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# Early development of the optic nerve in the turtle *Mauremys leprosa*

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ABSTRACT

We show the distribution of the neural and non-neural elements in the early development of the optic nerve in the freshwater turtle, *Mauremys leprosa*, using light and electron microscopy. The first optic axons invaded the ventral periphery of the optic stalk in close relationship to the radial neuroepithelial processes. Growth cones were thus exclusively located in the ventral margin. As development progressed, growth cones were present in ventral and dorsal regions, including the dorsal periphery, where they intermingled with mature axons. However, growth cones predominated in the ventral part and axonal profiles dorsally, reflecting a dorsal to ventral gradient of maturation. The size and morphology of growth cones depended on the developmental stage and the region of the optic nerve. At early stages, most growth cones were of irregular shape, showing abundant lamellipodia. At the following stages, they tended to be larger and more complex in the ventral third than in intermediate and dorsal portions, suggesting a differential behavior of the growth cones along the ventro-dorsal axis. The arrival of optic axons at the optic stalk involved the progressive transformation of neuroepithelial cells into glial cells. Simultaneously with the fiber invasion, an important number of cells died by apoptosis in the dorsal wall of the optic nerve. These findings are discussed in relation to the results described in the developing optic nerve of other vertebrates.



## 1. Introduction

In the visual system of vertebrates, the axons of retinal ganglion cells (RGCs) project to several mesencephalic and diencephalic structures maintaining a high degree of retinotopic order along the optic pathway. During development, the growth cones (GCs) advance by specific routes changing their morphology in different cellular context (Bovolenta and Mason, 1987). In amphibians and fishes, the GCs advance exclusively in the peripheral region of the optic nerve (Easter et al., 1981; Cima and Grant, 1982; Springer and Mednick, 1986; Taylor, 1987), while in birds and mammals the axonal growth

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*Abbreviations:* CSPGs, chondroitin sulfate proteoglycans; EMCs, extraventricular mitotic cells; GC(s), growth cone(s); GP(s), glial precursor(s); RGC(s), retinal ganglion cell(s)

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shows a ventral to dorsal gradient (Williams and Rakic, 1985; Williams et al., 1986; Colello and Guillery, 1992; Reese et al., 1994; Drenhaus et al., 2000).

The growth of the newly generated axons could be associated with environmental structures including the extracellular matrix (Thanos and Mey, 2001), neighboring axons (Navascués et al., 1987; Drenhaus et al., 2000; Thanos and Mey, 2001), and the neuroepithelial and glial processes, which provide adhesive cues to GCs (Silver and Sidman, 1980; Silver and Rutishauser, 1984; Navascués et al., 1985; Horsburgh and Sefton, 1986; Maggs and Scholes, 1986; Williams et al., 1986; Thanos and Mey, 2001). Cell death, implicated in the early development of the optic nerve (Navascués et al., 1985; Horsburgh and Sefton, 1986; Moujahid et al., 1996; Rodríguez-Gallardo et al., 2005), might also play a role in the development of the optic pathway. Once the optic fibers are present in the optic nerve, they could induce neuroepithelial cell division, cell migration, and subsequent differentiation to glial cells (Ulshafer and Clavert, 1979; Navascués et al., 1985, 1987, 1989). Together, these events play a decisive role in establishing the definitive cytoarchitecture of the adult optic nerve.

Data concerning the development of the reptile optic pathway are sparse (Dunlop et al., 2002; Francisco-Morcillo et al., 2004, 2006; Hidalgo-Sánchez et al., 2006). Recent studies conducted in our laboratory have analyzed the patterns of proliferation, differentiation, and cell death in the retina (Francisco-Morcillo et al., 2004, 2006; Hidalgo-Sánchez et al., 2006), and the sequence of the developmental changes in the number of fibers in the optic nerve (Hidalgo-Sánchez et al., 2006) in the turtle *Mauremys leprosa*. The turtle primary visual pathway begins to develop at about E16, when the first RGCs are observed in a region slightly dorsal to the optic nerve head (Francisco-Morcillo et al., 2006). The number of RGCs increases rapidly until E34, and at E40 the generation has finished (Hidalgo-Sánchez et al., 2006). RGCs die by apoptosis between E23 and E64 (Francisco-Morcillo et al., 2004; Hidalgo-Sánchez et al., 2006), with a reduction of about 42% with respect to the maximum (Hidalgo-Sánchez et al., 2006). Taken together, these results suggest that the mechanisms of development in the turtle optic nerve could be similar to that reported the birds and mammals.

Here we analyze, using light and transmission electron microscopy, the early development of the turtle optic nerve from the embryonic day 14 (E14; before the first optic fibers are generated in the retina) to E21 (the stalk lumen is eliminated) to check whether development follows the avian and mammalian vertebrate model. We focused our attention on four aspects: (i) the topographic distribution of GCs; (ii) the frequencies of GCs in the fascicles; (iii) morphology of the GCs; and (iv) proliferation, migration, and cell death of the optic nerve cells. The present work provides evidence that the arrival of optic axons at the optic stalk ventral wall is involved in the progressive transformation of neuroepithelial cells into glial cells. Simultaneously, in the dorsal wall an important number of cells die by apoptosis. The analysis of the distribution of GCs showed that they were found in nearly all fascicles, but with a ventral to dorsal gradient. Moreover, the GCs tended to be more irregular in superficial regions than in internal ones. These findings are discussed in relation to the results described in the developing optic nerve of other vertebrates.

## 2. Results

### 2.1. *Morphological characteristics of the early developing optic nerve*

At E14, before the arrival of the first ganglion cell axons, transverse section showed that the optic stalk presented a rounded structure, with a central lumen which corresponds to the optic ventricle (Figs. 1A, B). The wall of the optic stalk was composed by a pseudostratified neuroepithelium with the cell nuclei located at different levels. Close to the retina (distal portion), the dorsal wall was thinner than the ventral one (Fig. 1A), where elongated neuroepithelial nuclei were arranged radially. However, in the vicinity of the chiasm anlagen

**Fig. 1 – Cell proliferation, cell death, gliogenesis and fiber invasion during turtle optic nerve development. Transverse (A, B, D–L) and longitudinal (C) optic nerve sections obtained from E14 (A, B), E16 (C–E), E18 (F–I), E19 (J), and E21 (K, L) embryos. Dorsal is up and temporal is to the right. Semithin (A, B, D–I, K, L) and paraffin (C, J) sections stained by toluidine blue and TUNEL technique, respectively. (A, B) Before the arrival of the earliest optic axons (E14), the distal (A) and proximal (B) portions of the optic nerve showed abundant mitotic figures, in close contact with the ventricular lumen (large arrows), and a few extracellular spaces, located in the ventral (A) and ventro-lateral (B) regions (arrowheads). (C) TUNEL-positive bodies were found in the optic nerve at E16, in close proximity to the optic fibers (asterisks). (D, E) At E16, the optic fascicles (asterisks) were concentrated in the ventral periphery from the distal (D) to the proximal (E) regions. Panel E shows an extraventricular mitosis (large arrows). Melanosomes (arrowheads in panel D) and pyknotic profiles (small arrows in panel E) were clearly distinguished in the dorsal region. (F–I) At E18, transverse sections to the medial (F) and proximal (G–I) regions of the optic nerve showed densely packed glial precursors (GPs) both delimiting dorsally (large arrows) the optic fascicles (asterisks) and intermingled between them (arrowheads). Elongated pear-like (H) and wavy (I) nuclei of GPs (arrowheads) were observed in the ventral wall, suggesting that these cells are migrating towards to the ventral surface. Dead cells were also found in the dorsal wall (small arrow in panel F). (J) At E19, the dorsal wall of the optic nerve showed abundant TUNEL-positive nuclei (small arrows). (K, L) By E21, pyknotic bodies were abundant in the dorsal wall of the medial region (small arrows in panel L; dorsal region) and scarce in the ventral region of the distal and medial thirds (small arrows in panels L, K; ventral region). Notice that the nuclei of GPs were more elongated in ventral regions of the optic nerve. Radially oriented mitotic figures were clearly seen (large arrows). Thick cellular processes could be clearly distinguished (arrowheads), extended radially from the cell bodies and terminated in end-feet at the ventral surface. ov, optic ventricle; pe, pigment epithelium; nr, neural retina. Scale bars denote 40 μm in panel A (A, B, D, E), 28 μm in panel C, 32 μm in panel I (H, I), 35 μm in panel J (F–J), 50 μm in panel L (K, L).**