Experimental Eye Research 92 (2011) 274–281

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Retinotopy of visual projections to the optic tectum and pretectum in larval sea lamprey

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ARTICLE INFO

Article history:
Received 5 November 2010
Accepted in revised form 26 January 2011
Available online 3 February 2011

Keywords:
agnathans
retinotopy
biplexiform ganglion cells
optic tract
development

ABSTRACT

The sea lamprey has a complex life cycle with very different larval and adult stages. The eyes of larvae are subcutaneous, lack a differentiated lens and probably work only as an ocellus-like photoreceptor organ, while the well-developed adult eyes are capable of forming images. The larval retina differs greatly from the adult retina and presents a central region with differentiated photoreceptors and a lateral, largely undifferentiated part that grows in the second half of larval life. In the present study, we examined the retinotopy of projections from larval ganglion cells to the optic tectum and pretectum in sea lamprey by using retrograde tract-tracing techniques. In most regions of the tectum, application of the tracer neurobiotin (NB) resulted in labelled ganglion cells in the lateral retina, mostly in the contralateral eye. Ganglion cells of the lateral retina showed a very simple dendritic tree, possibly because of the lack of differentiation of most retinal layers in this region. The retinotectal projection is already retinotopically organized in larvae and follows a pattern similar to that observed in adult lampreys and other vertebrates. Application of NB to the central region of the tectum also led to labelling of a few ganglion cells in the central retina, which were clearly more complex than those in the lateral region, as they had dendrites that branched both in the outer and inner plexiform layers. Application of NB to the medial pretectum led to labelling of ganglion cells in the contralateral central retina. Occasional cells were also labelled in the lateral retina. The differential organization of larval retinal projections to the pretectum and tectum suggests a different role for these projections, which is consistent with the different involvement of these centres in visual behaviour, as determined in adult lampreys. The observations in larvae also reveal very different developmental timetables for these putative functions.

1. Introduction

The sea lamprey is a living species of the Agnatha, the oldest extant vertebrates. Sea lampreys have a complex life cycle that comprises an embryonic period followed by a non-feeding prolarval stage and a very long larval period, which lasts between 5 and 7 years. Larval lampreys are filter-feeding animals that remain burrowed in the river sediment. After the larval period, larvae undergo a complex metamorphosis and transform into active parasitic adults. The key phylogenetic position of lampreys, together with their unusual life cycle makes them an interesting "evo-devo" animal model. The development of the visual system of lampreys is exceptional among vertebrates, since the retina develops two characterised zones (central and lateral retina) at different stages before its complete development. Formation of the lamprey retina begins early on in embryogenesis with a small region (central retina) that becomes differentiated in prolarvae. However, only a single type of photoreceptor, ganglion cells and bipolar cells appear to be differentiated at this stage, and no amacrine or horizontal cells have been observed in the small central retina of larvae, which locates surrounding the optic nerve head (Meléndez-Ferro et al., 2002; Villar-Cerviño et al., 2006; Villar-Cheda et al., 2006). The late-developing peripheral retina (lateral retina) begins to grow in medium-sized larvae and remains largely neuroblastic until metamorphosis (de Miguel and Anadón, 1987; Meléndez-Ferro et al., 2002). Unlike in most vertebrate species, the differentiation of retinal cells in the lateral retina occurs over a very long period of time: ganglion cells develop gradually during the late larval period, whereas the differentiation of amacrine, horizontal, photoreceptor

Abbreviations: CR, central retina; IGCs, inner ganglion cells; INbL, inner neuroblastic layer; INL, inner nuclear layer; IPL, inner plexiform/optic fibre layer; LaR, lateral retina; LR, lateral region; NB, neurobiotin; OGCs, outer ganglion cells; OLM, outer limiting membrane; ONbL, outer neuroblastic layer; ONL, outer nuclear layer; OPL, outer plexiform layer; P, photoreceptor; PR, periventricular region.

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doi:10.1016/j.exer.2011.01.011

and bipolar cells only takes place during metamorphosis (Abalo et al., 2008; de Miguel and Anadón, 1987; de Miguel et al., 1989; Meléndez-Ferro et al., 2002; Rubinson and Cain, 1989; Villar-Cheda et al., 2006, 2008); the central and lateral retinas cannot be distinguished after metamorphosis. During metamorphosis, the eye also acquires the capacity to form images.

In larval lampreys, the optic nerve gives rise to two optic tracts, the axial and lateral optic tracts. The central retina gives rise to the axial optic tract, which projects to the pretectum, while the lateral retina gives rise to the lateral optic tract that projects to the optic tectum (de Miguel et al., 1990). Retinopetal fibres arising in midbrain neurons course in the axial optic tract (de Miguel et al., 1990) and the three retinopetal nuclei arise sequentially during the larval period (Rodicio et al., 1995). In adult lampreys, the retinal projections to both the pretectum and tectum have been described (Jones et al., 2009). The retinal projection to the optic tectum shows the same retinotopic pattern as in other vertebrates, whereas the retinal projection to the pretectum originates from a group of ganglion cells with dendrites that appear to contact photoreceptors (Jones et al., 2009). Electrophysiological studies in adult lampreys have demonstrated that the pretectum mediates the visual escape responses and the dorsal light response (Deliagina and Fagerstedt, 2000; Ullén et al., 1993, 1995), whereas the optic tectum mediates goal-oriented motor behaviour controlling eye, head and body movements (Saitoh et al., 2007). The retinopetal system of adult lampreys (Vesselkin et al., 1984) is similar to that of large larvae.

Because of the very different lifestyles of larval and adult lampreys, we decided to investigate the pattern of retinal projection to the optic tectum and pretectum, and the types of ganglion cell present in the retina during the larval period. The types of ganglion cells and their projections in larvae are compared with those of adults and the relationship between the retinal projection pattern and the development of the sea lamprey is discussed.

2. Materials and methods

Larval ($n = 32$; 90–130 mm in total body length) sea lamprey (*Petromyzon marinus* L.) were used in the present study. Animals were collected from the River Ulla (Galicia, northwest Spain) and maintained in fresh water with a bed of river sediment, until their use. Before experiments, all animals were deeply anaesthetized with 0.05% benzocaine (Sigma, St. Louis, MO) in fresh water. The experiments were approved by the Bioethics Committee of the University of Santiago de Compostela and conformed to the European Union (86/609/EEC) and Spanish (Royal Decree 223/1998) regulations for the care and handling of animals in research.

2.1. Retrograde tract tracing

The larval mesencephalon and caudal diencephalon were exposed by means of a longitudinal incision made in the dorsal skin of the head, caudally to the pineal fontanel. Small crystals of Neurobiotin (NB; 322.8 Da molecular weight Vector, Burlingame, CA) were applied with a minute pin (000) at different rostrocaudal and dorsoventral levels of the optic tectum as well as the pretectum. All NB applications were unilateral and of these 20 were restricted to the pretectal ($n = 5$) or tectal ($n = 15$) zone chosen; therefore, other 12 larvae were excluded from analysis by failure in the site of application or insufficient tracer transport. In the pretectum, NB was applied through the dorsal surface in order to avoid the lateral optic tract. The incision was then closed with Histoacryl® tissue adhesive (B. Braun Surgical, Tuttlingen, Germany). The animals were maintained at 4 °C, with appropriate aeration conditions, in lamprey Ringer solution (137 mM NaCl, 2.9 mM KCl, 2.1 mM $CaCl_2$, 2 mM HEPES) for 2 days, to allow transport of the tracer.

At the end of the incubation period, the larvae were deeply anaesthetised, and the heads were removed, then fixed by immersion in freshly prepared 4% paraformaldehyde in 0.4 M Tris buffer saline (TBS; pH 7.4) for 6 h. Samples were then rinsed in TBS and cryoprotected with 30% sucrose in TBS overnight, embedded in Tissue Tek (Sakura, Torrance, CA), frozen in nitrogen-cooled isopentane, and cut serially on a cryostat (20 μm thick) in transverse planes. Sections were mounted on subbed glass slides.

Sections were incubated at room temperature with fluorescein isothiocyanate (FITC)-labelled avidin-D (Vector, Burlingame, CA) diluted 1:1000 in TBS containing 0.3% Triton X-100 for 4 h. Sections were then rinsed in distilled water and coverslipped with mounting medium for fluorescence (Vectashield; Vector). In addition, some sections of heads of untreated larvae fixed as above were treated with FITC-labelled avidin-D to rule out the possibility that staining was due to endogenous brain biotin. No FITC staining was observed in these sections.

2.2. Fluorescence microscopy and image processing

Fluorescence photomicrographs were taken with a spectral confocal laser microscope (Leica TCS-SP2), converted to grey scale and adjusted for brightness and contrast with Adobe Photoshop 7 software.

2.3. Additional material

Haematoxylin–eosin stained sections from our laboratory collection were available for comparison.

3. Results

The application of NB to the pretectum or the optic tectum gave rise to labelled cells in the larval retina. In the larvae under study (body length 90–130 mm), the retina consisted of a small differentiated central retina and a large undifferentiated lateral retina (Fig. 1A). The central retina was mainly located dorsal to the optic nerve head, and consisted of photoreceptor (P), outer nuclear (ONL), outer plexiform (OPL), inner nuclear (INL) and inner plexiform/optic fibre (IPL) layers (Fig. 1B). The lateral retina was largely neuroblastic with a narrow primordial IPL on its vitreal side. In the region close to the central retina, the inner and outer neuroblastic layers (INbL and ONbL, respectively) were distinguishable (Fig. 1C). The optic nerve exit was located in the ventral half of the retina, near the centre of the dorsoventral axis and roughly in the middle of the anteroposterior axis. In larvae, the dorsal and ventral retina can be easily distinguished as the ventral retina lacks melanin in the retinal pigment epithelium (de Miguel et al., 1992). Since the sections analyzed in this study were transverse to the head, for description of results the anterior, posterior, dorsal and ventral parts were considered in relation to the optic nerve exit.

3.1. Retino-pretectal projection

Two regions were distinguished in the pretectum of larval lampreys: a periventricular region with densely packed cell bodies, and a lateral region that mainly consisted of neuropil, fibre tracts and a few scattered neurons (Fig. 2A). As reported by de Miguel et al. (1990), the lateral optic tract coursed superficially in the pretectum, while the axial optic tract was ventromedial to it (Fig. 2B) and the fibres gave rise to a terminal field in the pretectum.

To establish which cells give rise to the retinal projection to the pretectum, NB was applied to the lateral and to the dorsomedial surface of the pretectum. Application of NB to the pretectum from the lateral surface affected both the lateral and the axial optic tracts,