A Role for Tectal Midline Glia in the Unilateral Containment of Retinocollicular Axons

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Retinal fibers approach close to the tectal midline but do not encroach on the other side. Just before the entry of retinal axons into the superior colliculus (SC), a group of radial glia differentiates at the tectal midline; the spatiotemporal deployment of these cells points to their involvement in the unilateral containment of retinotectal axons.

To test for such a barrier function of the tectal midline cells, we used two lesion paradigms for disrupting their radial processes in the neonatal hamster: (1) a heat lesion was used to destroy the superficial layers of the right SC, including the midline region, and (2) a horizontally oriented hooked wire was inserted from the lateral edge of the left SC toward the midline and was used to undercut the midline cells, leaving intact the retinorecipient layers in the right SC. In both cases, the left SC was denervated by removing its contralateral retinal input. Animals were killed 12 hr to 2 weeks later, after intraocular injection of anterograde tracers to label the axons from the remaining eye. Both lesions resulted in degeneration of the distal processes of the tectal raphe glia and in an abnormal crossing of the tectal midline by retinal axons, leading to an innervation of the opposite (“wrong”) tectum. The crossover occurred only where glial cell attachments were disrupted.

These results document that during normal development, the integrity of the midline septum is critical in compartmentalizing retinal axons and in retaining the laterality of the retinotectal projection.

Key words: midline septum; GFAP; axon barrier; radial glia; axon guidance; brain compartmentalization

Glia, glial cells have supportive or attractive influences on axon growth (Singer et al., 1979; Whitehead and Morest, 1981; Silver and Rutishauser, 1984; Dodd and Jessell, 1988; Muller and Best, 1989; Vanselow et al., 1989; Aguayo et al., 1990; Hatten, 1990; Abbott, 1991; Schachner, 1991) and play an inhibitory or repulsive role during fiber extension (Patterson, 1988; Chiquet, 1989; Abbott, 1991; Schachner, 1991) and play an inhibitory or repulsive role during fiber extension (Patterson, 1988; Chiquet, 1989; Vanselow et al., 1989; Aguayo et al., 1990; Hatten, 1990; Abbott, 1991). Glia are associated with guiding fiber systems by glia.

Glycoproteins expressed on oligodendrocytes inhibit axon growth. Antibodies that neutralize this inhibition allow for fiber regrowth (Schwab and Schnell, 1991; Kapfhammer et al., 1992; Schwab et al., 1993), suggesting that differentiating oligodendroglia outline spatial domains that contain late-developing fiber systems (Schwab, 1990; Kapfhammer and Schwab, 1994; Schwegler et al., 1995). Glial cell-associated glycoconjugates form boundaries that delineate compartments in the developing brain (Steindler and Cooper, 1987; Steindler et al., 1989, 1990; Snow et al., 1990a; Schwab and Schnell, 1991; Schwab et al., 1993; Steindler, 1993). We focus here on the latter function, particularly on the compartmentalization of growing fiber systems by glia.

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A group of radial cells resides along the tectal midline (Jhaveri et al., 1992; Wu et al., 1995). These are distinct from other tectal glia in their timing of differentiation, morphology, and molecular composition. Damage to the midline surface in neonatal rodents results in a bilateral invasion (across the midline) of the tectum by retinal axons from a single eye (Schneider, 1973; Hsiao and Schneider, 1978; Jen and Lund, 1979); no crossing of the tectal midline by retinal axons is detected if the lateral tectum, but not the midline, is damaged (So and Schneider, 1978; Schneider et al., 1985). These results suggest that normally, a structure(s) along the midline compartmentalizes retinotectal axons to one side. Here, we document an involvement of the tectal raphe glia in mediating such a barrier function. We show that retinal axons can be induced to cross the tectal midline abnormally but only where the pial processes of midline glia have been disrupted.
MATERIALS AND METHODS

Syrian hamsters (Mesocricetus auratus) were bred in the animal facility at the Massachusetts Institute of Technology, or timed-pregnant females were purchased from Charles River Laboratories (Wilmington, MA). The Massachusetts Institute of Technology Committee on Animal Care approved protocols for all surgical procedures involving live animals.

Surgical procedures

Unilateral tectal lesions. Postnatal day 1 (P1) hamster pups were anesthetized by hypothermia, the skin overlying the tectum was incised, and the flat head of a pin was heated and applied for a few seconds to the cartilaginous skull overlying the right superior colliculus (SC). The right eye was also removed. The skin was sutured, and the pup was warmed and returned to the home nest.

Undercutting the midline glial cells. Animals were anesthetized by hypothermia on P1, and the skull covering the SC was exposed as described above. A small slit was made with a number 11 scalpel blade at the lateral edge of the skull covering the left SC; a hooked tungsten wire was inserted through the slit and pushed medially, below the pia, all the way to the midline. The wire was then moved in the horizontal plane to disrupt the pial processes of the raphé glia, while the retinoreceptor layers of the right tectum were maintained intact. The right eye was also removed at the same time to denervate the left SC. Earlier studies had shown that such denervation induces a larger number of retinal axons from the remaining left eye to cross the tectal midline and to abnormally innervate the left tectum (Schneider, 1973; So and Schneider, 1978). Animals were warmed, returned to the home nest, and killed after varying survival times (12 hr to 3 weeks). To control for effects of the eye removal, the midline undercutting surgery was done on a group of newborn animals without enucleating the right eye. To control for the lesion made in the left SC by the tungsten wire, surgery was performed on a group of animals as described above, except that the wire was advanced just short of the midline, and the midline (raphé) glia were not severed; in these cases the right eye was also removed, as for the experimental animals.

Horseradish peroxidase dye injection and retinotectal axon labeling

Hamsters with unilateral SC lesions were perfused 10–21 d after surgery. Pups (at least five at each time point) with a midline undercut were killed at P12 or P14; two animals were perfused on P7. Control animals were age-matched. The preceding experimental hamsters at the time of perfusion. One day before being killed, the animal was fully anesthetized with hypothermia (for the neonates) or with Chloropen (0.35 ml/100 gm body weight), and 1–3 μl of 40–50% horseradish peroxidase (HRP) (type VI; Sigma, St. Louis, MO) made up in 2% dimethyl sulfoxide was injected into the left eye, with use of a micropipette attached to a Drummond (Broomall, PA) micropipette dispenser or a picojector (World Precision Instruments, Sarasota, FL). Animals were killed with an overdose of anesthesia and perfused transcardially with an initial rinse of 0.9% sodium chloride containing 0.25% of the vasoconstrictor sodium nitrite. This was followed by 4% paraformaldehyde made up in 0.1 m phosphate buffer (PB), pH 7.4. The brains were not post-fixed but were immediately dissected out and stored in PB for <1 week. Brains prepared for light microscopy were cryo-protected in 30% sucrose (made up in PB, pH 7.4) and cut coronally at a thickness of 40–50 μm on a cryostat. Three parallel series of sections were collected; one was reacted with tetramethylbenzidine (Sigma) to visualize the HRP-labeled radial glia. The labeled brain was returned to 4% paraformaldehyde for 4–15 d at room temperature. Brains were then cut on a vibratome into 100-μm-thick transverse sections, and tissue was collected in 0.1 M PB, mounted onto microscope slides, and coverslipped in wet PBS. The edges of the coverslips were sealed with nail polish. Dihydroindocarbocyanine per chlorate (DiI) (Molecular Probes, Eugene, OR) dissolved in dimethyl formamide was injected into the left eye. Animals were allowed to survive for another 1–2 d, were overdosed with pentobarbital, and perfused with 4% paraformaldehyde, and 4-(4-dihexadecylaminostyryl)-N-methylpyridinium iodide labeling of midline radial glia

Midline undercutting surgery was performed on P1 pups, as above. On P2 (24 hr after surgery), the pups were reanesthetized by hypothermia, and a solution of 1.1'-dioctadecyl-3,3',3''-tetramethylindocarbocyanine perchlorate (DiI) (Molecular Probes, Eugene, OR) dissolved in dimethyl formamide was injected into the left eye. Animals were allowed to survive for another 1–2 d, were overdosed with pentobarbital, and perfused with 4% paraformaldehyde, and 4-(4-dihexadecylaminostyryl)-N-methylpyridinium iodide (DiA) (Molecular Probes) was placed in the dorsal wall of the ventricle at the level of the cerebral aqueduct of Sylvius. The labeled brain was returned to 4% paraformaldehyde for 4–15 d at room temperature. Brains were then cut on a vibratome into 100-μm-thick transverse sections, and tissue was collected in 0.1 M PB, mounted onto microscope slides, and coverslipped wet in PBS. The edges of the coverslips were sealed with nail polish. DiI labeling of retinal axons in the tectum was analyzed and photographed with a Nikon epifluorescence microscope using a rhodamine filter. DiA-labeled radial processes on the same section were photographed with use of a fluorescein filter.

Electron microscopy

Normal hamsters and hamsters that had received a unilateral heat lesion of the SC on P0 or P1 survived until P6, at which point they were perfused with 4% paraformaldehyde and 3% glutaraldehyde in PBS. The brains were removed and post-fixed in the same solution. Coronal sections through the midbrain were cut on a vibratome, rinsed in PBS, osmicated (1% osmium tetroxide in PB) for 1–3 hr at room temperature, and rinsed again in buffer. They were sequentially dehydrated in a series of ethanol and embedded in Epon 812. Semithin sections, stained with toluidine blue, provided orientation to trim the block along the tectal midline. Ultrathin sections were cut on a DuPont (Billerica, MA) MT-5000 ultramicrotome; they were mounted on formvar-coated copper mesh grids, stained with lead citrate and uranyl acetate, and viewed on a Zeiss 109 electron microscope.

RESULTS

Midline radial glia and retinotectal projections in P0 hamsters

In normal animals, the tectal midline is comprised of tightly bundled radial cells whose perikarya are positioned around the lumen of the ventricle and whose radial processes span the thickness of the colliculus and are embedded in the pial surface. We refer to these radial extensions as the pial processes of the midline glia. Details of the development of these cells have been provided...
by Wu et al. (1995). Here, we briefly review salient light microscope observations that are relevant to the present study.

On P0–P1, vimentin immunoreactivity revealed radial cells in the lateral tectum, as well as along the midline (Fig. 1A–C); the latter were particularly striking because of their density and the bundling of their processes. These midline cells retained their vimentin expression at least until P12 (Fig. 2D). No lateral radial cells stained positively with the GFAP antibody that we used in this study. On the other hand, the midline cells were intensely reactive for this intermediate filament protein, both in neonates and at least through the second week of postnatal life (Figs. 1D, 2B) but not in embryonic animals. As has been shown before for all normal rodents, HRP-labeled retinal axons that projected to the superficial gray layer (SGS) of the contralateral tectum were retained within the SC on one side, with no encroachment of the visual axons across the tectal midline (Fig. 2C,D). (Retinotectal axons that are deflected ipsilaterally at the chiasm normally approach the tectum from the rostral end, as do the ones from the contralateral eye; they do not cross the tectal midline.) The following experiments describe the relationship between the damage inflicted on the midline glia and the altered projection patterns of retinotectal afferents (Jhaveri, 1993a,b; Jhaveri and Hoffman-Kim, 1996).

**Disruption of the midline after heat lesions of the tectum**

As a consequence of heat damage at the tectal surface, the underlying tissue became necrotic, and a cyst formed in this region (Fig. 3). Eight days after lesion, HRP-labeled retinal axons could be followed into the region of damage and across the tectal midline into the contralateral SC. These axons took two paths to the opposite side. The first was a membranous bridge, which provided a substrate for the axons to travel over the cyst and into the contralateral SC (Fig. 3A,B). Immunostaining of sections immediately adjacent to the ones in which HRP-labeled retinal axons had been visualized in this bridge revealed that GFAP-positive processes (of unknown origin) lined this upper membranous route (Fig. 3D). In addition, retinal axons were also seen coursing along the surface of the remnant SC, subjacent to the necrotic tissue (Fig. 3A,B) (So, 1979; Harvey et al., 1986). This second route occurred in the region where the pial processes of the midline raphe cells had been disrupted (Figs. 3, compare A,B and C,D): most of the pial attachments of the raphe glia had pulled back from the surface or were degenerating. The neatly bundled GFAP-positive processes, normally attached at the pial surface of the midline, were no longer visible (Fig. 4). However, the glial cell somata, along with their more proximal radial processes, were still tethered at the ventricular surface (Figs. 4A,B, 5C). Higher magnification views (Fig. 5) showed that the disruption of the radial processes created a “gap” just below the pial surface (Fig. 5A,C) and that retinal axons were confined to travelling through this gap. Nevertheless, surface view reconstructions of the tectal midline illustrated that the gap in the midline glia was not coextensive with the region over which the axons crossed but that it spanned a larger rostrocaudal extent of the SC than the region within which retinal axon crossover occurred (see Fig. 11, quantitation for the midline undercut cases presented below).
Midline ultrastructure in the normal SC and in the tectal lesion cases

In normal animals, ultrastructural examination of the pial processes of tectal midline glia revealed electron-lucent, radially oriented profiles that were rich in glycogen granules and mitochondria (see spared radial fibers in Fig. 6, which shows a micrograph from a lesioned animal). The distal tips of these processes were enlarged, forming end feet that attached at the basal lamina. On P6, small unmyelinated axons, many of which were likely collateral arbors of normal retinotectal fibers, could be identified, clustered in the upper layers of the developing SC; near the midline, these fibers were abutted against the radial processes. In experimental animals that had been subjected to heat-induced unilateral tectal lesions, ultrastructural examination documented a close interaction between axons and non-neuronal cells. This was especially evident in the tissue bridge that formed above the necrotic collicular tissue; immature glial processes that invaded this region had the ability to actively extend long ramified processes. The axons and their growth cones were closely apposed to these processes (Fig. 7).

Undercutting the midline cells

A second approach to damaging the pial processes of the midline glia, one that retained the integrity of the retinorecipient tectal zones, was to undercut the radial cells. This technique involved the insertion of a tungsten wire at the lateral edge of the left SC and pushing it medially toward the midbrain septum to sever the distal portions of the raphe glia from the cell body and more proximal processes (see Materials and Methods). In most cases, the wire track encroached slightly into the medial portion of the right SC (Fig. 8A). The right eye was also removed at the time of surgery. This increased the number of retinal axons that crossed over into the denervated (left) SC.

Eighteen hours after surgery, the horizontal cut made by the passage of the tungsten wire could be visualized in histological material (Fig. 8). Degradation of the severed distal processes was evident in tissue immunostained with the anti-vimentin antibody (Fig. 8B); however, the lower portions of the radial glial processes (below the wire cut) and the glial perikarya (located near the ventricular surface) appeared to remain viable. Over the next couple of weeks, the edges of the tissue along the wire track came together and could be identified by immunostaining for GFAP (Fig. 9A, arrowheads). The left SC was shrunken because of the removal of its contralateral retinal input at the time of neonatal surgery. Labeled retinal axons from the left eye could be followed to their normal zone of termination in the SGS of the right SC and were also seen streaming across the tectal midline into the SGS of the left SC (Fig. 9B, D). Adjacent sections were stained for visualizing HRP-labeled retinal axons or GFAP-reactivity. These documented a correlation at the midline between the zone in which severed pial processes of the raphe glia had been disrupted (Fig. 9A, C, double arrows) and the region over which retinal axons were able to navigate across the midline (Fig. 9B, D).
should also be noted in Figure 9 that at the midline the ventralmost extent of retinal axons reached no further than the region beyond which the basal processes of the glia were intact. Sections from control animals (data not shown) documented that no re-crossing of the midline was seen when the wire stopped short of the raphe glia or when the eye was removed but the midline was not undercut.

In two animals, midline cells were undercut on P1, the right eye was removed, and a solution of DiI was injected in the left eye via a trans-scleral approach. The animals were perfused on P3, and
crystals of DiA were placed along the upper surface of the aqueduct of Sylvius to visualize the radial glia in the postnatal SC. Two days later, the DiA labeling revealed the full extent of the radial fibers that had survived the wire cut; in the left SC, they stretched dorsally from the ventricle, stopping abruptly just below the optic fiber layer, at the point where their distal processes had been severed by the wire (Fig. 10A, B). At least up to 2 d after surgery, there was no indication of regeneration of the severed pial processes. In the same brains, Dil-labeled retinal axons could be followed from the left eye to the right SC. It was clear that even within 2 d of undercutting the midline cells, a few retinal processes had begun to cross the tectal midline and to enter the wrong (left) SC (Fig. 10C, curved arrows).

Double exposure photography using fluorescein and rhodamine filters documented a complementary relationship between the region in which the abnormal crossover of retinal axons occurred and the area over which the cut processes of the midline glia were withdrawn (Fig. 10). Note that these first few retinal axons that ventured across the midline were not fasciculated but seemed to travel individually; nor did they necessarily show a preference for growing along the pial surface, as do the earliest retinal fibers that enter the SC in the normal embryo (Jhaveri et
Recrossing retinal afferents were, instead, distributed throughout the gap created by the undercutting (Fig. 10C). We were able to trace a few crossing axons retrogradely from their terminals in the left SC and found that they also had small collateral branches in the “correct” SC (data not shown). Thus, it appears that at least at this early stage, retinal ganglion cell axons fated to terminate in the right SC might expand their target area by branching into the left SC but without relinquishing their normal territory.

The recrossing of the tectal midline by retinal axons was robust several days after the midline glia were damaged. By P14, retinal axons from the left eye were found to occupy one-half to two-thirds of the mediolateral extent of the left SC (Fig. 9B,D); moreover, there was no obvious indication at this stage that they achieved this massive extra projection by a compensatory decrease in the density of projections to their appropriate termination zone in the right SC. If both eyes were left intact but the midline glia were cut, HRP-labeled retinal axons originating in the remaining left eye (B, D). The point at which the wire entered the left SC is delineated by gliosis (A, arrowheads), as seen with GFAP immunostaining. Double thin arrows in A and C indicate the point above which the midline processes were severed. In B and D, the distribution of HRP-labeled retinal axons is shown. Note that in both cases, the retinotectal projection to the right SC is targeted to normally retinoreceptive layers, and a significant contingent of labeled retinal axons can be traced across the tectal midline into the SGS of the left SC. Open arrows in B and D indicate the position of the tectal roof plate. Scale bar (in D), 200 μm.

Figure 9. Pairs of immediately adjacent sections through the SC of two P14 brains (A and B from one animal, C and D from the other) in which the midline processes were undercut on P1 and the left SC was denervated by removing the right eye. Coronal sections through the SC were immunostained with an anti-GFAP antibody (A, C) or were reacted with tetramethylbenzidine to visualize HRP-labeled retinal axons originating in the remaining left eye (B, D). The point at which the wire entered the left SC is delineated by gliosis (A, arrowheads), as seen with GFAP immunostaining. Double thin arrows in A and C indicate the point above which the midline processes were severed. In B and D, the distribution of HRP-labeled retinal axons is shown. Note that in both cases, the retinotectal projection to the right SC is targeted to normally retinoreceptive layers, and a significant contingent of labeled retinal axons can be traced across the tectal midline into the SGS of the left SC. Open arrows in B and D indicate the position of the tectal roof plate. Scale bar (in D), 200 μm.

DISCUSSION

The spatiotemporal deployment of a group of glial cells at the tectal midline would permit them to subserve the unilateral confinement of retinotectal axons in normal animals (Fig. 12A) (Raedler et al., 1982; Barradas et al., 1989; Harvey et al., 1993; Wu et al., 1995). Lesion of one SC leads to an abnormal crossing of retinal afferents but only if the tectal midline is disrupted (Mustari and Lund, 1976; Hsiao and Schneider, 1978; Jen and Lund, 1979; Schneider et al., 1985). Here, we use two early lesion paradigms to document the direct involvement of the tectal raphe glia in the containment of retinal axons. With both perturbations, glial processes degenerate and retinal axons cross the collicular midline (Fig. 12B).
Cells that colonize the midline of the neuraxis

There is considerable speculation about the role of specialized cells in forming barriers against migrating neurons or against growing axons (Joosten and Gribnau, 1989; Silver et al., 1993; Heyman et al., 1995; Brunso-Bechtold and Henkel, 1996). In the spinal cord, interactions between gene products of the bone morphogenetic protein 4, sonic hedgehog, and related genes (Liem et al., 1995; Jordan et al., 1997) may determine the formation of the roof plate and floor plate and affect growth patterns of sensory and motor projections (Ruiz i Altaba and Jessell, 1993; Dosch et al., 1997; Knecht and Harland, 1997; Mehler et al., 1997). Floor plate cells express a chemotropic factor that attracts axons of commissural neurons toward the midline (Tessier-Lavigne et al., 1988; Kennedy et al., 1994; Colamarino and Tessier-Lavigne, 1995a). Roof plate glia are implicated in blocking dorsal column fibers from contralateral encroachment (Snow et al., 1990a,b). Cells in the optic chiasm are involved in the guidance of temporal and nasal retinal axons (Silver, 1984; Godement and Mason, 1993; Wizenmann et al., 1993). Specialized glia also form boundaries in regions that lie off the midline (Steindler et al., 1990; Silver et al., 1993; Steindler, 1993; Heyman et al., 1995). However, apart from the work on the crossing of ventral commissural axons through the floor plate, the relationship between midline cells and the guidance of axons that encounter them has been correlative. Here, we show a causal relationship between disruption of the tectal midline glia and a rerouting of retinotectal afferents.

Permissive versus inductive mechanisms for retinal axon crossing

After heat damage to the developing SC, a membranous bridge forms over the necrotic tissue (So and Schneider, 1978; So, 1979; Harvey et al., 1986). This structure is rich in GFAP-positive glial processes, many of which have a close spatial relationship with growing tectal afferents (Figs. 3D, 6, 7). Glia that invade the bridge likely provide the substrate on which retinal axons navigate across the tectal midline. Because we never see a bridge without retinal axons coursing along it, its substrate is likely both permissive and instructive for the growth of retinotectal afferents. However, such an influence is not easy to quantify because of technical difficulties in retaining bridge tissue during histological processing. Retinal axons also cross via a second route, under the necrotic tissue, through the area in which the midline glia have been undercut. Midline reconstructions reveal that the region of damage and the region over which retinal axons cross are not coextensive. The axons occupy only a fraction of the gap in the roof plate and do not grow across any part of the tectal midline where the raphe glia are not disrupted. Collectively, these observations indicate that disruption of glial processes leads to the formation of a permissive, but not instructive, substrate for the growth of immature retinal axons (So and Schneider, 1978; Kapfhammer et al., 1992). Thus, additional signals must be involved in determining exactly where retinal axons travel within the disrupted zone (see below).

Specificity of glial barriers and putative molecular bases for the barrier

A critical question concerns the specificity of glial barriers in relation to different axon systems. Barriers that block growth of only certain fiber populations and ones that universally block all growing fibers may function in the brain. For instance, retinotectal axons do not cross the tectal midline, but contralaterally projecting fibers in the tectal commissure are able to successfully navigate across this region. This may be either because each system is differentially responsive to the midline glia, or because intertectal axons cross the midline before glial differentiation (Jhaeri, 1993a,b) and before the expression of putative inhibitory molecules along the tectal roof plate. Similarly, dorsal spinal commissural axons cross the roof plate after the ingrowth and

![Image](https://example.com/image.png)
unilateral compartmentalization of sensory axons in dorsal columns when putative axon-inhibitory molecules are no longer detected along the roof plate. These observations suggest that the barrier function of midline cells might be universal and that if axon systems were to bypass the blockade, they would be compelled to grow through before its functional maturation or after its ephemeral expression of inhibitory molecules has diminished.

One could argue that selective decussation of nasal (but not temporal) retinal axons at the optic chiasm belies this notion: axons from nasal and temporal retina approach the ventral midline at approximately the same time, but each interacts differentially with chiasm cells (Godement and Mason, 1993; Sretavan et al., 1994). However, ipsilateral deflection of temporal axons occurs early during chiasm formation (Drager, 1985; Reese and Colello, 1992; Baker and Reese, 1993); later, both temporal and nasal retinal axons project contralaterally, again invoking temporally variable factors in the decision to cross or not. Also, ipsilaterally directed corticocortical axons cross the tectal midline after deafferentation of the contralateral colliculus but in the absence of any damage to the midline glia (Mustari and Lund, 1976; Jen et al., 1978; Rhodes and Chalupa, 1978; Land et al., 1984); however, the expanded corticocortical projection crosses the midline at deeper levels of the SC (Jen et al., 1978; Harvey and Worthington, 1990), where retinal fibers do not normally reach.

The molecular bases for tectal midline barrier functions are unknown, but PGs have been incriminated. In vivo, some PGs are present in regions in which axons do not grow (Snow et al., 1990a,b; Cole and McCabe, 1991; McKeon et al., 1991; McCabe and Cole, 1992; Gonzalez et al., 1993; Pindzola et al., 1993; Silver et al., 1993; Heyman et al., 1995; Brunso-Bechtold and Henkel, 1996; Jhaveri and Hoffman-Kim, 1996; Reese et al., 1997). In the developing SC, sulfated PGs are detected in tissue harvested from the midline (where retinal axons do not grow) and also from the lateral tectum (where retinal axons do extend). Biochemical characterization shows that PG core proteins are similar at the tectal midline and laterally but that those along the midline are more heavily glycosylated (Hoffman-Kim et al., 1996, 1998). This suggests that the glycosaminoglycans, and not the protein cores, are likely participants in the barrier function.

In vitro observations of retinal explants growing on glial carpets also confirm that the glia harvested from the midline region are less supportive of retinal axon extension (Young et al., 1997). Thus, it is likely that PGs on glial cells or in the extracellular matrix surrounding glial cells subserve the avoidance of the tectal midline by retinal axons. Garcia-Abreu et al. (1995, 1996) document a differential PG content in glia harvested from the middle versus lateral thirds of the midbrain and show that tectal neurons exhibit differences in neurite outgrowth patterns on the two types of glia.

Figure 11. Schematic dorsal view reconstructions of the tectal midlines from animals (ages P12 and P14 at time of perfusion) that had the raphe glia undercut on P1. The number of sections through the rostral (R) to caudal (C) extent of each SC was counted; the extent of the midline is depicted along a line (arbitrary units). The region of each midline along which the distal processes of the raphe glia were observed as being intact is marked with hatching; the extent of each midline where the pial processes were disrupted is depicted with a thick straight line; the region of each midline over which retinal axons were seen crossing abnormally to the opposite side is marked with Xs. Numbers (in arbitrary units) representing the length of the rostrocaudal extent of the undamaged region for the gap (Gap) created by disrupting the midline glia and for the length of the midline over which retinal axons are seen crossing (Xng) are indicated. The length (in micrometers) for the disrupted region (Gap) and for the distance over which retinal crossover is observed (Cross) are provided at the bottom of each reconstruction.
the growth of specific afferent systems (Colamarino and Tessier-Lavigne, 1995b). Mice deficient in netrin (Serafini et al., 1996; Guthrie, 1997) or its receptor (Fazeli et al., 1997) show disrupted decussation of some axons but not of others, indicating a specificity of responses by different afferents to this class of molecules. A diffusible factor that repels commissural axons has been reported for the spinal cord roof plate (Augsburger et al., 1996), which must act in concert with chemoattractants released by the floor plate to guide the growth of the ventrally crossing fibers. For the hamster SC, evidence points to an inhibitory role for PGs (membrane bound or secreted into the extraneuronal matrix) in relation to retinal axon growth (see above). However, the observation that retinal axons do not completely fill the gap created by disruption of the midline glia (Fig. 11) suggests that, at least in animals with a damaged midline, a second diffusible chemorepellant substance released by these glia is also at work. If such a diffusible factor does emanate from the roof plate, perhaps it is not a very potent player during the normal development of the retinotectal fibers given the fact that many of these fibers encroach so close to the tectal midline. Finally, the recent discovery of genes such as rob1 (Kidd et al., 1998a,b) and sax3 (Zallen et al., 1998) demonstrates that the genetic make-up of afferent neurons, and not just the molecular composition of the extraneuronal environment, is an additional factor in determining whether or not successful decussation of axons can take place.

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