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The pineal complex of Senegalese sole (*Solea senegalensis*): Anatomical, histological and immunohistochemical study

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ABSTRACT

Many fish exhibit seasonal reproductive rhythms that are synchronized by environmental cues such as photoperiod and temperature. The pineal organ of teleost fish, a non-visual photosensitive structure, plays an important role in this synchronization because it transduces the photoperiodic and temperature information into neural and neurohormonal messages that reach the brain and the reproductive axis. In this study, we describe the anatomy and histology of the pineal complex of Senegalese sole, *Solea senegalensis*, a seasonal species of increasing importance for aquaculture. We have also analyzed the presence of photoreceptor and melatonin-producing cells using immunohistochemical techniques. The pineal complex of sole consists of the pineal and parapineal organs, and a highly lobulated dorsal sac. The sole exhibits a real metamorphosis during early developmental stages that induces an asymmetry of the rostral forebrain areas, including the pineal organ that shifts its photosensitive pineal vesicle towards the upper-right pigmented side, where both eyes are also placed. To the best of our knowledge, such a marked asymmetry in the position of the epithalamic pineal organ has never been described in fish. The pineal vesicle is hypertrophied, probably reflecting an adaptation to benthonic life. Melatonin-secreting cells, which were revealed using antisera against its precursor serotonin and against hydroxyindole-*O*-methyltransferase, appear to be direct photoreceptive cells because they adopt a distribution similar to that of the cone opsin- and rodopsin-immunostained cells, along the pineal stalk and vesicle. This work and other ongoing studies analyzing pineal projections and melatonin targets will give us an important basis for understanding the integration of the photoperiodic signal in this species.

1. Introduction

In teleosts, the pineal organ is a functional, non-image forming photoreceptive structure that transduces photoperiodic information into neural and neurohormonal messages (Ekström and Meissl, 1997, 2003; Falcón et al., 2007a). The neural message reaches the brain via axonal projections, and the neurohormonal signal is melatonin, a time-keeping molecule synthesized in pinealocytes and released rhythmically into the cerebrospinal fluid and blood during the night time (Zachmann et al., 1992; Ekström and Meissl, 1997; Falcón et al., 2007a). Melatonin rhythms not only codify daily time but also calendar time since their duration and amplitude change over the seasons in accordance with photoperiod and temperature, respectively (Reiter, 1993; García-Allegue et al., 2001). This hormone is directly involved in many rhythmic physiological processes, such as reproduction, feeding, growth, development and metamorphosis (Khan and Thomas, 1996; Vanecek, 1998; Wright, 2002; Danilova et al., 2004; López-Olmeda et al., 2006; Ziv and Gothilf, 2006; Falcón et al., 2007a,b).

Descriptive analysis of pineal histology, histochemistry, immunohistochemistry and/or ultrastructure has been carried out in species belonging to many fish orders (Ekström and Meissl, 1997; Falcón et al., 2007a). Pleuronectifom species represent interesting models for the analysis of the pineal organization because they exhibit a real metamorphic process during early developmental stages. This metamorphosis determines a shift in the plane of swimming and an asymmetry in the main sensory structures (eyes, olfactory organs), as well as in the anterior pole of the forebrain (Rodríguez-Gómez et al., 2000a; Fernández-Díaz et al., 2001). These transformations are accompanied by changes in their habitats where they encounter markedly different environmental light conditions. Thus, pre-metamorphic pelagic larvae swimming in the water column develop into benthonic juveniles and adults living on the sandy floor of the sea. However, little is known concerning the anatomical and functional organization of the pineal organ in pleuronectiform fish and only a

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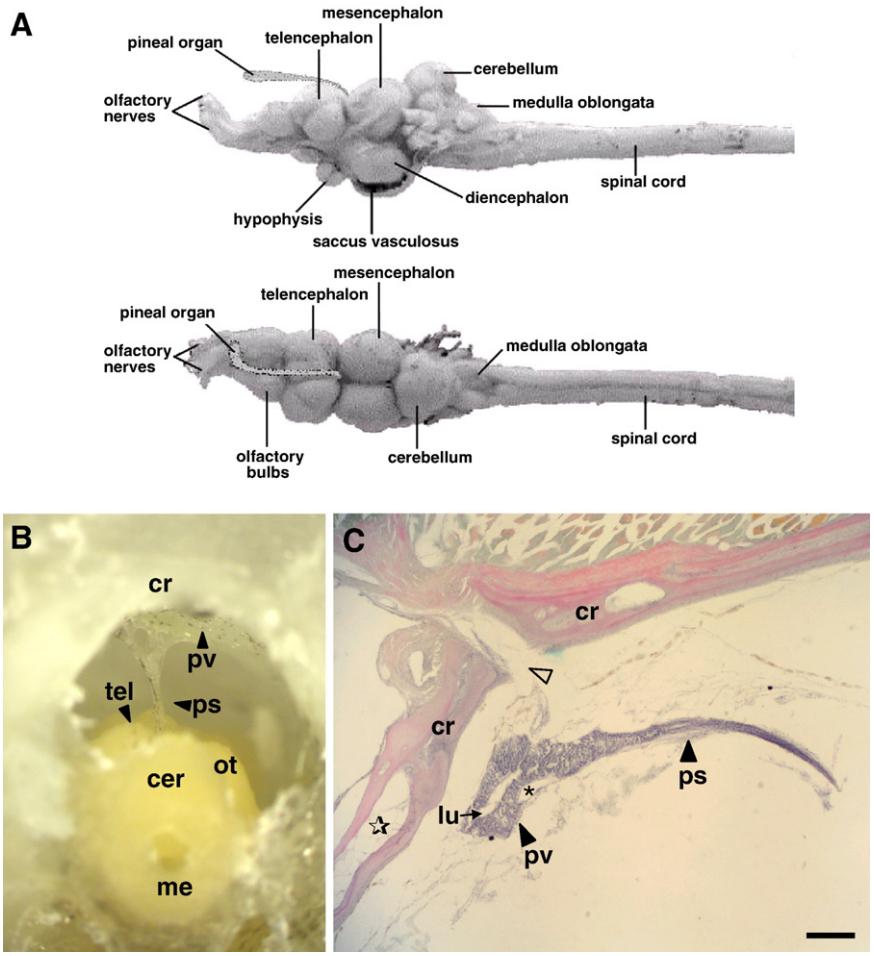

Fig. 1. Gross anatomy and histological section of the sole (*Solea senegalensis*) pineal organ. A. Lateral and dorsal views of the sole brain. The anatomical disposition of the pineal organ is represented. B. Paraffin-embedded decalcified brain of sole showing the midline pineal stalk extending into a large cerebral cavity and the right-turned pineal vesicle. C. Sagittal histological section of the pineal organ of sole showing a long pineal stalk and a prominent pineal vesicle closely apposed to the cranium bone. Note that the cranium bone is thinner at this level (white star) and exhibits a conspicuous fissure (white arrowhead) that could facilitate the access of light. Abbreviations: cer, cerebellum; cr, cranium; lu, pineal lumen; me, medulla; ot, optic tectum; ps, pineal stalk; pv, pineal vesicle; tel, telencephalon. *Represents blood vessels. Bar scale: 200 μm.

few studies have been undertaken in developing Atlantic halibut and turbot (Forsell et al., 1997, 2001, 2002; Vuilleumier et al., 2007).

The Senegalese sole, *Solea senegalensis*, is a pleuronectiform species with a high market value and an increasing importance for European aquaculture (Dinis et al., 1999). This nocturnal species exhibits marked circadian melatonin and locomotor rhythms (Bayarri et al., 2004), but also seasonal melatonin and reproductive rhythms (Imsland et al., 2003; Anguis and Cañavate, 2005; García-López et al., 2006a; Vera et al., 2007). The anatomy of the neuroendocrine systems involved in the control of reproductive process (i.e. hypothalamic GnRH, catecholamines, neuropeptide Y, serotonin and galanin) has been analyzed in the sole (Rendón et al., 1997; Rodríguez-Gómez et al., 1999, 2000b,c,d, 2001). In this paper, we present an anatomical, histological and immunohistochemical study of the pineal organ in this species. The aim of the anatomical and histological study was to determine if metamorphic process affects the organization and characteristics of the pineal organ in sole. The main objective of the immunohistochemical study was to identify melatonin-secreting cells and the presence of cone opsin-like and rod opsin-like photopigments in the pineal organ of sole but also to elucidate if it exhibits a low or a high number of photoreceptor cells as an adaptive mechanism to benthonic habitats.

2. Materials and methods

Adult specimens of Senegalese sole, of 150–200 g body weight, were purchased from a local fishery (Cupimar, San Fernando, Spain) in April 2005. Animals were kept in running seawater at a constant temperature and salinity of 19 ± 1 °C and 39 ppt, respectively, in indoor facilities receiving natural environmental light (approximately 13 L:11D) from the "Laboratorio de Cultivos Marinos" (University of Cadiz, Puerto Real, Spain). Animals were treated according to the European Union regulation concerning the protection of experimental animals. For histological and immunohistochemical analysis, all animals were processed at the same time of day (local time, 11.00 to 14.00 h GMT+2 h).

Fig. 2. Histological sections of the pineal vesicle of the sole (*Solea senegalensis*). A. Transverse section at the posterior pole of the pineal vesicle, which appears in the midline (dotted line). B. At the level of the rostral olfactory bulbs, the pineal vesicle leaves the midline (dotted line) and turns 90° to the right side of the head. Transverse section. C. Transverse section through the rostral olfactory bulbs of another sole specimen showing a prominent pineal vesicle clearly shifted to the right hemisphere. Dotted line represents midline. D. Detail of the pineal vesicle showing large blood vessels (asterisks). Transverse section. E. Detail of the pineal vesicle showing a folded epithelium and a prominent pineal lumen. Sagittal section. F. High magnification of the pineal vesicle showing at least three different pineal cell types: large blue-stained cells around the pineal lumen (white arrowheads), smaller cells with a dark purple-stained nucleus (black arrowheads), and cells exhibiting a large blue/grey-stained nucleus (arrow). Transverse section. Abbreviations: c, connective tissue; cr, cranium; lob, left olfactory bulb; lu, pineal lumen; nu, nucleus; pin, pinealocytes; ps, pineal stalk; pv, pineal vesicle; rob, right olfactory bulb. *Represents blood vessels. Bar scale: 200 μm in A,B, 100 μm in C,D,E and 10 μm in F. A–E, A–E, paraffin-embedded sections; F, semithin section.