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A Role for Oligodendrocytes in the Stabilization of Optic Axon Numbers

R. J. Colello[a] and M. E. Schwab

Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland

Differentiated oligodendrocytes express neurite growth inhibitory proteins at a time when these cells are involved in the myelination of recently formed fiber pathways. As the process of myelination follows the completion of neurite outgrowth and is concurrent with the stabilization of fiber numbers in a pathway, we set out to determine whether myelination and fiber tract stability could be causally related.

Myelin formation was prevented in the rat retinofugal pathway by x-irradiating the optic nerves during oligodendrocyte proliferation. Electron microscopic and immunohistochemical analysis of irradiated optic nerves at P15 showed that oligodendrocytes and myelin were virtually absent. Optic fiber numbers were determined at 2 weeks of age throughout the length of normal and x-irradiated nerves. In some cases, normal or irradiation-treated pups were intraocularly injected with FGF 5 d prior to the fiber count in order to promote neurite outgrowth.

Axon counts showed that the total fiber number in a myelin-free optic nerve was 10–30% higher than that of a myelinated nerve. Further, fiber numbers fluctuated by as much as 20% along the length of a myelin-free nerve but were relatively constant throughout the length of a myelinated nerve. Treatment of myelinated nerves with fibroblast growth factor (FGF) had no effect on either total fiber numbers or fiber number fluctuation. Conversely, fiber numbers in myelin-free/FGF-treated optic nerves were as much as 40% higher than in normals. Furthermore, total fiber numbers along the length of these nerves fluctuated by up to 34%.

These results indicate that, in the absence of myelination, optic fibers are able to form sprouts. This suggests that oligodendrocytes have a role in preventing sprouting and stabilizing the number of fibers in a pathway during development.

[Key words: glia, retinofugal pathway, optic fibers, myelination, x-irradiation, sprouting]

Myelination of fiber projections in the CNS represents one of the final stages of fiber tract formation. This process follows a number of sequential developmental events, composed of "progressive and regressive" phenomena, that help shape the adult fiber projections (Oppenheim, 1981; Cowan et al., 1984). In the visual system, for instance, the initial overproduction of neurons and their axons is followed by a period of cell death that eliminates many of these supernumerary neuronal elements (Lam et al., 1982; Crespo et al., 1985). This reduction of the fiber population is then followed by axon myelination (Skoff et al., 1980; Ng and Stone, 1982; Sefton and Lam, 1984). Fiber numbers within the resultant myelinated pathway are now stabilized and are little affected throughout the life of the animal.

Although the process of fiber number stabilization has been linked to the matching of afferent input to the available target field, the transformation of fiber pathways from a permissive to a nonpermissive environment may also help to stabilize fiber tracts by preventing sprout formation (for review, see Schwab et al., 1993). It has been shown that differentiated oligodendrocytes start to express proteins that strongly inhibit neurite growth at a time when these cells are involved in the myelination of recently formed fiber pathways (Caroni and Schwab, 1988a,b, 1989; Schwab and Caroni, 1988). As the expression of these inhibitor proteins follows the completion of fiber outgrowth (Caroni and Schwab, 1989), this suggests that the process of myelination could stop fiber growth and prevent the formation of sprouts and, thus, serve to stabilize fiber pathways. This mechanism of fiber stabilization by myelination would be distinct from the stabilization of fiber counts by afferent input/target field matching. It would represent a mechanism by which fiber pathways, through the prevention of sprout formation, remain stable in axon number through the life of the animal.

To test this hypothesis, we suppressed myelin formation in the developing retinofugal pathway of rats by x-irradiating the optic nerves at the time of oligodendrocyte proliferation. This treatment has been shown to prevent oligodendrocyte development and myelin formation (Gilmore, 1963a,b; Colello et al., 1994). Optic fiber numbers were then determined for normal (myelinated) and x-irradiated (unmyelinated) nerves during a period when the fiber counts have reached previously recorded adult values. In some instances, an intravitreal injection of fibroblast growth factor (FGF) was given at a time (P10) when adult axon numbers should have been established (Sefton and Lam, 1984; Crespo et al., 1985). FGF has been shown to have neurotrophic effects on retinal ganglion cells (Sievers et al., 1988). The resultant unmyelinated nerves were found to have fiber counts significantly higher than that of normal animals, and these counts fluctuated throughout the length of the optic nerve

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Correspondence should be addressed to M. E. Schwab, Brain Research Institute, University of Zurich, August-Forel-Strasse 1, CH-8029 Zurich, Switzerland.

[a]Present address: Department of Human Anatomy, University of Oxford, South Parks Road, Oxford OX1 3QX, UK.

suggesting that sprouting and neurite growth did occur in these nerves in the absence of oligodendrocytes and myelin.

Materials and Methods

X-irradiation treatment. To suppress myelin formation, Lewis rat pups (Hannover, Germany) were exposed to x-irradiation on postnatal days (P) 0, 2, and 4. The pups were anesthetized by cooling in ice (Phifer and Terry, 1986) and placed in a plastic tray with their head supported upright and the entire body covered with a lead shield. To x-irradiate the retinofugal pathway, a slit was cut into the shield to correspond, in size, with the surface of the head overlying one optic nerve. Efforts were made to insure that neither the retinas or the retinal recipient nuclei were exposed to x-irradiation. A 5500 rad dose of x-rays was administered under the identical conditions of those previously described (Savio and Schwab, 1989; Colello et al., 1994). Briefly, the pup was placed 20 cm from a x-ray source set at 50 kV and 15 mA. The beam was filtered through a 0.25 mm aluminum screen.

To determine if oligodendrocytes were selectively targeted by x-irradiation, optic nerves were prepared for immunostaining for myelin basic protein (MBP), a differentiated oligodendrocyte marker, and glial fibrillary acidic protein (GFAP), an astrocyte marker (Dahl and Bignami, 1976; Omlin et al., 1982). Both normal and x-irradiated P15 rats were anesthetized with ether and perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. The optic nerves were dissected free, cut in the longitudinal plane at 15 μm with a cryostat and collected on 2.5% gelatinized slides. The sections were then pretreated with 95% ethanol/5% acetic acid for 30 min at 4°C, rehydrated and blocked with 5% bovine serum albumin in 0.1 M phosphate buffer, and incubated overnight at 4°C with a 1:500 diluted mouse monoclonal antibody against MBP (Boehringer Mannheim) or a 1:250 diluted rabbit monoclonal antibody against GFAP (Dako, Copenhagen). The primary antibodies were detected with either a biotinylated anti-mouse or anti-rabbit secondary antibody (Vector). The avidin–biotin–peroxidase complex (Vector, Burlingame, CA) was used to localize the biotinylated antibody. GFAP was also detected using an anti-rabbit FITC-linked secondary antibody (Miles, Naperville, IL) Histochemical detection of peroxidase activity was carried out in 0.1 M phosphate buffer containing 0.025% 3,3-diaminobenzidine (Sigma, St. Louis, MO) and 0.02% hydrogen peroxide. Sections were dehydrated, coverslipped, and viewed with an Olympus Vanox T microscope.

FGF treatment. X-irradiated or untreated littermate rats received a single intravitreal injection (50 ng/1 μl saline) of bFGF (Collaborative Research) in order to enhance neurite outgrowth. As FGF is also known to increase cell survival in the CNS (Walicke, 1988), it was important that administration was made after retinal ganglion cell counts had stabilized (Potts et al., 1982). Thus, FGF administration was made (under ice anesthesia) to P10 pups.

Ganglion cell counts. The number of ganglion cells on the x-irradiated side was compared with that of ganglion cells on the nonirradiated control side to evaluate whether the x-irradiation and the loss of oligodendrocytes has any affect on the outcome of natural retinal ganglion cell death. At P13, rat pups ($n = 3$) that received unilateral x-irradiation of the optic nerve were anesthetized with 0.3 ml of 3.5% chloral hydrate and placed into a stereotaxic apparatus. From predetermined coordinates of the lateral geniculate nucleus, four injections of horseradish peroxidase (HRP) were made along the extent of this region. Each injection was 0.15 μl of a 40% HRP (Sigma) solution in 2% DMSO. The total injection series was performed on both sides of the brain, within a 60 min period. Forty-eight hours later, the pups were again anesthetized and perfused with phosphate-buffered saline followed by 1% paraformaldehyde in 0.1 M phosphate buffer at pH 7.3. The retinas were then dissected, whole-mounted (see Stone, 1983) and finally reacted for HRP by the Hanker-Yates method (Hanker et al., 1977). The number of HRP-labeled ganglion cells per field area (0.075 mm^2) was determined at 40× using a camera lucida setup attached to a Zeiss Axiophot microscope. Counts were made from 12 fields found within a nasal-to-temporal strip of retina. This represented approximately 2% of the total retinal area.

Tissue preparation for electron microscopy.. At 15 d postnatal, both normal and x-irradiated rats were anesthetized with ether and transcardially perfused with a mixture of 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2. Subsequently, the cranium and lower jaw were removed and the head was immersed in the same fixative overnight.

Primary fixation was then followed by tissue preparation for electron microscopy. The optic nerves were separated from the eyes at the level of the eye/nerve junction and the nerves and chiasmatic region were dissected free from the brain and rinsed in phosphate buffer, postfixed in buffered 1% osmium tetroxide (pH 7.2), block stained for 30 min with 5% uranyl acetate in 50% ethanol, dehydrated, and embedded in Epon/Araldite. Thin sections (approximately 100 nm) of selected regions of the optic nerve were cut in a plane perpendicular to the outgrowth of optic fibers, collected on Formvar-coated grids, stained with uranyl acetate and lead citrate and viewed with a Zeiss EM-902 electron microscope.

Quantification of optic nerve fibers. Optic nerve fiber numbers were determined by counting fibers within a known area of the optic nerve and extrapolating this figure to the total areal extent of the nerve.

To determine the total area of the optic nerve, low-power electron micrographs (150×) were taken and measured using a Sigmagraphics bit pad and SIGMA-SCAN version 3.10 software. The area occupied by blood vessels was measured separately and subtracted from the total nerve area. This was done to ensure that the total area measurement was not influenced by any effect x-irradiation may have on blood vessel formation.

Fiber counts were made from 16 high-power micrographs (4500×) taken of nerve regions underlying the intersections of a regular grid superimposed upon a composite photograph of the whole nerve. This sample represented roughly 10% of the total nerve area. Axonal profiles were distinguished from glial profiles by the presence of microtubules, a light cytoplasm, the absence of ribosomes, and a regular, round to oval outline.

The statistical significance of the fiber counts obtained above was determined using the SPSS statistical program software version 6.0 (SPSS, Inc.). The follow tests were used to analyze the data presented: the *t* test was used to determine whether any differences exist between the sample means of ganglion cell counts in retinas of normal nerves and retinas from x-irradiated nerves, an analysis of variance was used for testing the differences among the sample means of the four groups of axon counts, the Tukey's test was used in making comparisons among individual means in the four groups of axon counts, and the *f* test was used to determine the variance in axon counts within animals between two groups.

Results

Ganglion cells in the rat are generated from E14 to E21 (Reese and Colello, 1992), with the peak number of axons in the optic nerve being reached at E20 (Sefton and Lam, 1984; Crespo et al., 1985). This is followed by axon elimination, which results in adult axon numbers at P5–P10 (Sefton and Lam, 1984; Crespo et al., 1985). The first GalC-positive, inhibitory oligodendrocytes appear shortly after birth (Caroni and Schwab, 1989); the first compacted myelin is present at P6 (Skoff et al., 1976). X-irradiation treatment was performed during the period of axon elimination and during the main proliferative period of oligodendrocytes (Miller et al., 1985). All evaluations of optic axon fiber numbers were made at postnatal day 15, that is, 1 week after the elimination of supernumerary axons has ended and when the number of axons has stabilized to the adult fiber numbers (Sefton and Lam, 1984).

Myelin and oligodendrocytes are absent in x-irradiated optic nerves

Gross examination of P15 optic nerves x-irradiated on P0, P2, and P4 reveals a near transparent fiber tract, unlike that of the opaque unexposed nerve (not shown). The retina attached to an exposed nerve is comparable in size to that of an normal retina and has a ganglion cell layer containing retinal ganglion cells with soma sizes comparable to that found in the retina on the untreated side (see Colello et al., 1994). Although capillary area was not included in the total areal measurement of the nerve it was interesting to note that x-irradiation had little effect

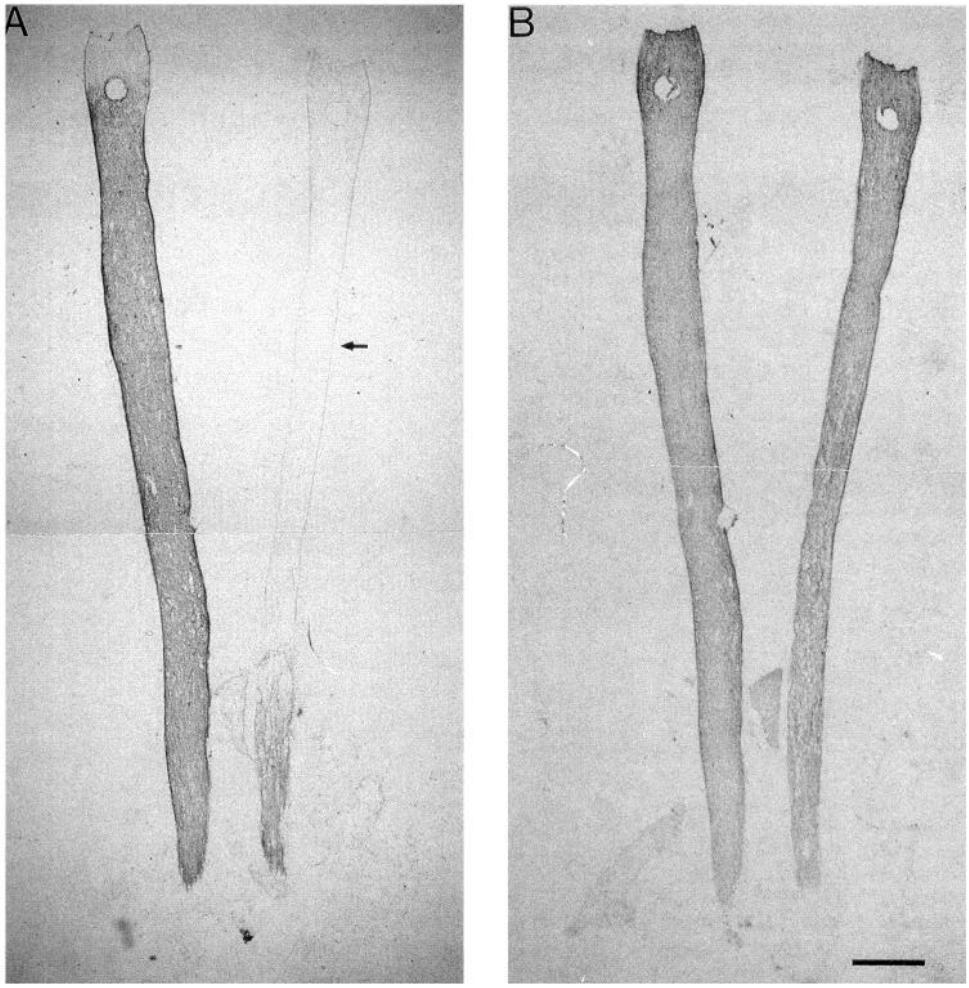

Figure 1. Longitudinal section of the optic nerves from a P15 rat unilaterally x-irradiated at postnatal days 0, 2, and 4. Antibody staining for MBP (*A*) shows that the normal nonirradiated optic nerve (*left side*) expresses this protein throughout its length, except within a region nearest the eye (*top*), where oligodendrocytes are normally not found. The x-irradiated nerve (*arrow*) shows a virtual absence of MBP expression, except for a <1 mm region of the nerve nearest the chiasm (*bottom*). Antibody staining for GFAP (*B*) shows that both the normal and x-irradiated nerves express similar levels of this protein throughout their length. Scale bar, 0.5 mm.

on the areal fraction of the nerve occupied by blood vessels (5.0% in x-irradiated vs 5.5% in normal nerves).

The prevention of oligodendrocyte development in the P15 nerve by x-irradiation was confirmed by the virtual absence of myelin basic protein (MBP) staining (Figs. 1*A*, 2*B*). At this age, only the region closest to the chiasm of the x-irradiated optic nerve was MBP positive. This is the result of a delayed myelination of the x-irradiated nerve that commences at this time (Colello et al., 1994). In contrast to the x-irradiated nerve, the normal nerve on the untreated side was heavily stained for MBP along its length (Figs. 1*A*, 2*A*). Staining was conspicuously absent from a segment of the nerve nearest the eye (top of left nerve in Fig. 1*A*). This represents a region of the nerve that remains unmyelinated (Hildebrand et al., 1985). Unlike the staining for MBP, the astrocyte-specific intermediary filament component, GFAP, was found in both normal and irradiated nerves at comparable distributions and intensity (Figs. 1*B*, 2*C,D*). The formation of an astrocytic network, as shown with immunofluorescence, can be readily seen in both the normal (Fig. 2*C*) and the treated (Fig. 2*D*) nerves.

Electron microscopic analysis of an x-irradiated P15 optic nerve confirmed that oligodendrocyte development and myelin formation have been prevented. Figure 3*B* shows the total absence of myelin in an irradiated nerve, in contrast to an unexposed nerve of the same age (Fig. 3*A*) Interestingly, the increase in axon diameter found normally during myelination was reduced in x-irradiated nerves. The mean diameter of the axon population was 40% smaller than that found in normal nerves (see Colello et al., 1994).

Optic nerve fiber numbers are increased by x-irradiation

Optic axon fiber counts were made at two or three locations along the length of normal and x-irradiated P15 optic nerves: near the eye (the intraorbital segment, "o"), midnerve (m), and within the prechiasmatic (c) region of the nerve. Counts were also made in these regions of normal and x-irradiated animals given intravitreal injections of FGF at P10. Each of the above conditions was represented by three animals from different litters. The results are summarized in Figure 4.

In normal, myelinated nerves, 91,000 fibers are present (SD = 1527, n = 3), a finding that is in agreement with previous published findings (Sefton and Lam, 1984). This number is stable throughout the length of the nerve; the fluctuation of fiber counts within a normal nerve is at most 5%, and as little as 2% between regions (see Fig. 4). The administration of one injection of 50 ng of bFGF into the eye of a normal rat did not influence the total optic nerve fiber numbers or fiber count fluctuation along the nerve; total fiber counts were 90,000 (SD = 1527, n = 3), almost identical in number to those found in the nerves of non-FGF-treated animals (using a Tukey's multiple comparisons procedure we found that there was no significant difference in numbers between normal nerves and FGF-treated

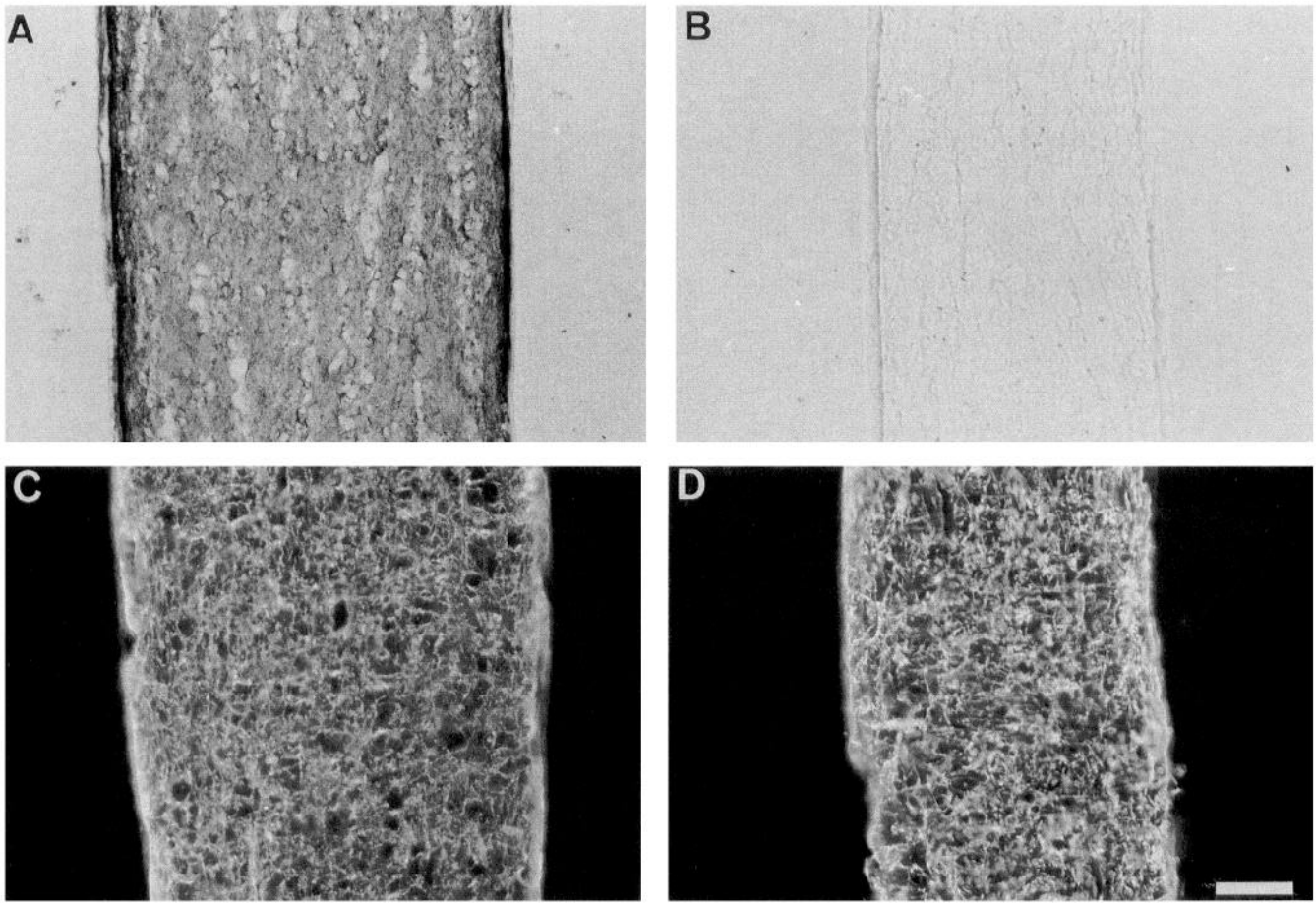

Figure 2. High-power micrographs of Figure 1 showing the extent of immunostaining for MBP (*A, B*) and GFAP (*C, D*) in either the untreated, normal nerve as shown in *A* and *C* or the x-irradiated nerve as shown in *B* and *D*. Scale bar, 0.1 mm.

normal nerves ($p > 0.05$), while there was a significant difference if irradiation treatment was present ($p < 0.05$; see below). Also, the fluctuation of fiber counts along the FGF-treated nerves was comparable, and not significantly different, to the non-FGF counterparts (3–5%). These results showed that FGF administered to a nonirradiated animal at P10 had no effect on the number of optic nerve fibers at P15.

In x-irradiated, myelin-free nerves (Fig. 4), the mean number of optic nerve axons was 111,000 (SD = 9536, $n = 3$). A repeated-measures analysis of variance (ANOVA) was carried out on the numbers of axons and provided strong evidence that the number of axons observed was significantly higher in the irradiation-treated cases independent of any effect of FGF treatment ($F = 79.2$, df = 1 and 8, $p = 0.00002$). Interestingly, the comparison of fiber counts along the nerve also revealed a greater fluctuation; axon numbers differed up to 20% between intraorbital, midnerve, and prechiasmatic segments. The highest fiber counts were not restricted to one segment of the nerve, but rather, in some rats the highest counts were close to the eye, in others close to chiasm (Fig. 4).

A very dramatic effect was observed in rats injected with FGF whose optic nerves had been irradiated (Fig. 4). In these cases, the total number of fibers seen in the nerve was up to 40% higher (mean number = 126,000, SD = 6110, $n = 3$) than that of normals. Further, there was up to 34% fluctuation of fiber numbers along the length of these nerves. In a comparison of mean fiber numbers in the FGF^- and the FGF^+ irradiation-treated groups, it was found that FGF treatment significantly increased sprouting. The repeated measures ANOVA provided evidence of a significant interaction effect between the irradiation and FGF treatments ($F = 7.6$, df = 1 and 8, $p = 0.025$). Examining the mean levels shows that the difference between the axon numbers for irradiated-treated and non-irradiation-treated nerves was larger when FGF is present ($p < 0.01$) than when FGF is not ($p < 0.05$). This was again determined using the Tukey's multiple comparisons procedure. Finally, the average fluctuation of optic nerve axon numbers was approximately sixfold higher in myelin-free/FGF-treated P15 optic nerves than that of their normal counterparts. Using an F test to compare the variance within animals between two treatment groups (−irrad/+FGF and +irrad/+FGF), we found evidence for a higher variation in axon numbers along the nerves in the x-irradiation treatment group versus the nonirradiated group ($p < 0.05$). Further, as shown in Figure 4, the axon numbers vary along the individual nerves, that is, the highest fiber counts were not located within any specific region of the nerve.

Retinal ganglion cell numbers are unaffected by x-irradiation

To determine whether the high fiber counts found in the x-irradiation–treated nerve were the result of increased ganglion cell numbers, counts were made of cells retrogradely labelled with HRP in retinas on both the normal and treated side (Table 1). In all animals examined, ganglion cell numbers were similar in both retina. Cell counts were always higher in central retinal

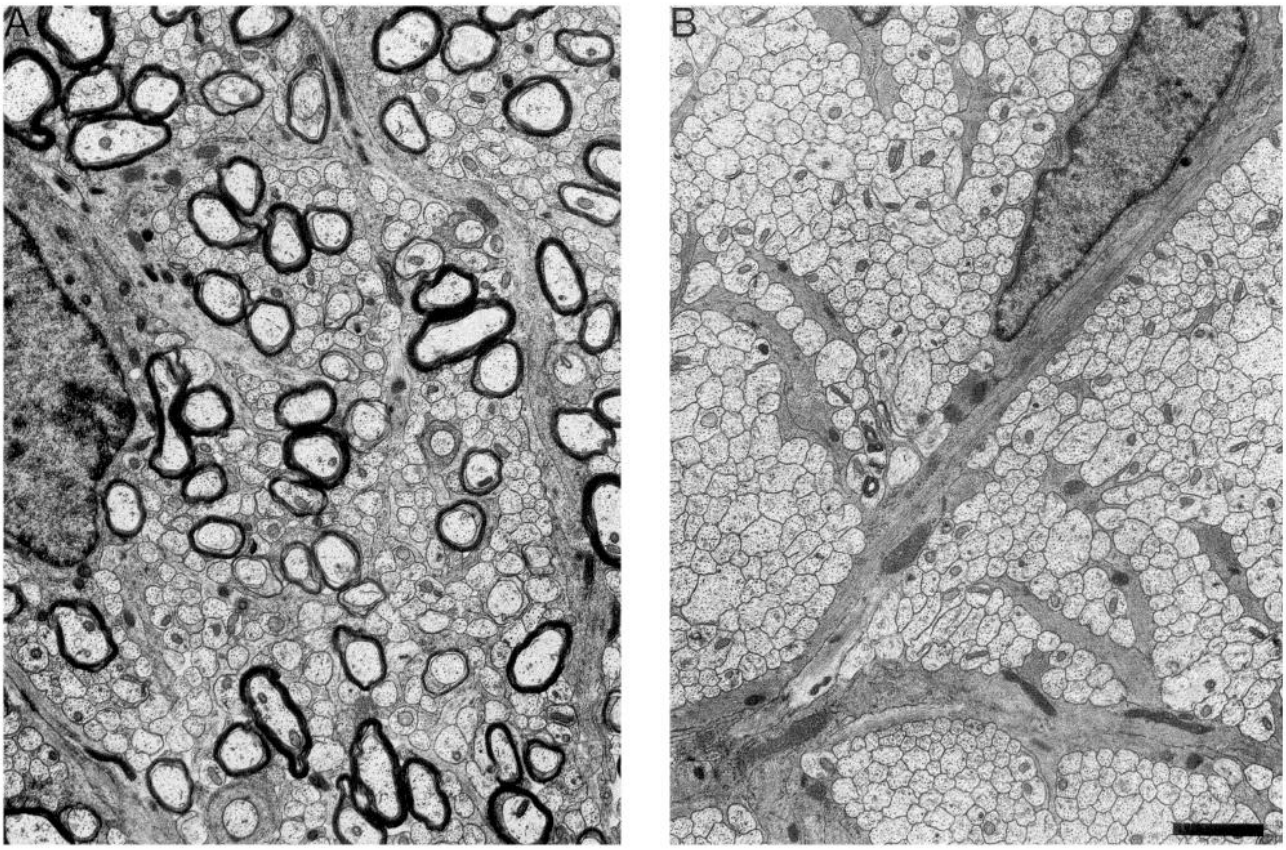

Figure 3. Electron micrographs of optic axons within the normal (*A*) and x-irradiated (*B*) nerve of a P15 rat pup that had been unilaterally x-irradiated as described in Figure 1. Note the absence of myelin ensheathment in the x-irradiated nerve. Scale bar, 2 μm.

fields as compared to those in peripheral retinal fields. An ANOVA on the ganglion cell numbers for the normal and irradiated rats provided no evidence of any difference between these two groups ($F = 0.75$, df = 1 and 4, $p = 0.43$). There was also no evidence of differences between rats within either the normal or irradiated groups. The maximum difference in ganglion cell counts between retinas in the same animal was never greater than 3%. Counts between animals were never greater than 6%.

Discussion

Total fiber numbers within the rat optic nerve become stabilized to their adult values toward the end of the first postnatal week, a time that overlaps with the onset of myelination (Sefton and Lam, 1984; Crespo et al., 1985). By preventing oligodendrocyte development and myelin formation, we found that fiber counts within an unmyelinated optic nerve were significantly higher than counts within a myelinated nerve. Further, we found that while an intravitreal injection of FGF at P10 was without effect in normal myelinated optic nerves, it elevated the total fiber numbers within an unmyelinated nerve to up to 40% above normal. Interestingly, this elevation in fiber counts was not constant throughout the length of the nerve, as was the case in normal nerves, but instead showed large fluctuations.

Table 1. Effects of optic nerve x-irradiation on ganglion cell numbers

	Normal	X-irradiated
Rat 1	190.6 ± 19.7	191.8 ± 16.6
Rat 2	190.9 ± 23.5	185.9 ± 36.2
Rat 3	196.3 ± 20.8	192.3 ± 21.7

Data are the number of ganglion cells per field (0.075 mm²) in P15 retinas in the presence or absence of x-irradiation treatment (similar treatment to Fig. 1) to the optic nerve. Twelve fields were examined in each retina and the values shown above represent the mean number of ganglion cells per field ± SD.

The presence of extranumerary fibers within an unmyelinated optic nerve could be accounted for by several processes. (1) An increased survival of retinal fibers during the period of axon elimination could result from the x-irradiation treatment and/or the loss of oligodendrocytes affecting the rate of natural cell death observed in the retina during development (Potts et al., 1982; Perry et al., 1983). Contrary to this, we found that retinal ganglion cells numbers were the same regardless of x-irradiation treatment to the optic nerve. Further, the large fluctuations of axon numbers along the nerves cannot be explained by such a mechanism. (2) The number of either retinoretinal or centrifugal fibers could be increased. A number of points, however, argue against this interpretation. First, although retinoretinal fibers are present within the fiber population during development, they represent a transient population and are eliminated shortly after birth (Bunt and Lund, 1981). Centrifugal axons are present in the normal nerve and survive throughout adulthood. However, this population contributes little to the total numbers of fibers in the adult nerve, that is, approximately 100 fibers (Itaya and Itaya, 1985; Labandeira-Garcia, 1988). Although this number could be higher in irradiation-treated animals, it is very improbable that it could account for the large number of extra

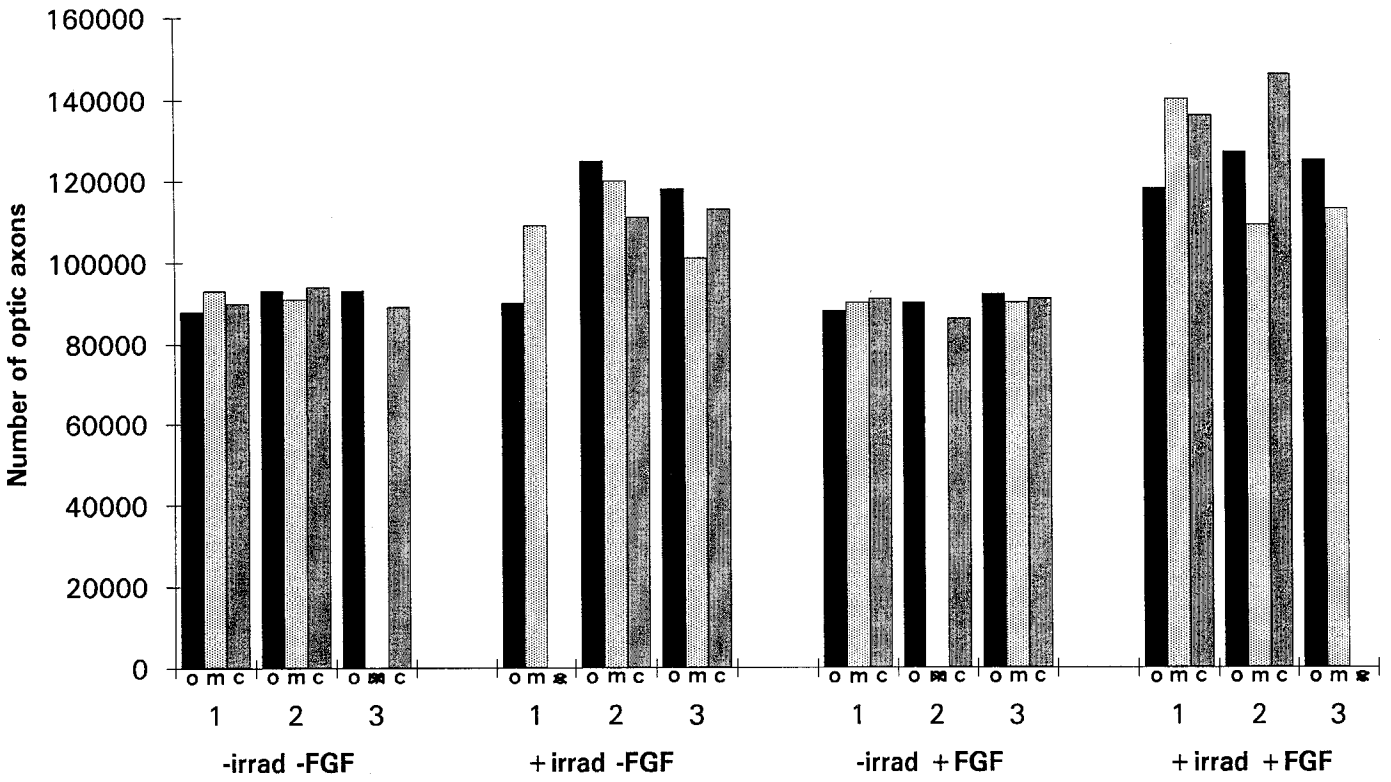

Figure 4. Histogram showing the number of axons present in the intraorbital (*o*), midnerve (*m*), and intracranial (*c*) segments of the optic nerve for rat pups treated with or without x-irradiation and with or without an intraocular injection of FGF. In cases where fiber counts were not possible in a region of the nerve (due to the obliqueness of sectioning) a × has been placed through the letter representing that region. Each animal in a group has been numbered *1*, *2*, or *3*. Notice both the increase and fluctuation of axon numbers in nerves absent of oligodendrocytes. All counts have been rounded to the nearest thousand. The SE of differences between any two of these four group means is 2244.06.

axons observed in the FGF/x-irradiated cases. Such fibers also cannot account for the fluctuations observed, particularly in one animal where fiber counts were elevated in both the intraorbital and prechiasmatic segments but were near normal in the midnerve. (3) Sprouting of side branches by the retinal axons. Random sprouting along the axons in the optic nerve could account for the increase and the fluctuation of the axon numbers observed. The increase of fibers in response to an injection of FGF into the eye is consistent with this hypothesis, especially since FGF receptors are present on rat retinal ganglion cells (Ferguson et al., 1990). FGF has also been shown to increase the survival of lesioned retinal ganglion cells (Sievers et al., 1987; Cadelli and Schwab, 1991), and FGF promotes retinal ganglion cell fiber outgrowth *in vitro* (Bähr et al., 1989). This correlates well with the recent finding that GAP-43 expression is elevated in both developing spinal cords and adult spinal cords demyelinated by x-irradiation treatment (Kapfhammer and Schwab, 1994). As GAP-43 protein is a major component of growth cone membranes and closely linked to axon growth and regeneration (Skene and Willard, 1981; Skene et al., 1986; Benowitz and Routtenberg, 1987), its elevated expression in myelin-free nerves implies a continued growth state. Similarly, irradiated optic nerves stained for GAP-43 tend to express this protein at elevated levels as compared to normal, myelinated optic nerves (R. J. Colello and M. E. Schwab, unpublished observations).

The following model therefore emerges. In the intact, myelin-containing optic nerve, sprouting is absent and axon numbers are totally stable after P10, the end of the axon elimination period. Trophic stimulation of retinal ganglion cells with bFGF does not lead to sprouting in the nerve, suggesting a stabilizing role, perhaps by nonpermissive or an inhibitory effect of the normal nerve environment. X-irradiation at birth removes this inhibitory effect and allows sprouting to occur.

The mechanism by which the normal optic nerve microenvironment suppresses sprouting is open to speculation. Whether this is causally related to the absence of oligodendrocytes and myelin or whether this effect can be attributed to other changes induced by x-irradiation needs to be investigated further. As shown here oligodendrocyte development and myelin formation were completely prevented by x-irradiation treatment. Astrocytes, as judged by GFAP immunofluorescence, were present and produced a process network as in normal nerves. The absence of oligodendrocytes from x-irradiation–treated nerves is of particular interest because these cells express membrane proteins (NI 35/250) that are potent inhibitors of neurite outgrowth (Caroni and Schwab, 1988a; Schwab and Caroni, 1988; Bandtlow et al., 1990). Further, oligodendrocytes secrete components (J1-160/J1-180) with both adhesive and antiadhesive properties for neurons (Pesheva et al., 1989). Interestingly, the neurite growth inhibitors NI 35/250 appear in the developing optic nerve during the first postnatal week, that is concomitant with the end of axon growth and well before the appearance of compact myelin (Caroni and Schwab, 1989). Moreover, these inhibitory proteins are present throughout the life of an animal (Caroni and Schwab, 1988b). They may well play a role in the effects observed here, although more complex mechanisms including oligodendrocyte–astrocyte interactions cannot be excluded.

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