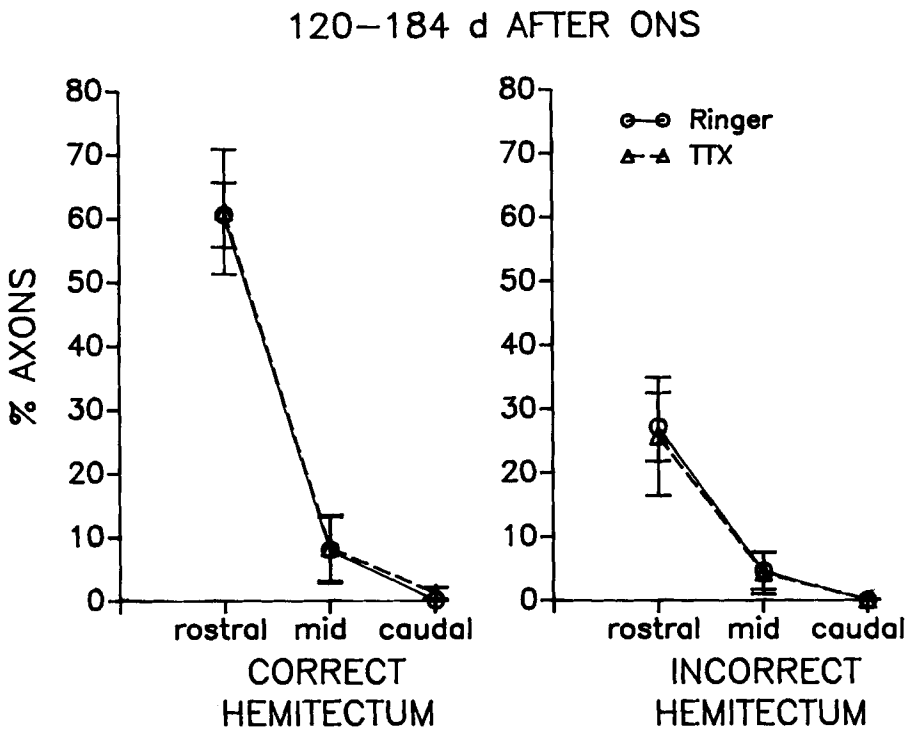


Fig. 11. Graphs to illustrate the distribution of the dorso-(ventro-)temporal axons over the 6 sample sites in tectum (defined in Fig. 1) at 120 to 184 days after optic nerve section (ONS). The vertical bars indicate the standard deviation. The distribution of the TTX-silenced axons over tectum parallels that of the axons from the Ringer-injected eye. Both show a drastic decline of axon numbers from rostral over mid-tectal to caudal tectal regions (compare with Fig. 5).

find these arbors. In normal fish we found these arbors to arise from small caliber axons and to be always weakly stained. These arbors could easily have escaped our observation here since we drew (for practical reasons) our attention only to those arbors in the cluster that were heavily labeled and distinct enough to trace them into their finest processes. However, we cannot exclude the possibility that regenerating axons may fail to form such small-sized arbors. If so, then this should apply to both active and activity-blocked axons since we discovered them neither in the TTX- nor in the control group.

Misplaced arbors and topographic refinement

The remaining alternative, that terminal arbors of TTX-silenced axons are inappropriately positioned, can neither be fully supported nor rejected by our experiments. However, our findings do allow us to place an upper bound on the degree of this hypothetical arbor displacement. Our findings suggest that the arbors cannot be extremely far from their optimal location, since axons from a small sector in retina are accumulated in a well-defined cluster over the retinotopically appropriate region. This differs to the distribution of radioactively labeled axon processes in tectum reported by Meyer ('83b). Following an ablation of a sector of retina 90 days after optic nerve section, a distinct label-free sector appeared in the tecta innervated by active axons but not in tecta innervated by TTX-silenced axons, suggesting that the projection established by TTX-silenced axons was nearly as diffuse as at early regeneration stages in the control group. Based on this report we had expected that the areas subtended by TTX arbors should be considerably larger than the areas occupied by active axons. The comparison of the terminal arbor fields of the TTX-silenced and active axons, however, showed that the fields of the TTX arbor clusters were less than 15% larger. In their recent report Olsson and Meyer ('87) applied WGA-HRP to the retina to anterogradely label a small group of axon endings. They compared the regions over which the endings of TTX-blocked and active axons were distributed. At 120 days after optic nerve section, the labeled terminals of the TTX-blocked axons did not remain as widely dispersed as at 30 days but instead had condensed into a small retinotopic cluster. They concluded that a progressive topographic refinement had occurred despite axonal impulse blockade (Olsson and Meyer, '87). Appropriate targeting under TTX was also shown for axons that had been deflected to innervate an ipsilateral tectum that had been deprived of retinal afferents for several months (Meyer, '87). Again, the region to which the TTX-silenced axons grew and where they terminated was retinotopic and no larger than the territory occupied by axons with normal activity. These data suggest that axons can progressively improve their targeting in the absence of activity. These reports together with our present results therefore suggest that there is a condensation of arbors to appropriate territories if sufficient time is allowed. Activity also appears to be dispensible for certain other rearrangements of axon terminals such as expansion and compression of the retinotectal map in fish (Meyer and Wolcott, '87) or the gradual disappearance of mislocated arbors in the rat retinocollicular projection (O'Leary et al., '86).

How these anatomical data relate to the results of electrophysiological recordings is difficult to judge. Electrophysio-

logical recordings from tecta innervated by axons that had been silenced by TTX while they regenerated showed that the multiunit receptive fields (MURFs) were 28–30° (Schmidt and Edwards, '83). They thus were 2.5 to 3 times larger than the MURFs of axons regenerated with normal activity. It is not known how arbor size or the relative tectal positions of arbors from neighbouring ganglion cells influence the MURF sizes. Therefore it remains open how the size of the arbor clusters found in our study relates to MURF sizes. Another difficulty in comparing the electrophysiological and the anatomical data lies in the difference

TABLE 2. Distribution (in %) of Regenerating TTX-Blocked and Control Axons from Temporal Retina over Tectum: Late Regeneration Stages

	Correct hemitectum			Incorrect hemitectum		
Region	1	2	3	4	5	6
TTX	61 (9.8)[1]	8.2 (5.2)	1.1 (0.9)	25.6 (9.3)	4.2 (3.3)	0
Ringer	60.5 (5.1)	7.9 (5.2)	0	21.7 (5.4)	4.5 (2.9)	0

[1]Standard deviation in brackets.

in the time at which the terminal field sizes were assessed. We determined the size of the terminal arbor clusters when we were able to delineate them clearly, which is not earlier than 120 days after optic nerve section, and thus 80 to 90 days later than Schmidt's and Edward's ('83) electrophysiology. At times of 30–40 days (at which their recordings were performed), axons with long side branches carrying growth cones and filopodia predominated and they were seen in various regions of the tectum. Terminal arbors of near-normal morphology were seen exclusively at retinotopic sites at 80–90 days, but their number was small, and they coexisted with axons and branches with growth cones. Whether the progressive changes in axonal morphology seen with anatomical methods would correlate with a change in MURF sizes cannot be answered. Slight misplacements of terminal arbors, however, remain undetected by anatomical techniques. Such misplacements could be the cause of the enlarged MURFs and they may persist as long as the axons remain deprived of their normal activity.

We should note that neither our nor earlier investigations have ruled out the possibility that electrical activity mediated by other ion channels may be used for the interaction of the axons with one another and their target.

Activity-dependent segregation of retinal axons

Although the current results weaken the importance of Na-channel-mediated activity for the formation of ordered projections, there is ample evidence that such activity-mediated axon-axon and axon-tectum interactions can induce drastic terminal arbor rearrangements such as during the formation of eye specific domains in doubly-innervated tecta of frogs (Constantine-Paton, '82), fish (Boss and Schmidt, '84), and chick (Fawcett and O'Leary, '85), and the formation of ocular dominance columns in the cortex of binocular mammals (Shatz and Stryker, '88; Stryker and Harris, '86). Activity, however, does not only force axons from different eyes to separate territories, but it also can push axons from neighbouring ganglion cells from one eye apart, as shown in this and earlier studies. Regenerating axons derived from a sector in retina may end in isolated patches instead of terminating side by side (Meyer et al., '85; Stuermer, '88a). This patch formation was not seen in tecta belonging to TTX-injected eyes. Such patches could represent a local aggregation of slightly mislocated arbors, which could reinforce each other via mutual interactions. Or they may be based on an segregation of ganglion cells of different response properties, such as "on" and "off" cells, and may be attributable to their different firing characteristics. It is unclear why such patches form only during optic nerve regeneration and not during normal development.

Evaluation of mechanisms involved in the formation of retinotopic maps

Our current results together with a variety of others indicate that considerable map refinement takes place in the absence of impulse activity during optic nerve regeneration in fish if sufficient time is allowed (Meyer, '87; Meyer and Wolcott, '87; Olsson and Meyer, '87). These findings then suggest that cytochemical cues (Sperry, '63; Gierer, '87) and perhaps activity-independent axon-axon-interactions may suffice for the reformation of well-organized projections. They leave open the possibility that activity might accelerate this process and may improve the map further. But they have also shown that activity can induce abnormalities of the map due to patch formation.

Embryonic development of the retinotectal map

The importance of activity for the establishment of the proper organization of the mammalian visual system has been demonstrated convincingly (Shatz and Stryker, '86; Stryker, '86; Sretavan et al., '87). Whether activity plays an important role during the development of the retinotectal map in lower vertebrates, however, has not been answered unequivocally. Retinal axons in the Xenopus embryo navigate to and terminate at their retinotopic target sites in tectum when the embryos are paralyzed with TTX (Harris, '84). The mapping precision was as good as 100 μm or better and not different from normal active embryos. Whether further map refinements require activity remained open.

In zebrafish embryos the embryonic retinotectal map is quite precise (Stuermer, '88c) and the individual arbors are smaller relative to the tectum than in Xenopus (Sakaguchi and Murphy, '85). The precision of the map and the arbor sizes were not altered when zebrafish were paralyzed by TTX-injections (Stuermer, '88d). With progress in development the arbors enlarge, but the tectum enlarges more rapidly relative to the arbors such that the total coverage of individual arbors is 8 times smaller in the adult than in the embryo (Stuermer, '88c). Several steps are involved in the formation of the adult map, among them the shifting of the axon terminals during the ongoing growth of the eyes and the tectum (Stuermer and Easter, '84, '84b). It remains to be seen whether activity blockade would have any affect on this sort of map refinement.

ACKNOWLEDGMENTS

We are most grateful to Drs. Michael Stryker and Tim Allsop for their valuable comments on the manuscript. We thank R. Groemke-Lutz for her skillful photographical reproductions.

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