Development of the optic nerve in *Xenopus laevis*

II. Gliogenesis, myelination and metamorphic remodelling

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SUMMARY

We studied the time of origin, development and location of glial elements in the developing optic nerve of *Xenopus* with light and electron microscopy. The first cells acting as a primitive glia are ependymal cells lying dorsal to the chiasmatic optic nerve (CON) at Nieuwkoop & Faber (1956) NF stage 39. Later (stage 47/48), immature astrocyte cell bodies migrate from the periphery of the middle optic nerve (MON) into the central fibre mass along cytoplasmic processes extending from the outer glia limitans. Shortly thereafter, oligodendrocyte cell bodies appear in the centre of the fibre mass and myelination begins, first in the middle of the MON, spreading from the centre distally towards the chiasm and proximally to the retina. In late tadpoles myelinated fibres appear first in the CON then in the retinal optic nerve (RON) increasing markedly in juveniles and adults. Segment-specific patterns of glia and myelination appear during optic nerve development. During metamorphic climax, the optic nerve shortens (Cullen & Webster, 1979), a process involving myelin and axon remodelling primarily in the MON. Neither the profound changes during metamorphosis, nor the processes of gliogenesis and myelination significantly alter the underlying chronotopic ordering in the tadpole nerve. In juvenile and adult optic nerves, however, as myelination and gliogenesis increase, and as more axons mature and grow in diameter, the dorsoventral chronotopic arrangement of axons becomes less apparent.

INTRODUCTION

In the previous paper (Cima & Grant, 1982) we reported on an early chronotopic ordering of fibres in the developing nerve of *Xenopus* with oldest fibres in dorsocentral sectors while the youngest, newly arriving axons, occupy a ventral peripheral position in most parts of the nerve. Such ordering, we believe, reflects the temporal addition of new fibres from the growing retinal margin coupled to the rule of fibre following, with all new fibres growing over the surface of older fibres as they leave the retina and enter the optic nerve (Grant & Rubin, 1980; Cima & Grant, 1982). Maintenance of near-neighbour associations by optic fibres growing in the developing optic nerve of zebra fish has also been suggested by Bodick & Levinthal (1980).

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Though the fibre pattern is established in the embryo, some changes in the pattern occur in the early tadpole between stages 42 and 49. Whereas the fibre patterns in the RON and MON are relatively similar in early stages, they differ by stage 49, after glia and myelin appear in the MON.

The optic nerve also undergoes massive remodelling as it shortens to one third its length during metamorphosis (Cullen & Webster, 1979). This too, may affect fibre organization in juvenile and adult. If these later events do, in fact influence fibre arrangements, then the organization of juvenile and adult optic nerves may reflect these secondary factors and obscure earlier acting morphogenetic factors responsible for fibre ordering. For example, the absence of ordered fibre arrangements in the adult optic nerve of *Rana* (Maturana, 1960) could result from metamorphic remodelling.

A morphometric analysis at the light and EM microscope level of the developing optic nerve through metamorphosis reveals region-specific patterns of glial cell types along the nerve. In addition, myelination is not uniform along the nerve, beginning in the middle optic nerve (MON) and extending bidirectionally, distally towards the chiasm and proximally towards the retina. These changes, coupled to the shortening of the optic nerve during metamorphosis, do not significantly modify chronotopic fibre in the tadpole optic nerve, but later, as the juvenile grows to the adult, the increased levels of gliogenesis and myelination do appear to mask the original chronotopic order.

**Materials and Methods**

The procedures for both light and electron microscopy have been described in the previous paper (Cima & Grant, 1982). Some sections and photographs used for the analysis of early development were also used to identify glial elements and myelin figures. As in the previous study, several different regions of the optic nerve, RON, MON and CON were sampled in each of two or three embryos or tadpoles at particular stages.

*Myelination quantification*

Myelinated fibre counts were made from low magnification (9500 ×) electron micrograph montages. Total myelinated fibre counts were made of each segment of each optic nerve on animals stage 50 through juveniles 4 weeks postmetamorphosis (4 wPM). Juveniles 8 months post-metamorphosis (8 mPM) and adult optic nerves were quantitated over one half of their cross-sectional area and total fibre counts were calculated by extrapolation. Optic nerves from three different animals were used for each count, at each stage. No correction was made for sections through nodes of Ranvier at any stages since the percentage error would be small and consistent through all stages of development.
RESULTS

Neuroglial fine structure in amphibian optic nerves resembles mammalian glial cell types and consists of astrocytes, oligodendrocytes and microglia (Gaze & Peters, 1961; Stensaas, 1977; Reier & Webster, 1974). Astrocytes and oligodendrocytes have been identified in *Xenopus* optic nerve but microglia have not been unequivocally reported. Although developing nerve contains primitive, intermediate and mature glial elements, we have used mature glial characteristics to distinguish glial cells.

Astrocytes have an oval or angular nucleus with a small amount of heterochromatin beneath the nuclear membrane. The cytoplasm is dark (light in young astrocytes), containing numerous 9–10 nm filament bundles (a diagnostic feature), few microtubules, homogeneous dense bodies, glycogen, rough and smooth endoplasmic reticulum and puncta adherentia between their long cytoplasmic processes. Oligodendrocytes possess a deeply staining nucleus with aggregates of heterochromatin adjacent to the nuclear membrane. Their cytoplasm is electron dense because of numerous free and bound ribosomes, granules, smooth and rough endoplasmic reticulum and Golgi. In contrast to astrocytes, they are free of glycogen and filaments but possess aligned microtubules in cytoplasmic processes.

Gliogenesis in CON and MON

There is no evidence of glial development any place along the embryonic primary fascicle. Optic stalk cells may behave as a primitive glia inasmuch as these do surround axon bundles at early stages (Cima & Grant, 1980; Cima & Grant, 1982). In the early embryo (NF stages 28–35/36, Nieuwkoop & Faber, 1956), the primary optic nerve consists of loosely arranged axons and growth cones in association with long expanded profiles of cytoplasmic processes coming from adjacent cells in the rostrodorsal border (Cima & Grant, 1982). By stage 37–38, however, a definitive optic nerve with more compactly arranged axons is seen.

Between stages 37/38 and 40 fine cytoplasmic processes in the CON fibre mass extend from cells which, by virtue of position and morphology, may be ependymal glia (Turner & Singer, 1974). The cells occupy positions in the diencephalon corresponding to that of the ependymal layer bordering the ventricle, dorsal and nasal to the CON. They are distinguishable from optic stalk cells by their darker cytoplasm. They possess large, irregular nuclei, numerous ribosomes, granules, mitochondria, microtubules and microfilaments. Cytoplasmic extensions from these cells penetrate the primary bundle, segregating it into smaller fascicles, a process that continues through development. By stage 50, numerous ependymal cell processes extend laterally and ventrally across the width of the CON forming septa that divide the nerve into stacks of fascicles (Fig. 1). If these ependymal cells are indeed acting as glia, they
represents the first signs of glial cell penetration of the primary fascicle. Only late in tadpole development are glial cell bodies seen in the centre of the CON.

Definitive gliogenesis begins first in the MON. Between NF stages 39/40 and 46 the MON contains a cell-free bundle of large and small unmyelinated fibres, usually penetrated by a single, long cytoplasmic process extending from squamous stalk cells at the periphery (Cima & Grant, 1982). At stage 46/47, a few cell bodies characterized by very pale cytoplasm and a nucleus containing amorphous chromatin can be seen close to the periphery of the nerve (Fig. 2A–C). These glial cell precursors seem to migrate into the MON fibre mass from the surrounding optic nerve sheath between stages 46 and 50. At stage 46 we first see pyramidal cells at the periphery with cytoplasmic processes extending into the mass of fibres (Fig. 2A). Processes from these cells are also continuous with the outer glia limitans covering the optic nerve surface. The cells resemble small glioblasts with almost homogenously distributed chromatin in small nuclei and a modest cytoplasmic heterogeneity. In Fig. 2B we see a cell body just inside the fibre mass with thin cytoplasmic processes continuous with the limiting membrane and in Fig. 2C a cell is seen in the centre of the MON.

The first neuroglia contain a few filaments and some granular material,
Optic nerve gliogenesis and myelination

Fig. 2. Stage 46/47 MON showing immature astroglial cells in positions suggesting migration from periphery into centre of optic nerve. (A) Precursor glial cell body at the periphery of the nerve. (B) Cell body of glial precursor deeper into fibre mass, still attached to glial limitans at the periphery (arrow). (C) Immature glial cell body in centre of fibre mass.

probably ribosomes. By stage 48, some centrally located cells appear to be fully differentiated astrocytes (Fig. 3). Numerous astrocytic processes extend from cell bodies in the centre to the periphery where they form a continuous glia limitans (Fig. 3B). The cytoplasm contains dense quantities of filament bundles, glycogen granules and oval-shaped nuclei with heterochromatic masses.

In later stages, well-differentiated astrocytes with filaments, dense, homogeneously granular nuclei and junctional connexions with adjacent cell processes can be seen. During larval development these, along with other glial elements, continue to accumulate in the more central parts of the fibre mass, forming a dense cellular core in older nerves (Fig. 4). Astrocytic processes penetrate the fibre mass segregating it into fascicles containing myelinated and unmyelinated axons.

Shortly after the appearance of astrocytes, oligodendrocyte precursors can be identified among optic fibres in the MON at stage 49. The nuclei of these cells are indented with irregularly distributed chromatin granules. The cyto-
Fig. 3. Mature astrocytes, stage 48/49 MON. (A) Fibrous astrocytes in centre of fibre mass. (B) Glia limitans, stage 48/49. Astrocytic processes in fibre mass (small arrows) extends to periphery where it spreads over optic nerve surface forming, in combination with other similar processes, the glia limitans (large arrows).

Fig. 4. MON of 8 month postmetamorphic juvenile. Central core of glial cell bodies (astrocytes and oligodendrocytes) with numerous processes continuous with the glia limitans at the surface of the optic nerve. Blood vessels and additional membranes make up the outer optic nerve sheath.
Optic nerve gliogenesis and myelination

Fig. 5. Oligodendrocyte in the MON. Stage 59. A mature dark oligodendrocyte (DO) with lobulated nucleus and dark-staining cytoplasm. Astrocyte processes (Ap) surround the cell body.

plasm is lighter than in the mature cell (Fig. 5) with rough endoplasmic reticulum and Golgi, but without filaments. The immature form may be equivalent to the ‘light immature oligodendrocyte’ described by Vaughn (1969) in the optic nerve of the rat. The more mature form lacks long cell processes, but contains a dense cytoplasm, a lobate nucleus with heterochromatin characteristic of the dark oligodendrocyte.

The larval MON between the optic foramen and the chiasm, the intracranial MON (ICMON), exhibits a different pattern of glial organization. This most distal part of the MON is a transition zone where glial elements gradually shift from the centre towards more dorsotemporal regions of the nerve (Fig. 6). The diameter of the optic nerve progressively decreases (it assumes an ellipsoidal shape) because fibres become more compressed in their trajectory to the chiasm. Fibre size patterns acquire a distinct dorsoventral asymmetry as the nerve approaches the CON (Cima & Grant, 1982). Large myelinated and unmyelinated fibres in the ICMON associate with glial cell bodies in the more dorsal regions which gradually merge into cell bodies of the ependymal region of the CON. Ventral regions filled with small axons are free of glia and myelin.

The numerous dark-staining bodies seen in these sections are degenerating myelin masses typical of the metamorphosing nerve (see below). Note that fewer such masses appear in sections close to the CON where no degenerating myelin is seen.
Fig. 6. Intracranial segment of the distal MON, stage 64. (A) Cross section of optic nerve approximately 1 mm from CON. Most of the myelin debris (black bodies) is located in the dorsocentral portion of the nerve along with glial cell bodies (arrows). (B) Section 100 μm more medial to (A). Myelin debris and glial cell bodies now located more caudally than in (A). (C) Section approximately 150 μm medial to (B). Oligodendrocyte (white arrow) can be seen among the glial cells now shifted more caudally and dorsally than before. Myelin debris is located in extreme dorsal aspect. Note decrease in optic nerve diameter between (A) and (C). (D) Section of optic nerve approximately 60 μm medial to (C). Note the relatively small number of glial cell bodies and total absence of myelin debris. Large myelinated and unmyelinated fibres concentrated in the more dorsal central part of the nerve with smaller fibres concentrated in the ventral and nasal periphery.

**RON/PON**

As axons accumulate in the RON they become tightly packed. After stage 37/38 fine cytoplasmic processes extend from the periphery into the fibre mass. These processes, continuous with those surrounding the optic nerve, are derived from cells possessing dark granular cytoplasm and large nuclei with clumps of membrane-associated chromatin. They may also serve as a primitive glia.

After stage 46 fibres in the retinal optic fibre layer at the nerve head are fasciculated by cells and processes enriched with pigment granules (Fig. 7). Some cell bodies are seen at the periphery and centre of the optic fibre mass in the RON. Except for the presence of melanosomes, these immature cells contain a relatively pale cytoplasm with Golgi, mitochondria and few free ribosomes. The nuclei are irregular with chromatin clumps. At later stages (stage
Optic nerve gliogenesis and myelination

52) these cells have differentiated into pigmented fibrous astrocytes containing numerous melanosomes, filament bundles in a dense cytoplasm, and irregular nuclei with homogeneously distributed chromatin.

The fibre mass of the more distal PON is generally free of cell bodies until stage 50 when well-differentiated astrocytes appear. The glial population of the PON throughout larval development is a mixture of both pigmented and normal fibrous astrocytes.

A summary of the time of origin of glial elements in different regions of the optic nerve is shown in Table 1. Inasmuch as the staging of Xenopus development is variable and somewhat unreliable (Grant, Rubin & Cima, 1980), the stages shown here are only approximations. More frequent samples of animals reared under standard conditions of temperature and nutrition are required to pinpoint the precise stages at which these various glial elements arise.

Although neuroglial populations have not been quantified, the differences in glial cell density between RON/PON and MON is clear. Only a few astrocytes are seen in the RON/PON while the MON is densely populated with both types of macroglia. The RON does not contain any well-defined oligodendrocytic cell bodies at any time in development and it is only late in development, after stage 57, that a few astroglia and oligodendroglia are seen in the
Table 1. Neurological appearance and distribution

<table>
<thead>
<tr>
<th>Optic nerve segment</th>
<th>Astrocytes</th>
<th>Oligodendrocytes</th>
<th>Ependyma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larval</td>
<td>Post-M</td>
<td>Larval</td>
</tr>
<tr>
<td>MON</td>
<td>+ (48)</td>
<td>+</td>
<td>+ (48/49)</td>
</tr>
<tr>
<td>PON*</td>
<td>+ (50)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>RON*</td>
<td>+ (52/53)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CON</td>
<td>+ (57)</td>
<td>+ (Juv 8 mPM)</td>
<td>+ (57)</td>
</tr>
</tbody>
</table>

* Contain both pigmented and normal fibrous astrocytes.

Note: Stage of appearance in parenthesis. Larval = pre-metamorphic. Post-M = Post-metamorphic.

Table 2. Number of myelinated fibres at fixed points in the visual pathway at different stages

<table>
<thead>
<tr>
<th>Stage</th>
<th>RON</th>
<th>PON</th>
<th>MON</th>
<th>CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0</td>
<td>0</td>
<td>73 ± 15</td>
<td>27 ± 13 (37%)</td>
</tr>
<tr>
<td>52</td>
<td>0</td>
<td>18 ± 4 (6%)</td>
<td>322 ± 55</td>
<td>140 ± 22 (43%)</td>
</tr>
<tr>
<td>57</td>
<td>0</td>
<td>173 ± 35 (33%)</td>
<td>523 ± 92</td>
<td>366 ± 65 (70%)</td>
</tr>
<tr>
<td>Juv 4 WPM</td>
<td>73 ± 24 (4%)</td>
<td>710 ± 81 (51%)</td>
<td>1402 ± 120</td>
<td>860 ± 96 (61%)</td>
</tr>
<tr>
<td>Juv 8 mPM</td>
<td>121 ± 57 (3%)</td>
<td>970 ± 120 (28%)</td>
<td>3472 ± 210</td>
<td>2128 ± 141 (61%)</td>
</tr>
<tr>
<td>Adult</td>
<td>222 (3%)</td>
<td>1107 (15%)</td>
<td>7250</td>
<td>6975 (96%)</td>
</tr>
</tbody>
</table>

Note. Each number is the mean of three specimens with the standard deviation, except for the adult, where only one animal was counted. The number shown in parentheses is the percentage of that data point to the MON value of the same stage.

CON. Later, in juvenile optic nerves, more glial cell bodies appear in the CON, but their number, as in the RON, is never large and always much less than in the MON.

**Myelination of the optic nerve**

Myelination begins at stage 48/49 in the MON, concurrent with the appearance of oligodendrocyte cell bodies in the centre of the optic nerve. Of three different nerves examined at this stage, only one showed evidence of myelination in a few fibres (less than nine). Abnormal myelin-like figures are seen occasionally in sections of stage-47 optic nerves but it is uncertain whether these are axons undergoing myelination or abnormal myelin figures sometimes seen in sections of the optic nerve, even at early stages. Myelinated axons are found only in the middle segment of the MON at the onset of the myelination process and are not seen in all sections of the MON in the same nerve. The first myelinated fibres are usually of large diameter (1-0 μm).

By stage 50, the number of myelinated axons in the MON increases to about 73 (Table 2). Sample sections examined at 200 μm intervals along the length of the MON show little variation in the number or size of myelinated fibres.
Myelinated optic fibres are present in the CON at stage 50; their number, however, is about one third that found in the MON. Myelinated fibres in the MON are generally seen in the central portion whereas those in the CON are located in the dorsal aspect of the nerve. No glial cell bodies are seen in the CON at this stage.

By stage 52, a few myelinated fibres appear in the PON, distributed uniformly in the cross-sectional area of the nerve. The number of myelinated fibres in the CON increases, with diameters ranging from 0.65 to 3.5 \( \mu \text{m} \). Large unmyelinated fibres at this stage vary between 0.5 and 1.8 \( \mu \text{m} \) in diameter.

By stage 57, the percentage of myelinated fibres in the CON has risen to 70\% of MON values, while in the PON it has risen to 33\%. In most instances, the largest axons are myelinated. No fibres in the RON are myelinated at stage 57.

In the juvenile RON, the number of myelinated axons increases slightly absolute numbers of myelinated fibres increase in the PON although they decline relative to the MON in late juvenile and adult. Myelinated fibres increase in the CON reaching, in the adult, levels found in the MON. Many more smaller axons are myelinated in juvenile and adult optic nerves; most of these occupy peripheral positions (Fig. 4).

**Metamorphic remodelling**

During metamorphosis the optic nerve shortens from a maximum length of 4700 \( \mu \text{m} \) to 1700 \( \mu \text{m} \) (Cullen & Webster, 1979). This occurs primarily in the MON and is accompanied by extensive changes in myelinated fibres. We have confirmed their observations. In sections of the MON from stage 60 to 66 we have found redundant myelin loops both inside myelinated axons and in the extracellular space (Fig. 8). Similarly, myelin debris apparently phagocytosed, is found in both astrocytes and oligodendrocytes. In addition, we have found delaminating myelin lamellae and loops of unmyelinated axons in all areas of the MON. These changes vary in gradient fashion along the length of the MON from a high point in the centre extending distally and proximally several hundred microns to either end of the MON.

The RON and intracranial segment near the CON, on the other hand, do not undergo metamorphic remodelling typical of the MON. Serial sections of the stage-64 MON at the light level from the cranial fossa towards the brain show that the affected area is progressively restricted to more dorsal regions of the nerve (Fig. 6). The nerve in the intracranial segment close to the chiasm shows no change in myelination.

There are a few small regions of condensed myelin debris in both the PON and CON of the metamorphosing animal. Such debris is found in all regions of the optic nerve before and after metamorphosis in practically all specimens regardless of age and may represent spontaneous events which occur throughout the animal’s life.
Fig. 8. Metamorphic remodelling in stage 61 MON. (A) Low-magnification section of the MON showing redundant myelin loops and myelin debris (arrows). (B) High magnification micrograph of abnormal whorl of unmyelinated axons. Note cross-sectional profiles of microtubules in central whorls (arrows). Such unmyelinated spirals are seen in the centre and periphery of the nerve. (C) High-magnification micrograph of redundant myelin loops seen intra-axonally (arrow) and extracellularly (small arrows).
DISCUSSION

Both gliogenesis and myelination begin in the larval optic nerve long after the primary fascicle is established, tectal innervation has occurred and visual function is initiated (Gaze, Keating & Chung, 1974). They begin at stage 46/47 about the time the visuotectal map becomes organized. The origin and patterning of glia and myelination are not uniform along the developing optic nerve; region-specific patterns of gliogenesis and myelination are seen in RON, MON and CON. Such heterogeneity differentially affects the pattern of fibre growth in the nerve and secondarily modifies chronotopic ordering of fibres, particularly as newly metamorphosed juveniles mature into adults.

Gliogenesis and myelination in MON

Definitive gliogenesis and myelination begin first in the MON, sometime after stage 46. In the embryo and early larva all cell bodies in the MON are located at the periphery, as small groups of squamous cells derived from the optic stalk. These precursors seem to migrate from the periphery into the centre of the fibre mass between stages 46–50. Before migration, their cell bodies, attached to the glia limitans, spin out several long radiating cytoplasmic processes into the central fibre mass. The perikaryons migrate into these processes very much like the migration of neuroepithelial cell bodies in the ventricular layer during neurogenesis.

These first glial cells in the MON are precursors of fibrous astrocytes. As they mature, they send out many cytoplasmic extensions, fasciculating groups of axons at random. Shortly thereafter, oligodendrocyte precursors are seen along with a few large myelinated fibres. Later arrival of oligodendrocytes also occurs in the developing optic nerves of the rat and chick (Skoff, Price & Stocks, 1976; Arees, 1978). The number of glial cell bodies and cytoplasmic processes increases during development by proliferation and/or migration of peripheral cells from the glia limitans, or as a result of proliferation of central glia, forming a complex cellular core in the nerve, with astrocytes far more numerous than oligodendrocytes (Skoff et al. 1976). The area occupied by glial elements in the adult optic nerve varies between 35 and 50% in Necturus (Kuffler & Nicholls, 1976), and the monkey (Minckler, McLean & Tso, 1976). If this is also true for Xenopus, it represents a significant cellular volume in the optic nerve core that begins to build up at stage 48. The myelin layers surrounding large axons in the centre also contribute to the non-axonal volume.

The process of myelination is bidirectional in the optic nerve. Starting initially in the central MON at stage 48/49 after oligodendrocytes differentiate, myelination first progresses distally to the CON, subsequently to the PON and RON in the proximal direction. The stage at which myelin first appears is imprecise because staging Xenopus tadpoles is unreliable (Grant et al. 1980). This probably
explains why Gaze & Peters (1961) detected myelin at stage 49, while Wilson (1971) reported the first myelinated fibres at stage 46.

According to Wilson, myelinated fibres between stages 46 and 52 range in diameter from 0.3 to 0.6 μm, while unmyelinated fibres range from 0.1 to 2.7 μm. Others, however, have noted that large axons are the first to myelinate in the optic nerve or other parts of the nervous system (Matthews, 1968; Friede & Samorajski, 1967; Arees, 1978). A critical axon diameter is not necessary for myelination since later in optic nerve development, small fibres are also myelinated (Wilson, 1971). In our material, myelin first appears around the largest axons. Either large fibres are selected for myelination and/or fibres also increase in diameter as a result of myelination. Since most large axons appear in the dorsocentral regions of the tadpole MON, most myelin will accumulate in these regions leaving the ventral periphery relatively free of myelin.

Gliogenesis and myelination in CON

No glial cell bodies are seen in the CON until stage 57, the beginning of metamorphic climax. Instead, it appears that ependymal cells lying dorsal to the CON behave as a primitive glia as early as stage 37/38; they extend fine cytoplasmic processes into the CON fibremass and subdivide it into fascicles (Cima & Grant, 1982). We suggest that when pioneering axons first grow into the CON and embryonic diencephalon, they do so on the surfaces of dorsally lying ependymal cells or their cytoplasmic processes. Hence, the oldest axons collect in the dorsal CON. Later-arriving axons will grow on the surfaces of these pioneers and, accordingly, will occupy a more ventral position. As more fibres accumulate in the CON in this manner, a chronotopic gradient of fibres (and fibre sizes) develops (Cima & Grant, 1982). Throughout development, new fibres arriving at the CON from the intracranial MON tend to collect more ventrally because they grow over the surfaces of pre-existing fibre bundles. The glial cells developing in the intracranial MON (astrocytes and oligodendrocytes) are found in progressively dorsal positions amidst large myelinated and unmyelinated fibres as the nerve extends towards the CON (Cima & Grant, 1982). The glial cell bodies in the ICMON become continuous with dorsal ependymal cells in the CON.

Oligodendrocyte cell bodies are not found nearer than 150–200 μm from the CON during early larval life, yet myelinated fibres are seen as early as stage 50. The source of myelin in the CON may be the ependymal cell (Turner & Singer, 1974), although in late tadpoles and juveniles, oligodendrocyte cell bodies in the CON may account for more extensive myelination noted after metamorphosis.
Optic nerve gliogenesis and myelination

Gliogenesis and myelination in RON/PON

Gliogenesis in the RON/PON begins when a few pigmented astrocyte precursors are seen at stage 49/50. Definitive astrocytes accumulate in the RON during metamorphosis though the significant increase occurs during juvenile growth. No oligodendrocytes appear in the RON/PON at any time in development. Most cell bodies in the RON are long and thin, aligned parallel to the long axis of fibre bundles in the nerve. No central core of glial cell bodies or myelin develops in the tadpole or juvenile RON.

Myelin in the RON/PON may arise from oligodendrocytes near the MON/PON interface whose processes extend into proximal segments. On the other hand, astrocytes in the RON/PON may myelinate optic fibres inasmuch as astrocytes myelinate cat optic fibres at the lamina cribosa, a region devoid of oligodendrocytes (Wendell-Smith, Blunt & Baldwin, 1966; Blunt, Baldwin, & Wendell-Smith, 1972).

Similar non-homogeneous patterns of myelination and glial cell distribution have been noted in the neonatal rat and rabbit (Skoff, Toland & Nast, 1980) which means that these optic nerves possess an heterogeneous organization along their length. Region-specific patterns of myelination may reflect locally specific axon–glial interactions.

Gliogenesis, myelination and chronotropic ordering of fibres

Glial and myelin pattern heterogeneity along the nerve may have a differential effect on axon growth and chronotropic ordering of fibres. In contrast to both the RON and CON, a glial–myelin matrix begins to accumulate in the central core area of the larval MON at stage 48, maximally in the middle, grading off proximally towards the retina and distally towards the CON. In the intracranial MON, the glial–myelin matrix shifts dorsally towards the CON where it eventually joins with the dorsal ependymal cells adjacent to the CON. Prior to stage 48 chronotropic ordering of fibres is relatively similar along the optic nerve from RON to CON (Cima & Grant, 1982). We suggest that the developing glial–myelin matrix affects subsequent growth of new fibres into the MON, and ultimately into the CON.

According to this hypothesis, many young fibres entering the centre of the larval MON from the relatively glial free PON encounter a growing cellular–myelin matrix and will, as a result, acquire a peripheral trajectory, taking a path of least resistance among the smaller unmyelinated fibres. Later-arriving fibres, by following their immediate neighbours, would also be diverted to more peripheral regions of the optic nerve as they follow the peripheral trajectory established by previous fibres; small, incoming fibres will collect at the periphery. It should be noted that gliogenesis begins before ventral retina attains its dramatic growth spurt at stage 52 (Beach & Jacobson, 1979). After this stage most incoming fibres come from ventral retina and will be diverted into the
ventral periphery. We suggest that the original chronotopic ordering established in the embryonic primary fascicle is enhanced by two factors arising later in development, a disproportionate increase in the number of fibres arriving from ventral retina, and a peripheral trajectory of centrally growing fibres as they enter the MON. Both these processes may contribute to the formation of the distinct crescent-shaped peripheral band of small incoming fibres in the ventral MON and CON, at least in tadpole stages (Cima & Grant, 1982). Since gliogenesis and myelination begin much later in the RON and CON, no central core develops in either region and the trajectory of fibres in these regions is essentially unchanged from earlier stages.

During metamorphosis, there is no gross change in fibre pattern though the MON undergoes considerable shortening as myelin degenerates and fibres assume an abnormal appearance. Both the RON and CON exhibit no signs of metamorphic remodelling. Evidently, the factors responsible for these changes are effective only over a specific region of the optic nerve.

After metamorphosis, however, fibre number doubles as juveniles grow into adults (Wilson, 1971). Both gliogenesis and myelination increase through the nerve and fibre patterns undergo some change. Many more fibres mature, increase in diameter and myelinate, and as many more intermediate and small fibres become myelinated, the dorsoventral asymmetry of fibre size is lost, to be replaced by a peripheral–central gradient of small to large myelinated fibres characteristic of juvenile and adult nerves. In spite of these changes and the profound remodelling changes during metamorphosis, optic fibre organization retains, for the most part, a chronotopic ordering, reflecting in large measure, the temporal ordering of ganglion cell birthdays at the growing retinal margin. Here we have a case of temporal ordering of ganglion cell birthdays that is translated into spatial ordering of optic nerve fibres. The question arises whether the second rule, that of fibre following on near neighbours, is sustained throughout development since changes in fibre trajectories do occur in the MON. If, indeed, the fibre-following rule holds, then we would predict that fibre order in the optic nerve is retinotopic as well as chronotopic. The final paper in this series is directed to this question.

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