# The torus longitudinalis in the gilthead seabream: an undescribed fiber tract link with the valvula cerebelli

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Summary. In this paper, we present a brief anatomical description of the torus longitudinalis and the valvula cerebelli in a percomorph fish, the gilthead seabream (Sparus aurata) based on the analysis of serial brain sections stained by paraldehyde fuchsin, Groat's hematoxylin-picroindigocarmin and cresyl violet. The existence of a small undescribed fiber tract directly bridging the ventral torus longitudinalis and the granular layer of the rostral valvula cerebelli is also reported. This small fiber tract was observed in its integrity on a few transverse sections providing that the angle of sectioning was appropriate. The existence of the anatomical link between the TL and the VC described in this paper might sustain the role of the TL as part of an ascending cerebello-tectal circuit, at least in gilthead seabream. However, these fibers might also represent fibers of passage originating from other brain structures.

**Key words:** Torus longidinalis, Valvula cerebelli, Gilthead seabream, Fiber tract

# Introduction

The torus longitudinalis (TL) represents a paired midline structure of the dorsal mesencephalon that protrudes into the ventricle in close proximity to the valvula cerebelli. Unique to actinopterygians, the torus longitudinalis has been implicated in visual activity and eye movements (Nothmore, 1984). On the other hand, the valvula cerebelli (VC) constitutes the most anterior subdivision of the cerebellum and lies within the mesencephalic ventricle, ventral to the optic tectum (Finger, 1983). This cerebellar structure is also exclusive to ray-finned fishes and has been implicated in the processing of electrosensory information (Finger et al., 1981; Nieuwenhuys, 1982). Some controversy exists in the literature about the existence of connections between

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the TL and the VC. Although it has been reported that the VC projects to the TL (Ito and Kishida, 1978). afferent or efferent connections between both structures have not been confirmed in other studies (Wullimann and Northcutt, 1989; Wullimann, 1994; Wullimann and Roth, 1994). On the other hand, the TL receives oculomotor inputs and is part of a sensory circuitry involving the tegmentum, the hypothalamus, the ventral thalamus and the pretectum (Wullimann, 1994). In this way, it has been proposed that the TL represents a main relay center in premotor circuitry descending from the telencephalon to the brainstem, rather than part of an ascending cerebellotectal circuitry (Wullimann, 1994; Wullimann and Roth, 1994). In this paper, we present an anatomical description of the torus longitudinalis and the valvula cerebelli in a percomorph fish, the gilthead seabream (Sparus aurata) and report the existence of a small fiber tract that bridges the ventral TL and the rostral VC.

# Materials and methods

Adult male and female gilthead seabream, Sparus aurata, ranging in body length from 30 to 40 cm (2-3 years old) were purchased from a local fishery (C.I.C.E.M. El Toruño, Puerto de Santa María, Cádiz, Spain). Six animals were anesthetized with phenoxyethanol and perfused via the aortic bulb with 0.9% saline solution followed by Bouin's fixative. The brains were removed, postfixed in sublimated Bouin-Hollande's fixative for 72 h before embedding in paraffin, and serial transverse sections (7  $\mu$ m-thick) were obtained on a rotary microtome. For sectioning, brains were oriented in order to obtain transverse sections perpendicular to the mid-sagittal and horizontal planes. Brain sections were stained with paraldehyde fuchsin, Groat's hematoxylin-picroindigocarmin or 1% cresyl violet (Gabe, 1968) and analyzed on a Olympus Provis photomicroscope. Sagittal sections were also performed and analyzed.

# Results

In gilthead seabream, the TL started slightly caudal

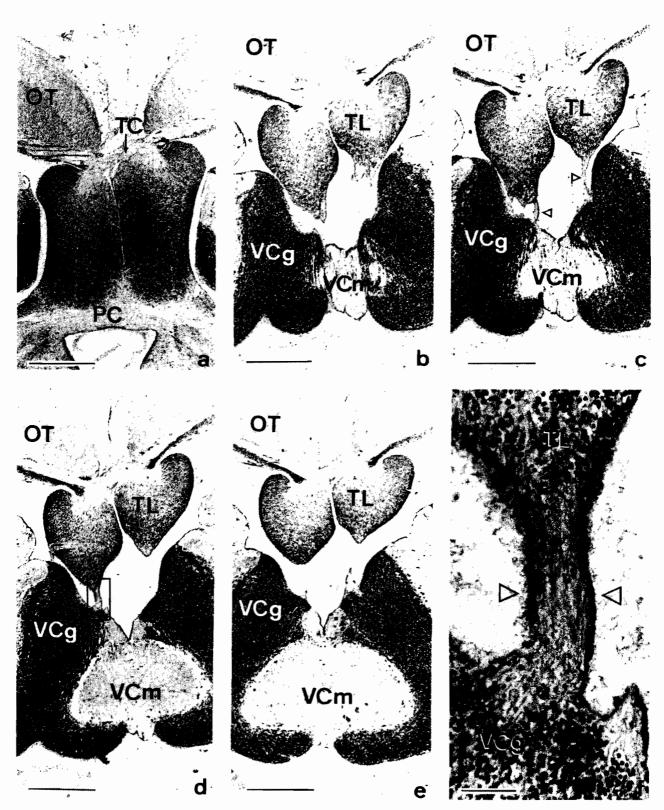


Fig. 1. Serial transverse sections through the torus longitudinalis and the rostral valvula cerebelli of the gilthead seabream showing the small fiber tract linking both structures (triangles). Cresyl violet staining. Distances between sections are as follows: a-b,  $560 \mu m$ ; b-c,  $35 \mu m$ ; c-d,  $105 \mu m$ ; d-e,  $105 \mu m$ ; a. Represents the rostralmost section and figure 1e the most caudal one. f. shows a high magnification of the framed area presented in d. OT: optic tectum; PC: posterior commissure; TC: tectal commissure; TL: torus longitudinalis; VCg: granular layer of the valvula cerebelli; VCm: molecular layer of the valvula cerebelli. Scale Bars: a-e,  $500 \mu m$ ; f,  $50 \mu m$ .

to the rostral part of the posterior commissure and disappeared at the caudal end of the nucleus of the medial longitudinal fascicle. In its anterior part, this structure was bordered by the nerve fibers of the tectal commissure dorsally and by those of the posterior commissure ventrally (Fig. 1a). The rostral TL was fused ventrally to the diencephalon and its small, round cells appeared more densely packed in the lateral zone than in the medial zone. Some medium-sized cells could also be found lateral to the medial zone. When progressing caudally, the cell density became higher, although the cells in the center of the TL remained slightly more scattered (Fig. 1b). The cells at the periphery of the TL appeared organized in laminae, which was more evident in the dorsolateral region (Fig. 1c). Bundles of fibers could be seen in the dorsal aspects of the TL and medium-sized cells were distributed ventromedially to these fibers. The medial separation between the two lobes of the TL became more evident and finally, two heart-shaped lobes appeared excised from the region of the posterior commissure (Fig. 1b). At this level, the VC expanded medially, occupying the place left by the TL, and separated it from the caudal diencephalon (Fig. 1b,c). More caudally, the TL was greatly reduced in size and the VC also expanded dorsally (Fig. 1e). Although the gilthead seabream is a non-electroreceptive teleost, the VC was a very prominent, folded structure that filled the tectal ventricle almost completely. As in other teleosts, a molecular and a granular layer could be observed (Fig. 1b-e), but medial and lateral subdivisions of the VC were not evident.

In the process of this neuroanatomical study, it was possible to clearly identify the existence of a small connective, vascular and axonal bridge between the ventral TL and the granular layer of the VC (Fig. 1c,d). Figure 1 shows a sequence of serial sections demonstrating this anatomical connection (Fig. 1 c,d), as well as levels slightly rostral (Fig. 1b) and caudal (Fig. 1e) to it. The overall rostro-caudal extent of this bridge was estimated at  $105~\mu m$ . In most other brains analyzed, the small size of this fiber tract and the plane of sectioning prevented us to really observe this direct link between the TL and the VC. Nerve fibers running through this small tract could be clearly observed in Fig. 1f.

# Discussion

The existence of the anatomical link between the TL and the VC described in this paper might sustain the role of the TL as part of an ascending cerebello-tectal circuit, at least in gilthead seabream. Some physiological data support the existence of direct connections between the TL and the VC in teleosts. In two marine percomorphs and in the goldfish, it has been reported that the neurons of the ventral TL exhibit bursts of activity associated to saccadic eye movements and that ablation or intersection of the VC abolished this phenomenom (Nothmore et al., 1983; Nothmore, 1984). Furthermore, in the carp, Cyprinus carpio, Ito and Kishida (1990) reported that

the VC projects ipsilaterally to the TL by means of anterograde tracing experiments. This result was confirmed by a subsequent study in which, following HRP injections into the rostral part of the valvula, orthogradely-labeled axons were observed to penetrate the TL (Ito and Yoshimoto, 1990). However, when HRP injections were performed more caudally in the valvula, no labeled terminals were observed in the TL (Ito and Yoshimoto, 1990). Interestingly, the fiber tract observed in gilthead seabream links the ventral TL to the rostral part of the VC.

On the other hand, after HRP injections into the TL of Cyprinus carpio, labeled cells were observed beneath the nucleus lateralis valvulae but not in the VC (Ito and Yoshimoto, 1990). In *Pantodon*, Dil deposition into the TL showed the existence of labeled cells in the nucleus lateralis valvulae and the dorsal tegmental nucleus as well as labeled fibers in the granular layer of the VC but no perikarya were detected in the valvula (Wullimann and Roth, 1994). These authors stated that the existence of these labeled fibers in the VC could represent an artefact resulting from the labeling of axonic collaterals of neurons of the dorsal tegmental nucleus or the nucleus lateralis valvulae, which project both to the TL and the VC (Wullimann and Northcutt, 1988, 1989). The axons running between the granular layer of the rostral VC and the ventral TL of the gilthead seabream could also represent fibers of passage originating from other sources. In particular, axons linking the TL and the VC might be mesencephalic fibers ascending to the TL through the VC. This latter possibility could also explain why HRP injection into the rostral valvula gives rise to labeled terminals in the TL (Ito and Yoshimoto, 1990) or why Dil implantation in the TL results in labeled axons in the granular layer of the VC (Wullimann and Roth, 1994). The corpus cerebelli might be another candidate source because its efferent axons have been described to terminate in the TL and the granular layer of the VC (Wullimann and Northcutt, 1988).

Further studies should be directed to elucidate if these nerve fibers linking the ventral TL and the granular layer of the VC of gilthead seabream are present in other teleosts and constitute a direct connection between these structures or only represent fibers of passage originating from other brain structures.

Acknowledgements. This study was supported by grants of DGICYT PB93-1209 and the Junta de Andalucia.

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Accepted October 2, 1997