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Research report

The segmental organization of the developing shark brain based on neurochemical markers, with special attention to the prosencephalon

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Abstract

Brain regionalization has been extensively studied in tetrapods, teleosts and cyclostomes. In contrast, it has not been investigated in elasmobranchs, despite their key phylogenetic position to understand brain evolution in jawed vertebrates. In this study we provide a schematic view of the segmental pattern of the developing shark brain based on mapping of the expression of *Pax6* and neurochemical markers such as calretinin, tyrosine hydroxylase, serotonin, and glutamic acid decarboxylase. By correlating the cytoarchitectonic limits with the specific location of these markers, we identify transverse and longitudinal boundaries and domains, which suggest a segmental pattern, reminiscent of the one described in other vertebrates. Taken together, these data provide an initial scheme, which will be further tested and refined using a broader range of genetic markers involved in patterning and differentiation.

Keywords: Pax6; Pallial–subpallial boundary; Elasmobranchs; GABA; Calretinin; Tyrosine hydroxylase; Serotonin; *In situ* hybridization; Immunohistochemistry

1. Introduction

The homeobox gene *Pax6* encodes a highly conserved transcription factor, which plays multiple roles in early forebrain, and more generally in central nervous system patterning, including boundary formation, neuron specification and axon guidance [10,20]. Comparison between the patterns of *Pax6* expression in zebrafish and mammals has highlighted a high degree of conservation but also some differences. In order to gain insight into the ancestral gnathostome pattern, we have analyzed the distribution of Pax6 protein and its mRNA in the brain of developing and postembryonic sharks. Additionally, with triple and double immunofluorescence techniques, we have compared *Pax6* expression with that of some markers for neuronal differentiation such as glutamic acid decarboxylase (GAD), calretinin (CR), tyrosine hydroxylase (TH) and serotonin (5-HT). Our results provide a schematic view of the segmental pattern of the developing shark brain, extending and supporting the data available in early dogfish embryos [5].

2. Materials and methods

Embryos and juveniles of the dogfish *Scyliorhinus canicula* and the shyshark *Haploblepharus fuscus* were kindly provided by the "Aquário Vasco da Gama" and "Oceanário" (Lisbon, Portugal) and the "Aquarium Finisterrae" (A Coruña, Spain). Embryos were staged according to Ballard et al. [2]. Stages from 25 to 34 (prehatching) and juveniles were used. After deep anaesthesia with MS-222 (Sigma), embryos were fixed by immersion in phosphate buffered 4% paraformaldehyde, while juveniles were intracardially perfused with this fixative. Whole embryos and juvenile brains were serially sectioned on a cryostat. Series were processed for the PAP or ABC method (single labeling) or for double or triple immunofluorescence. As primary antibodies we have used polyclonal (rabbit anti-Pax6, Chemicon; sheep anti-GAD65/67, kindly provided by Dr. E.

Abbreviations: Cb, cerebellum; ET, eminentia thalami; fr, fasciculus retroflexus; H, habenula; HL, hypothalamic lobe; Hy, hypothalamus; IR, inferior reticular formation; LC, locus coeruleus; Nflm, nucleus of the medial longitudinal fascicle; OB, olfactory bulb; OM, olfactory mucosa; OT, optic tectum; P, pallium; pc, posterior commisure; por, preoptic recess; PT, pretectum; PTh, prethalamus; PTu, posterior tubercle; sn, substantia nigra; Sp, subpallium; SR, superior reticular formation; TEL, telencephalon; TGm, mesencephalic tegmentum; Th, thalamus; VTA, ventral tegmental area; zli, zona limitans intrathalamica.

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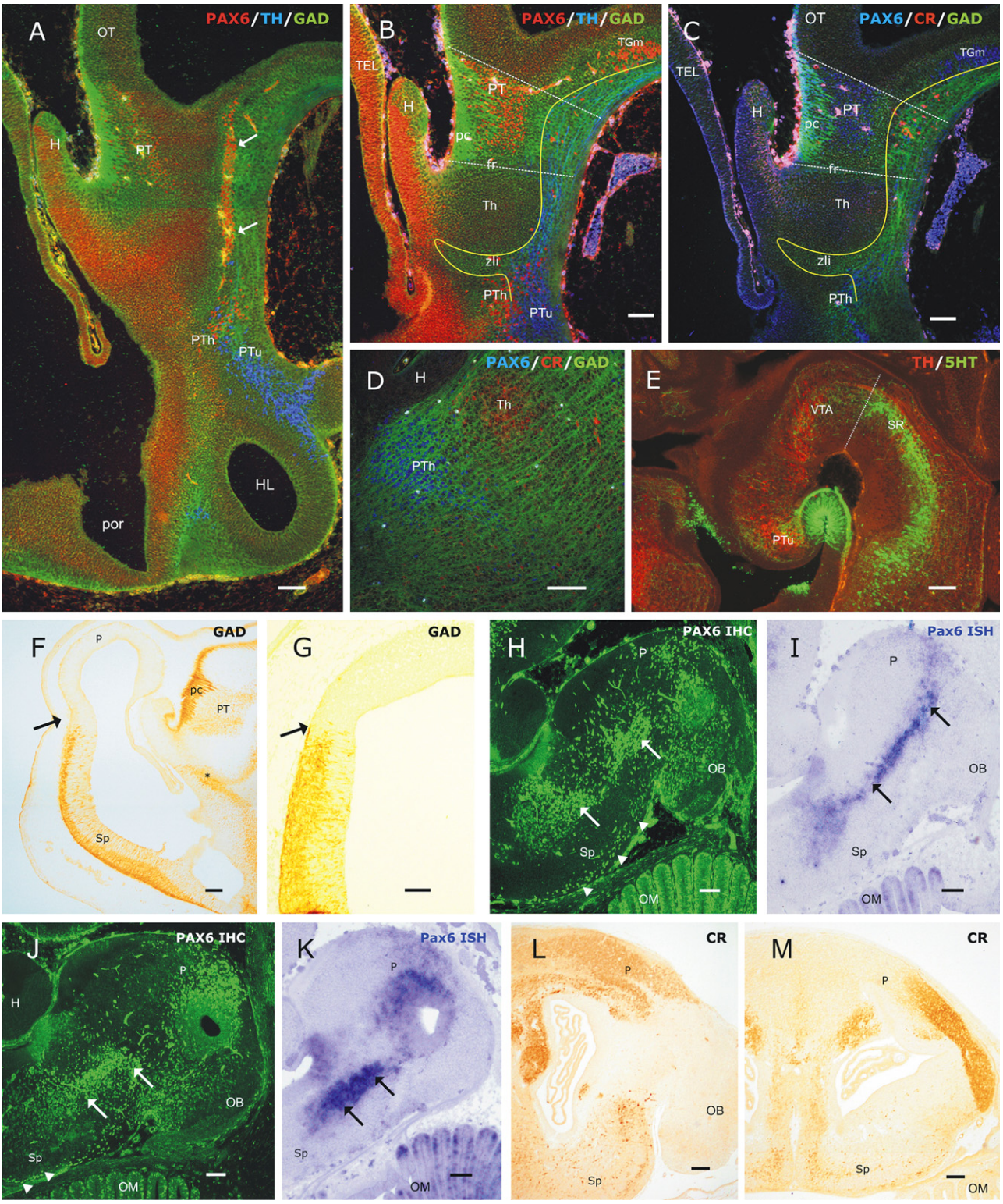

Fig. 1. (A–M) Brain sections of embryos of shyshark (A–D and H) and dogfish (E–G, I and J) processed for immunohistochemistry (IHC) for Pax6 (A–D and H), GAD (A–D, F and G), TH (A, B and E), 5-HT (E) and CR (C, D and J) and for *in situ* hybridisation (ISH) for Pax6 (I). (A–C) Triple labeled sagittal sections at median (A) and paramedian (B and C) levels of stage-29 embryos. Note the column of Pax6-ir cells along the mesencephalon, synencephalon and posterior prosencephalon related with the alar–basal boundary (arrows in A). B and C are adjacent sections. (D) Detail of a parasagittal section of the caudal prosencephalon of a stage 31 embryo showing the CR-ir cell group of the thalamus adjacent to the Pax6 population of the prethalamus. Rostral is at left. (E) Double labeling for TH and 5HT on a sagittal section of a stage-31 embryo. The sharp limit between the TH-ir cells of the ventral tegmental area and the 5-HT-ir cells of the reticular formation marks the mes–rhomb boundary (dotted line). (F and G) GAD immunoreactivity in sagittal sections of the telencephalon of embryos at stages 26 (F) and 28 (G) showing the massive presence of GAD-ir cells in the subpallium and their absence in the pallium at these stages. Arrows indicate the pallial–subpallial boundary. Note abundant GAD-ir fibres in the posterior commissure and cells in both the pretectum and related to the zona limitans intrathalamica (asterisk in F). (H–K) Transverse sections