Organization of the catecholaminergic system in the short-lived fish *Nothobranchius furzeri*

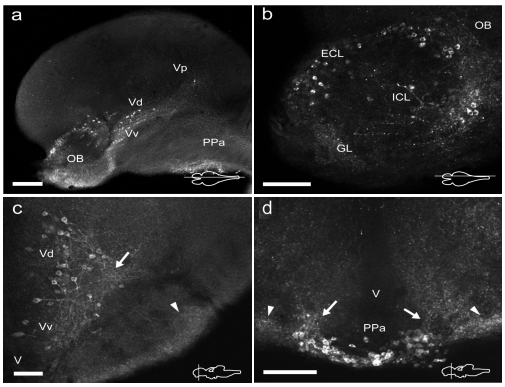
Borgonovo J. (1,2,3), Ahumada-Galleguillos P. (1,2), Oñate-Ponce A. (1,2,3), Allende-Castro C. (1,2,3), Henny P. (4), Concha M.L. (1,2,3)

(1) Program of Integrative Biology, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, PO Box 70031, Santiago, Chile; (2) Biomedical Neuroscience Institute, Independencia 1027, Santiago, Chile; (3) Center for Geroscience, Brain Health and Metabolism, Santiago, Chile; (4) Department of Anatomy and Interdisciplinary Center of Neurosciences, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile.

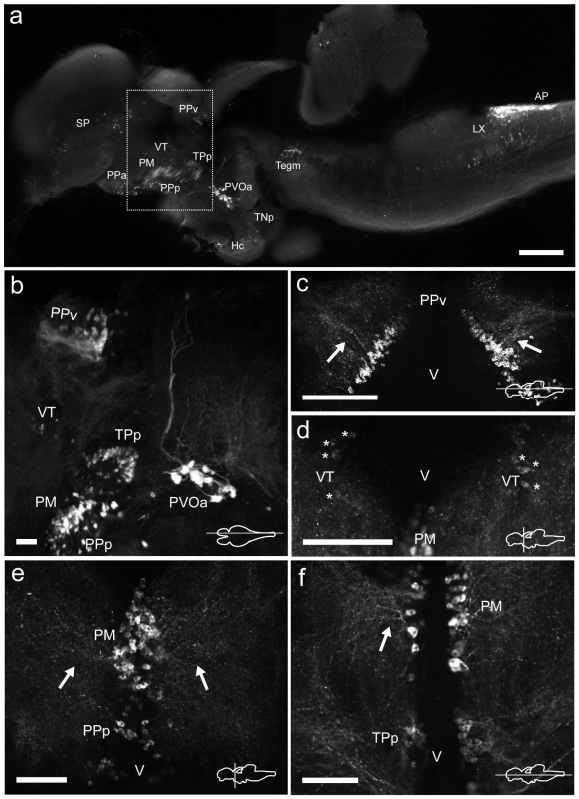
Introduction

The distribution of catecholamines appears to be highly conserved throughout evolution and constitutes one of the oldest phylogenetically neurochemical systems in the vertebrate brain. Despite the variation in the morphology and complexity of the brain, the catechoalminergic (CAergic) groups located at the caudal and rostral/isthmic rhombenecephalon and the dopaminergic groups of the diencephalon, olfactory bulb and the retina, are a shared feature of the vertebrate CNS. However, CAergic groups located in the midbrain are more variable and while they are commonly found in the tetrapod lineage, they represent an unusual characteristic among actinopterygians. Contrary, the existence of DAergic groups in the subpallium and pretectum is common among actinoptegyrians but is not a broadly features of amniotes. Some of these divergence could be attributable of adaptation of the brain function to the different types of vertebrate lives. The present study aims to provide a detailed description of the distribution of the TH immunoreactive neurons in the brain of the adult *Nothobranchius furzeri* as a basis for identified the CAergic groups functionally homologous to mammals and facilitate the use of killifish as biomedical model organism to study the aging-related deterioration of the CAergic system.

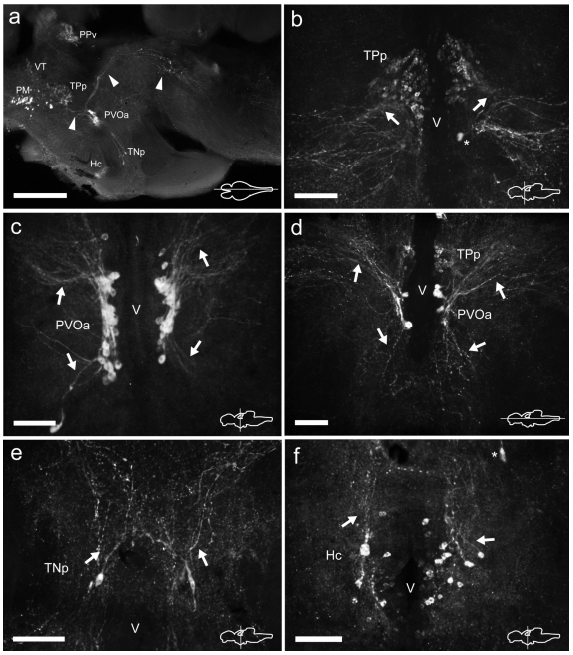
Results



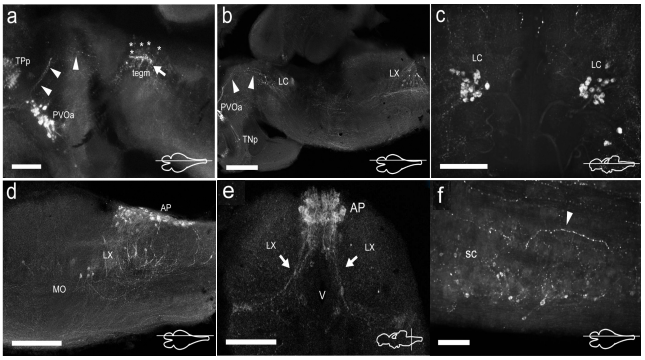
TH+ neurons and fibers in the telencephalon and anterior preoptic area. (a) Para-sagittal section showing a panoramic view of TH+ neuronal groups in the olfactory bulb (OB), subpallium and in the anterior preoptic nucleus (PPa). (b) Para-sagittal section showing the TH+ groups in the OB. (c) Transverse section at the level of ventral telencephalic areas showing TH+ groups in the ventral (Vv) and dorsal (Vd) zones. The arrow and arrowhead indicate the local TH+ processes of ventral/dorsal zones and the intense innervation at the ventrolateral edge of the telencephalon, respectively (d) Transverse section showing the TH+ neurons at the PPa. Arrows and arrowheads indicate the TH+ dorsocaudal projections of neurons and the lateral intense neuropil seen at each side of this cell group, respectively. Dorsal is to the top. ECL: external cellular layer, GL: glomerular layer, ICL: internal cellular layer, V: ventricle, Vp: posterior zone of ventral telencephalon. Scale bars, 250µm (a) and 100 µm (b-d).



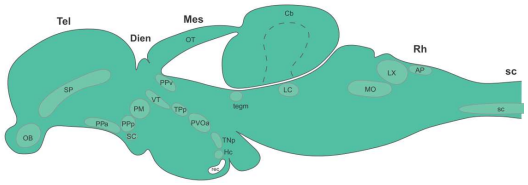
TH+ neurons and fibers in the diencephalon at the preoptic, thalamic and pretectal levels. (a) Para-sagittal section of the brain showing a panoramic distribution of TH+ groups. The dotted box delimits the groups analyzed in this figure. (b) Para-sagittal section showing the TH+ groups at the ventral periventricular pretectal nucleus (PPv), ventral thalamic nucleus (VT), parvocellular portion of preoptic nucleus (PPp), magnocellular preoptic nucleus (PM), periventricular nucleus of the posterior tuberculum (TPp) and paraventricular organ-accompanying cells (PVOa). (c) Horizontal section showing the TH+ neurons of the PPv sending projections towards to the optic tectum (arrows). (d) Transverse section showing TH+ neurons at the VT (asterisks). (e) TH+ cells of the PPp and the PM with their lateral projections as seen in a transverse section (arrows). (f) Horizontal section showing TH+ neurons and their projections (arrow) at the PM and its relative position with respect to the TPp. Dorsal is to the top in transverse sections. Rostral is to the top and left, in horizontal and para-sagittal sections, respectively. Scale bars. AP: area postrema, Hc: caudal hypothalamus, LX: vagal lobe, PPa: anterior preoptic nucleus, SP: subpallium, Tegm: tegmentum, TNp: posterior tuberal nucleus, V: ventricle. Scale bars, 500 µm (a) and 100 µm (b-f).



TH+ neurons and fibers in the diencephalon at the level of the posterior tuberculum and hypothalamus. (a) Para-sagittal section showing a panoramic view of the distribution of all TH+ groups in the diencephalon. Arrowheads indicate TH+ fibers travelling from the paraventricular organ-accompanying cells (PVOa) towards the telencephalon and hypothalamus (bottom left), and across the mesencephalon to turn posteriorly towards the medulla (top). (b) Transverse section showing the TH+ group in the periventricular nucleus of the posterior tuberculum (TPp). Arrows show the lateral tuft of projections emerging from the nucleus. The asterisk indicates a soma of the PVOa. (c) Transverse section showing the large pear shape neurons of the PVOa. Arrows indicate the ascending and descending processes. (d) Relative position of the TPp and the PVOa in a horizontal section. Arrows point to ascending/descending processes of the PVOa. (e) Transverse section showing TH+ neurons at the posterior tuberal nucleus (TNp). The arrows show the dorsal projections of the cells. (f) Transverse section showing the TH+ neurons at the caudal hypothalamus (Hc). The arrow indicates the orientation of processes. The asterisk indicates a cell of the TNp. Dorsal is to the top in transverse sections. Rostral is to the top and left, in horizontal and para-sagittal sections, respectively. PM: magnocellular preoptic nucleus, PPv: ventral periventricular pretectal nucleus, TPp: periventricular nucleus of the posterior tuberculum, V: ventricle, VT: ventral thalamic nucleus. Scale bars, 500 µm (a) and 100 µm (b-f).



TH+ neurons and fibers in the mesencephalon, rhombencephalon and spinal cord. (a) Para-sagittal section showing the group of mesencephalic TH+ neurons at the level of the oculomotor nucleus (asterisks indicate their lightly stained somas) and the intense TH+ plexus (arrow). TH+ fibers travelling from the PVOa across the mesencephalon to turn posteriorly towards the medulla are indicated with arrowheads. (b) Para-sagittal section showing the TH+ group at the locus coeruleus (LC) and the caudal group in the vagal lobe (LX). (c) Horizontal section showing the TH+ group of the LC. (d) Para-sagittal section showing the TH+ groups at the medulla oblongata (MO), LX and area postrema (AP). (f) Transverse section showing the TH+ neurons at the AP. Arrows indicate the ventral processes. (g) Para-sagittal section showing scattered small TH+ neurons in the spinal cord (SC). The arrowhead points to a TH+ fiber in the ventral region of the SC. Dorsal is to the top in transverse sections. Rostral is to the top and left, in horizontal and para-sagittal sections, respectively. Scale bars, 500 µm (a,d), 250µm (b,g) and 100 µm (c,h).



Summary diagram illustrating the localization of the CAergic neuronal groups of the brain of *Nothobranchius furzeri*. Dien: diencephalon, Mes: mesencaphalon, Rh: rhombencephalon, SC: spinal cord, Tel: telencephalon.

Discussion

The distribution of TH+ neurons in the brain of *Nothobranchius furzeri* is consistent with the basic pattern reported in the literature for other teleosts. However, it shows some peculiarities such as the CAergic neurons in the mesencephalic tegmentum, which is an unusual trait among the actinopterygians. It remains to be determined the evolutionary significance of this group and whether it reflects a functional specialization. Despite the species specific characteristics, the killifish shares the same CAergic groups that are present in all vertebrates including mammals, such those found in the olfactory bulb, the preoptic region, the hypothalamus, the locus coeruleus, medulla oblongata and the retina. These conserved features together with the aging phenotype shared with mammals, make *Nothobranchius furzeri* a suitable model organism to investigate the physiological and pathological deterioration of the CAergic system related to age.

Funding: FONDAP 15150012, P09-015-F, PIA ACT192015.