ELSEVIER

Journal of Chemical Neuroanatomy 19 (2000) 17–32

Journal of CHEMICAL NEUROANATOMY

www.elsevier.com/locate/jchemneu

Localization of tyrosine hydroxylase-immunoreactivity in the brain of the Senegalese sole, *Solea senegalensis*

F.J. Rodríguez-Gómez [a], M.C. Rendón-Unceta [a], C. Sarasquete [b], J.A. Muñoz-Cueto [a,*]

[a] *Faculty of Marine Sciences, Department of Animal Biology, Plant Biology and Ecology, University of Cádiz, Polígono Río San Pedro, 11510 Puerto Real, Cádiz, Spain*

[b] *Institute of Marine Sciences of Andalucía (CSIC), Polígono Río San Pedro, Apdo. Oficial, 11510 Puerto Real, Cádiz, Spain*

Received 22 November 1999; received in revised form 13 March 2000; accepted 13 March 2000

Abstract

The localization of catecholamines in the brain of the Senegalese sole was determined by immunohistochemical techniques using antibodies against tyrosine hydroxylase. Although the general pattern of distribution of catecholamines is consistent with that reported in other teleosts, some remarkable differences are observed. The most rostral tyrosine hydroxylase immunoreactive (TH-ir) cells were identified in the olfactory bulbs, in which a clear asymmetry in the number and location of TH-ir perikarya and fibers was observed. The number of TH-ir cells is manifestly higher in the right olfactory bulb, especially in the internal cell layer. TH-ir fibers are also much more abundant in the right bulb, principally in the glomerular and internal cell layers. Other TH-ir cell masses were identified in the ventral telencephalon, preoptic area, caudoventral hypothalamus, posterior tuberculum, synencephalon, isthmic region and rhombencephalon. Surprisingly, no ir cell bodies were identified in the ventromedial thalamic nucleus, which exhibits a large number of TH-ir cells in other teleosts. The presence of TH-ir fibers in the brain of sole is particularly evident within and around the nuclei in which immunoreactive cells are found. However, other zones such as the dorsal telencephalon, posterior commissure, optic tectum, torus semicircularis, reticular formation or inferior olive also displayed TH-ir fibers. TH-ir axons also enter the infundibulum, reaching the proximal pars distalis of the adenohypophysis. The distribution of TH-ir cells and fibers is compared with that observed in other teleosts and is discussed in a comparative context.

Keywords: Catecholamines; Olfactory bulbs; Asymmetry; Reproduction; Pleuronectiformes; Teleost

1. Introduction

It has been clearly established that catecholamines, acting as neurotransmitters and/or neurohormones, can influence different behavioral and physiological processes in the central nervous system (Björklund and Hökfelt, 1984). Tyrosine hydroxylase (TH) is the rate-limiting enzyme of catecholaminergic cellular synthesis. This enzyme, together with dopa decarboxylase, dopamine-β-hydroxylase and phenylethanolamine-*N*-methyltransferase, constitute the synthesizing enzymes for dopamine (DA), noradrenaline and adrenaline. In the teleost brain, DA and noradrenaline represent the predominant catecholamines, whereas adrenaline seems to be present at very low levels (Caroff et al., 1986; Hornby and Piekut, 1989).

The organization and activity of catecholaminergic systems in the brain of teleosts have been analyzed extensively by means of histofluorescence (Parent et al., 1978; Watson, 1980; Parent, 1983; Kah et al., 1984a), immunohistochemistry (Yoshida et al., 1983; Halpern-Sebold et al., 1985; Hornby and Piekut, 1990; Hornby et al., 1987; Alonso et al., 1989; Sas et al., 1990; Ekström et al., 1990; Meek and Joosten, 1993; Batten et al., 1993; Manso et al., 1993; Muñoz-Cueto et al., 1997; Briñón et al., 1998), biochemistry (Nilsson, 1989; Saligaut et al., 1992, 1993; Senthilkumaran and Joy, 1995; Linard et al., 1996a) and in situ hybridization tech-

* Corresponding author. Tel.: +34-956-016023; fax: +34-956-016019.

E-mail address: munoz.cueto@uca.es (J.A. Muñoz-Cueto)

0891-0618/00/$ - see front matter
PII: S0891-0618(00)00047-8

niques (Boularand et al., 1998). Up to date, information on the distribution of catecholamines have been obtained in a range from early developed teleosts (e.g. eels and mormyrids; Roberts et al., 1989; Meek and Joosten, 1993; Meek et al., 1989; Boularand et al., 1998), via intermediately originated groups (e.g. ciprinifoms and gymnotiforms; Hornby et al., 1987; Sas et al., 1990), to more recently developed teleosts (e.g. gasterosteiforms and perciforms; Ekström et al., 1990; Batten et al., 1993; Muñoz-Cueto et al., 1997). Flatfishes or pleuronectiforms belong to the most modern teleosts, i.e. the percomorpha, and seem to represent the most advanced and recently developed group of fishes within this order. However, to our knowledge, no data are currently available on the brain distribution of catecholamines in pleuronectiform fishes. This fact is, in part, a consequence of the absence of specific neuroanatomical tools to perform this kind of studies.

The Senegalese sole, *Solea senegalensis*, is a pleuronectiform fish exploited in extensive and intensive aquaculture in the south European and African coasts (Drake et al., 1984; Dinis, 1992; Fehri-Bedoui, 1997). This species hatches as externally symmetric pelagic larva but has a characteristic external asymmetry during adult benthonic life. In the Senegalese sole, as in other flatfishes (Prasada Rao and Finger, 1984; Briñón et al., 1993), the external asymmetry of sensory organs (e.g. olfactory organ, eyes) is also extended to the central nervous system, with the right olfactory nerve and bulb being grossly larger than the left one (Rodríguez-Gómez et al., 2000).

In this paper we examine the immunohistochemical distribution of tyrosine hydroxylase in the brain of the Senegalese sole using a brain atlas for this species recently developed in our laboratory (Rodríguez-Gómez et al., 2000; Rodríguez-Gómez et al., in preparation). The major aim of this study is to extend the information on the brain distribution of catecholamines to highly evolved teleosts. This study also provides basic information on the possible role of catecholamines in the neuroendocrine regulation of adenohypophyseal cell functions in this commercially important species.

2. Materials and methods

Adult specimens of Senegalese sole, *S. senegalensis* ($n = 12$) were purchased from a local fishery (Cupimar, S.A. San Fernando, Spain) and kept in the laboratory in running sea water. All animal manipulations were conducted according to the 'Principles of laboratory animal care' (NIH publication number 86-23, revised 1985) and the Spanish laws. Specimens were deeply anesthetized with 2-phenoxiethanol (Sigma, St. Louis) and perfused via the aortic bulb with 0.6% saline solution, followed by Bouin fixative (4% *para*-formaldehyde in 0.1 M phosphate buffer, pH 7.4, 0.2% picric acid). Brains with the pituitary attached were then removed carefully and further postfixed in the same fixative for 6 h in darkness at 4°C. After fixation, the brains were cryoprotected overnight in 0.1 M phosphate buffer containing 15% sucrose, embedded in Tissue Freezing medium (Jung, Nussloch), frozen in cold isopentane and kept at −80°C until processed. Serial transversal sections (16 μm thick) were cut in a cryomicrotome and mounted on gelatin-coated glass slides.

Immunohistochemical staining was performed using the peroxidase–anti-peroxidase (PAP) method. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide in Coons buffer (CBT, 0.01 M veronal, 0.15 M NaCl, 0.1% Triton X-100) for 30 min. Before immnunostaining, sections were transferred for 5 min to CBT and saturated in CBT with 0.5% casein for 30 min. Sections were incubated overnight in a humid chamber at room temperature with mouse anti-tyrosine hydroxylase monoclonal antibody at 1:1000 dilution in CBT 0.5% casein (Chemicon International Inc.). Sections were washed in CBT and incubated for 90 min at room temperature with goat anti mouse-IgG (Chemicon International Inc.) diluted 1:100 in CBT. After washing in CBT, sections were incubated for 90 min at room temperature with mouse PAP complex (Chemicon International Inc.) diluted 1:300 in CBT. Finally, sections were washed with CBT followed by Tris–ClH (0.05 M, pH 7.4) and peroxidase activity was detected in Tris–HCl 0.05 M, pH 7.6, containing 0.048% 4-chloro-1-naphthol (Sigma, St Louis, MO), diluted previously in 20 ml of ethanol 100°, and 0.05% hydrogen peroxide. To confirm the specificity of the immunostaining, controls were performed by replacement of primary antibody with normal mouse serum and omission of primary and secondary antibodies. Some series were counterstained with neutral red to facilitate the identification of cell masses. The sections were mounted on an aqueous mounting medium for microscopy (Aquatex, Merck). For the description, we have subdivided the immunoreactive cells into three categories, small (5–15 μm), medium-sized (16–25 μm) and large (26–40 μm).

The precise location of tyrosine hydroxylase immunoreactive cells and fibers was identified with the help of a sole brain atlas previously drawn up in our laboratory (Rodríguez-Gómez et al., 2000; Rodríguez-Gómez et al., in preparation). Nomenclature of brain areas is adapted from Muñoz-Cueto et al. (in press). This nomenclature follows the cytoarchitectonic description of Northcutt and Davis (1983) for the telencephalon. For most of the diencephalon, the nomenclature developed by Braford and Northcutt (1983) was generally used, with additional elaborations by Wullimann and Meyer (1990). However, for the

preoptic area and hypothalamus the cytoarchytectonic scheme of Peter and Gill (1975) was adopted, with some modifications according to Braford and Northcutt (1983) and Wullimann and Northcutt (1988). We have used the description of Northcutt (1983) and Wullimann and Northcutt (1988) for the mesencephalon. For the cerebellum, the neuroanatomical nomenclature follows the revision of Finger (1983) and that of Wullimann and Northcutt (1988) in *Lepomis cyanellus* and *Carassius auratus*. Finally, the terminology used here for the rhombencephalon is primarily adopted from McCormick (1982) and Prasada Rao et al. (1987).

3. Results

3.1. Tyrosine hydroxylase immunoreactive cell bodies

Tyrosine hydroxylase-containing cells were observed in the telencephalon, diencephalon, at the boundary region between the mesencephalon and rhombencephalon (isthmus region), as well as in the rhombencephalon proper, of the Senegalese sole.

In the telencephalon, the most rostral tyrosine hydroxylase-immunoreactive (TH-ir) cell bodies are observed in the olfactory bulbs (Fig. 1A, Fig. 2A–D, Fig. 3A; Table 1). The TH-ir cells appear in the internal cell layer (ICL) of the olfactory bulbs as well as in the secondary olfactory fiber layer (SOF) and external cell layer (ECL, Fig. 1A and Fig. 2A). These positive cells are small, rounded or ovoid in shape, and most of them exhibit a distinct dendritic process exiting from the cell body (Fig. 2C and Fig. 3A). TH-ir mitral cells of the ECL are slightly larger in size than those of the ICL (Fig. 2C). The olfactory bulbs of the Senegalese sole are asymmetric in shape and size, the right olfactory bulb being grossly larger than the left one (Fig. 1A and Fig. 2A). This asymmetry also applies to the location and number of catecholaminergic cells. In the right bulb, most of the ir cells are present in the ICL, while in the left bulb a large majority of TH-ir neurons appear in the SOF and ECL, the ICL being almost devoid of cells (Fig. 1A and Fig. 2A). Although direct quantification was not contemplated in the present study, the number of immunostained perikarya is manifestly higher in the right bulb than in the left one (Fig. 2B and D). In the cerebral hemispheres, four cell masses of the ventral telencephalon contain TH-ir cell bodies (Fig. 1B, Fig. 3B and C) the ventral part (Vv), central part (Vc), lateral part (Vl) and dorsal part (Vd). The TH-ir cells located in the ventral telencephalon are similar in morphology to those of the olfactory bulbs but their dendritic processes become less obvious (Fig. 3B and C). Although TH-ir cells in Vl and Vd constitute discrete cell groups, a continuous strip of TH-ir cells and fibers can be observed from Vv to Vc (Fig. 3B and C).

The diencephalon of the Senegalese sole contains eight TH-ir cell populations, lying in a medial position close to the ventricular wall. In the preoptic area, the most rostral ir cell group is in the anteroventral part of the parvocellular preoptic nucleus (NPOav). This cell mass appears bordering the areas ventral and ventrolateral to the rostral preoptic recess (Fig. 1C). Its cells are small and rounded in shape and more caudally they appear separated from the ventricle by the cells of the parvocellular part of the parvocellular preoptic nucleus, in which no TH-ir cells were detected (Fig. 3D). More caudally, another population of small TH-ir neurons appears in the anterior periventricular nucleus (NAPv) dorsal to the TH-ir cells of the NPOav (Fig. 1D). These cells lie at a certain distance from the ventricular wall and have characteristic laterally- to lateroventrally directed processes (Fig. 3E). At the same transverse level, just ventral to the positive cells of the NAPv, the TH-ir cells of the suprachiasmatic nucleus (NSC) are found (Fig. 1D and Fig. 3E). In this nucleus only one to two large neurons by section can be observed in each hemisphere. These cells have thick dendrites and exhibit an intense immunostaining (Fig. 3E). The caudalmost TH-ir cells of the preoptic area appear in the posterior periventricular nucleus (NPPv). These small and medium-sized cells, rounded and fusiform shaped, lie lateral to the third ventricle and dorsal to the rostral hypothalamus (Fig. 1E and Fig. 3F). The remaining diencephalic TH-ir cell masses appear in the synencephalon, the posterior tuberculum of the thalamus and the caudoventral hypothalamus. In the synencephalon, a column of TH-ir cells expands laterally from the ventricle, lying ventral to the posterior commissure fibers (Fig. 1F and G, Fig. 4B). These small and densely packed cells belong to the ventral part of the periventricular pretectal nucleus (PPv) and migrated slightly laterally in caudal sections. Two TH-ir nuclei can be observed in the posterior tuberculum of the thalamus — the nucleus of the paraventricular organ (nPVO) and the posterior tuberal nucleus (NPT, Fig. 1F, Fig. 4B). The former lies lateral and ventrolateral to the paraventricular organ and contains medium-sized and large cells, rounded and ovoid in shape (Fig. 4B). The TH-ir cells of the NPT are found ventrally to nPVO positive neurons and are slightly smaller in size (Fig. 4B). Finally, in the caudal hypothalamus, a population of TH-ir cells appears along the dorsal edge of the posterior recess, laterally to the ventricle (Fig. 1F and Fig. 4C). These small cells are confined within the limits of the inferior part of the lateral tuberal nucleus (NLTi).

In the hindbrain of the Senegalese sole, three TH-ir cell groups can be distinguished, located in the isthmal tegmentum and rhombencephalon. In the isthmus region, the transition zone between the mesencephalon and rhombencephalon, a population of large multipolar

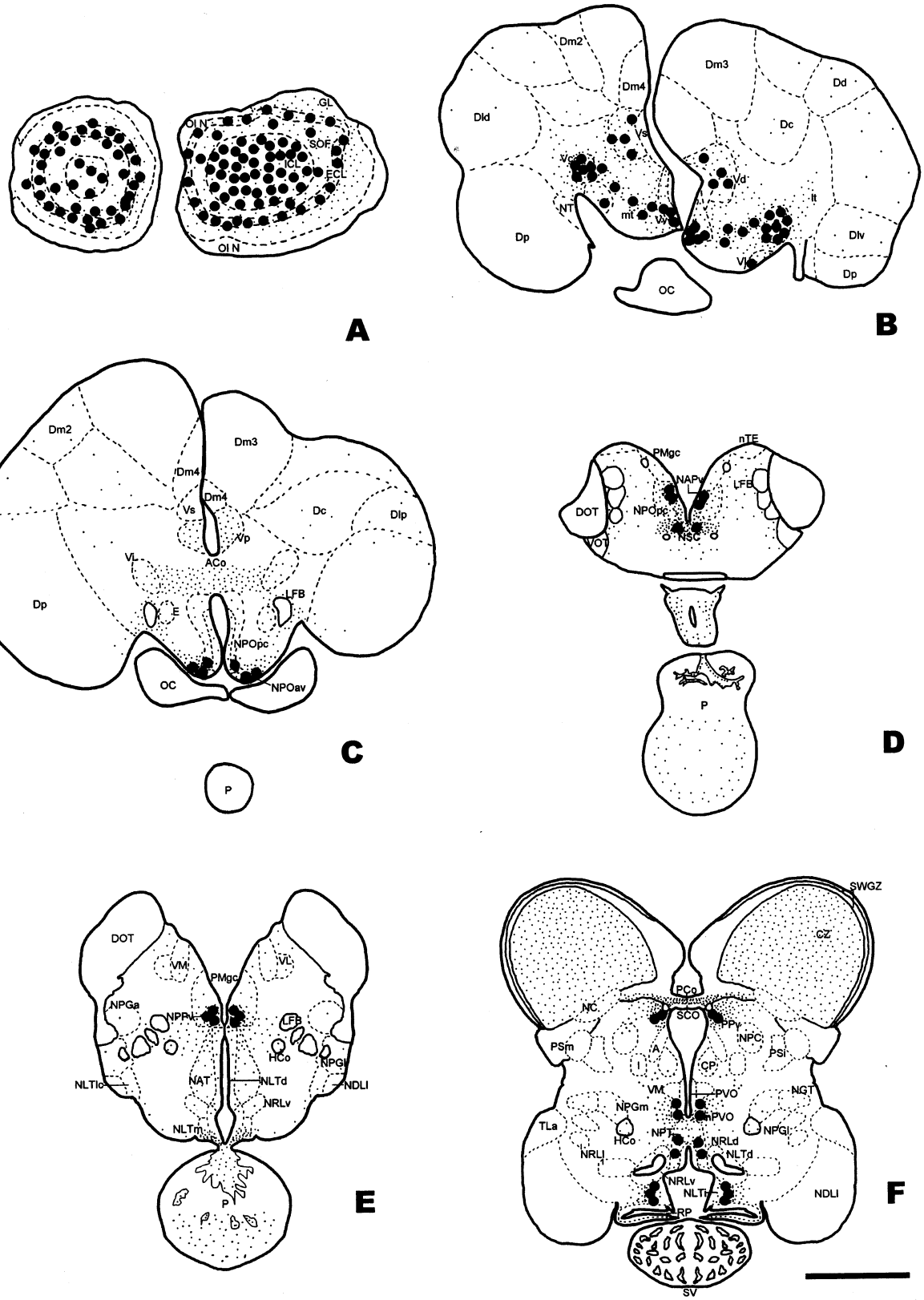

Fig. 1. Series of schematic transverse sections through the brain of *S. senegalensis* showing the distribution of tyrosine hydroxylase-immunoreactive perikarya (large filled circles) and fibers (small dots). A constitutes the rostralmost section and L the caudolmost one. Bar scale represents 1 mm. For abbreviations, see Table 1.

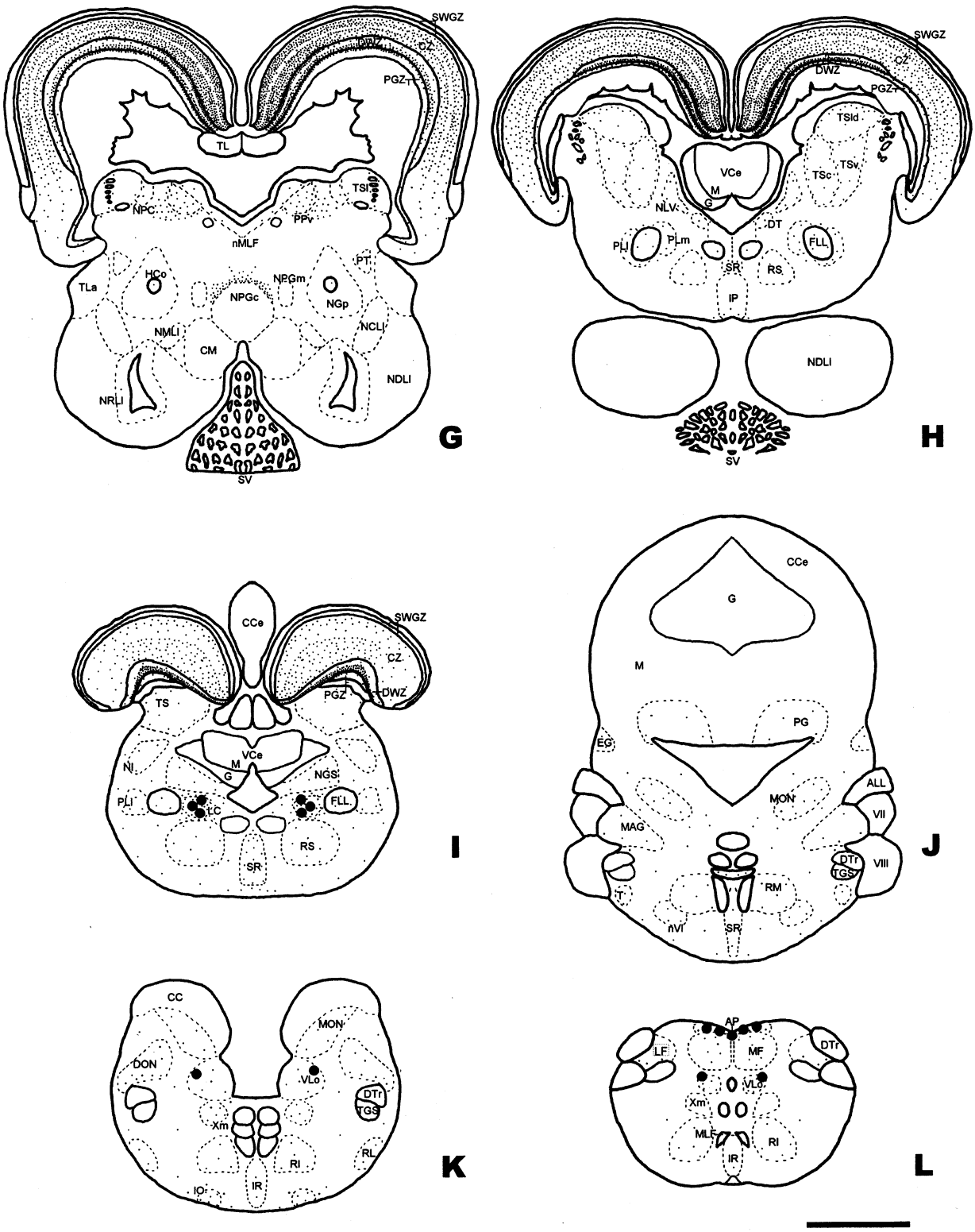

Fig. 1. (*Continued*)

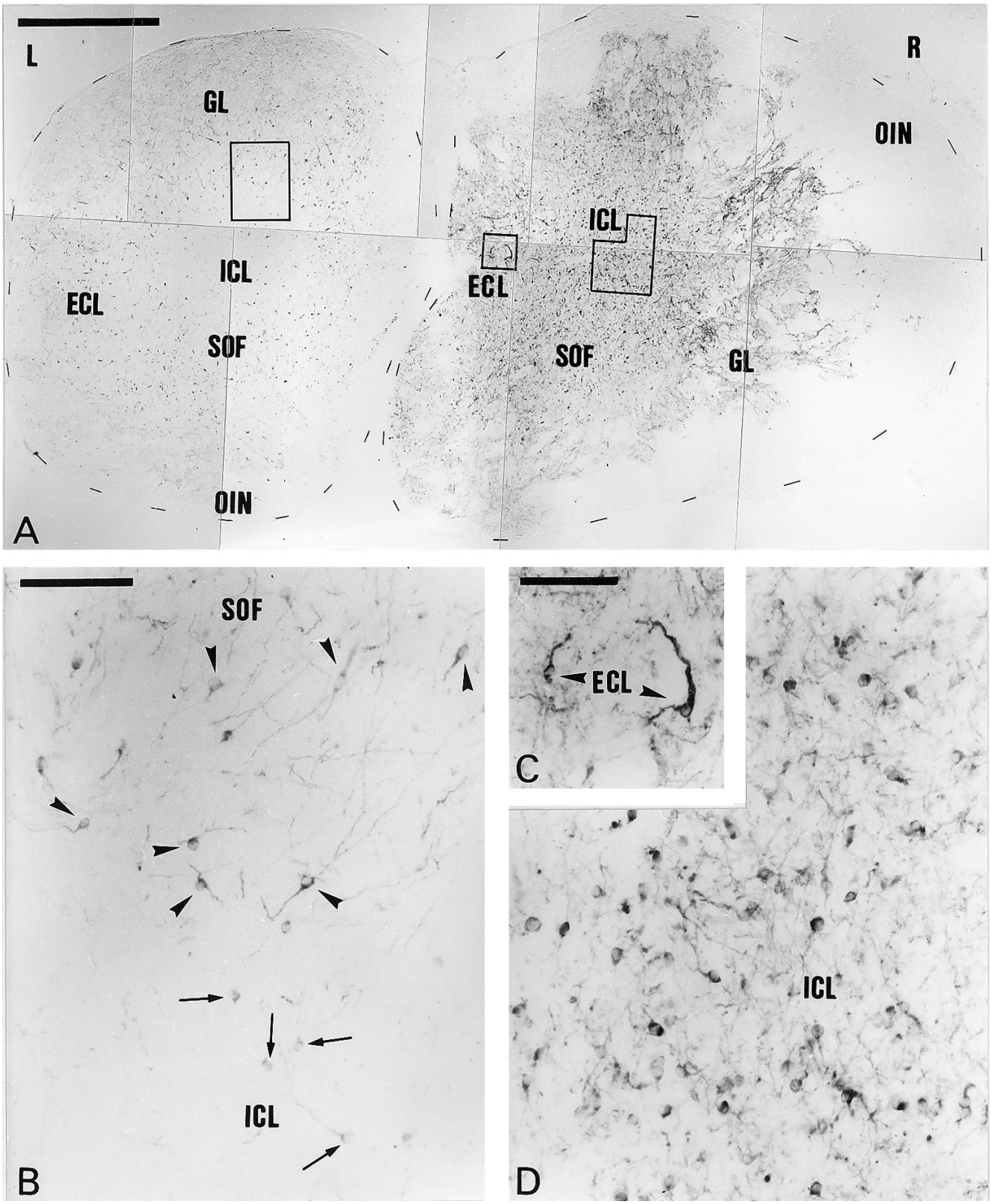

Fig. 2. Photomicrographs of transverse sections through the olfactory bulbs of *S. senegalensis*, showing TH-ir perikarya and fibers. (A) Section approximately at the level of the Fig. 1A. Note the large size, as well as the intense immunostaining of the right olfactory bulb (R) in relation to the left (L). Bar scale represents 500 μm. (B) Higher magnification of the framed area in the left olfactory bulb of A. Few weakly immunostained cell bodies are observed in the secondary olfactory fiber layer (arrowheads) and internal cell layer (arrows). Bar scale represents 50 μm and magnification is the same in D. (C) Higher magnification of the squared area in the right olfactory bulb of A, showing TH-ir cells in the external cell layer (arrowheads). Bar scale represents 50 μm. (D) Higher magnification of the framed area in the right olfactory bulb of A, showing abundant TH-ir perikarya and fibers in the internal cell layer. For abbreviations, see Table 1.

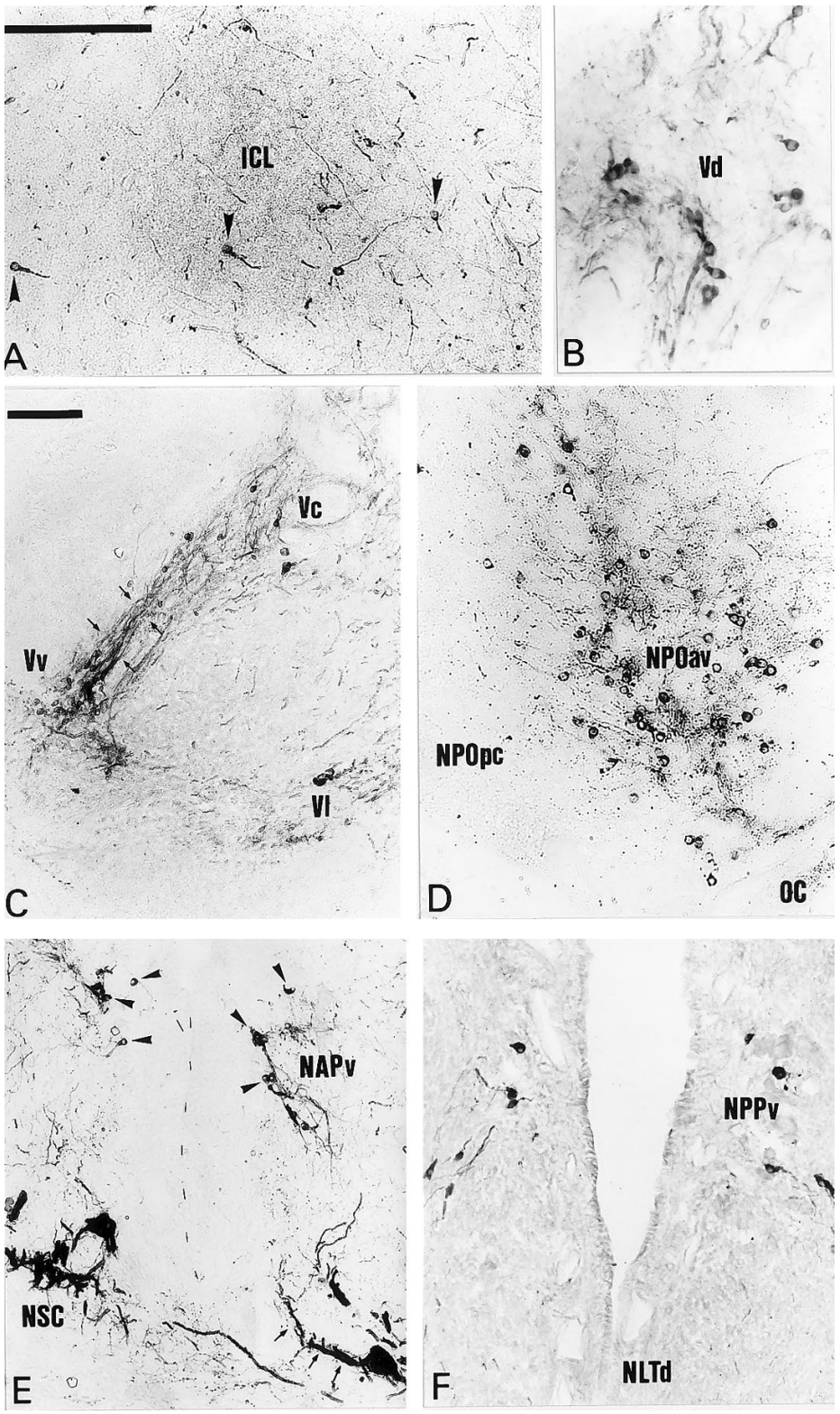

Fig. 3. Photomicrographs of transverse sections through the forebrain of *S. senegalensis*, showing TH-ir perikarya and fibers. (A) Internal cell layer of the olfactory bulbs. In some cells, dendritic processes can be observed exiting from the cell bodies (arrowheads). Bar scale is 100 μm and magnification is the same in B and D. (B) Ventral telencephalon. TH-ir cells and fibers in the dorsal nucleus (Vd). (C) Ventral telencephalon. TH-ir cells and fibers in the ventral (Vv), central (Vc) and lateral (Vl) nuclei. Numerous TH-ir fibers are observed in the medial olfactory tract (small arrows). Bar scale represents 100 μm and magnification is the same in E and F. (D) Preoptic area. Abundant TH-ir cells in the anteroventral part of the parvocellular preoptic nucleus (NPOav). Note the absence of catecholaminergic cells in the parvocellular part of the same nucleus (NPOpc). (E) Preoptic area. Small TH-ir cells (arrowheads) of the anterior periventricular nucleus (NAPv) and large TH-ir cells of the suprachiasmatic nucleus (NSC), in which thick dendrites are observed exiting from the cell bodies (small arrows). (F) TH-ir cells and fibers in the posterior periventricular nucleus. For other abbreviations, see Table 1.

ir cells appears in the locus coeruleus (LC), medially to the fasciculus longitudinalis lateralis (Fig. 1I). The large perikarya of these cells are clearly immunostained but the processes are lightly stained and can be traced for only a short distance from the cell bodies (Fig. 4D). The rhombencephalon contains catecholaminergic cell groups exhibiting immunoreactivity to TH in the vagal lobe (VLo) and area postrema (AP). The few ir cells of the VLo appear mainly in the dorsolateral zone of this nucleus and show a characteristic ovoid or elongated shape (Fig. 1K and L, Fig. 4E). Most of these cells are small and lightly stained, but medium-sized and large cells can also be observed. The TH-ir population of the area postrema is constituted by a compact cluster of small cells that line the dorsomedial edges of the caudal medulla (Fig. 1L and Fig. 4E).

3.2. Tyrosine hydroxylase-immunoreactive fibers

The presence of TH-ir fibers in the brain of *S. senegalensis* is particularly evident within and around the nuclei in which immunoreactive cell bodies are found. In the olfactory bulb, TH-ir fibers appear distributed mainly in the internal cell layer and the glomerular layer (Fig. 1A and Fig. 2A, Fig. 3A). As found with TH-ir cell bodies, TH-ir fibers were much more abundant in the right olfactory bulb than in the left one (Fig. 2A). This asymmetry becomes more evident in TH-ir fibers of the glomerular layer, but also in the internal cell layer. Numerous TH-ir fibers are observed in the ventral telencephalon, many of them running within the medial (Fig. 1B and Fig. 3C) and lateral olfactory tracts (Fig. 1B). These fibers are present in all nuclei that exhibited TH-ir cells (Vv, Vl, Vc, Vd) but also in other cell masses such as the postcommissural part of the ventral telencephalon, which is devoid of TH-ir cells (Fig. 1C). The dorsal telencephalon exhibits a weak density of TH-ir fibers, which are more evident in the central, laterodorsal and dorsal zones (Fig. 1B and C). Fine varicose TH-ir axons are seen dorsal and ventral to the fibers of the anterior commissure and around the lateral forebrain bundles (Fig. 1C).

In the diencephalon, the presence of TH-ir fibers is significant especially around the preoptic area (Fig. 1C–E). In this region, the immunostained perikarya were identified in NPOav, NSC, NAPv and NPPv (Fig. 1C–F, Fig. 3D–F), all these nuclei showing a large number of TH-ir axons. At this level, immunoreactive axons from the preoptic cells run ventrally and seem to enter the infundibulum, reaching the proximal pars distalis (PPD) of the adenohypophysis (Fig. 1D and E, Fig. 4A). In the hypothalamus, we observed most of the ir fibers within the medial tuberal zone (Fig. 1E and F, Fig. 4C). Furthermore, in the posterior tuberculum, TH-ir fibers are concentrated mainly within and around the nuclei with positive neurons, i.e. nPVO and NPT (Fig. 1F and Fig. 4B), although ir projections near the commissural preglomerular nucleus are also evident (Fig. 1G). In the dorsal aspect of the diencephalon, TH-ir axons are observed in the posterior commissure and pretectum (Fig. 1F and Fig. 4B). In this area, a tract of TH-ir axons originating in the cells of PPv can be seen; this tract extends laterally toward the optic tectum (Fig. 1F and G, Fig. 4B).

TH-ir cell bodies are not observed in the mesencephalon whereas ir fibers can be found, especially in the dorsal zone. In the optic tectum, the central zone (CZ) and the deep white zone (DWZ) displayed higher densities of labeled axons, which appear more concentrated in the medial region of CZ and the interne region of DWZ (Fig. 1F–I). We have also found TH-ir projections in the medial area of the mesencephalic tegmentum and in the torus semicircularis (Fig. 1G–I). At the transition between the midbrain and the hindbrain, most of the TH-ir fibers appear in the locus coeruleus, which also contained TH-ir cells (Fig. 1I and Fig. 4D). In the rhombencephalon, TH-ir axons are seen lateral to the medial longitudinal fasciculus, as well as in the reticular formation, the vagal lobe and the area postrema (Fig. 1I–L). Fine immunostained varicosities are also present in the inferior olivary region (Fig. 1K).

4. Discussion

We have described in this study the CNS distribution of TH immunoreactive systems in the Senegalese sole, *S. senegalensis*, using a brain atlas recently developed in this species (Rodríguez-Gómez et al., 2000; Rodríguez-Gómez et al., in preparation). To our knowledge this study represents the first description of the distribution of catecholamines in Pleuronectiformes, one of the most advanced order of euteleosts. The overall distribution of TH systems has been studied in Mormyriformes (Meek and Joosten, 1993), Anguilliformes (Roberts et al., 1989; Boularand et al., 1998), Cypriniformes (Yoshida et al., 1983; Hornby et al., 1987; Briñón et al., 1998), Salmoniformes (Manso et al., 1993; Anglade, 1994), Cyprinodontiformes (Halpern-Sebold et al., 1985), Siluriformes (Sas et al., 1990), Gasterosteiformes (Ekström et al., 1990) and Perciformes (Batten et al., 1993; Muñoz-Cueto et al., 1997). The general pattern of distribution of catecholamines in the brain of the Senegalese sole is consistent with that reported in these teleosts, but some remarkable differences are observed.

The rostralmost TH-ir cell population in the Senegalese sole appears in the olfactory bulbs. In this species, the right olfactory organ lies in the occular pigmented side and is larger than the left olfactory organ, which appears on the unpigmented side, partially turned toward the substratum. As a consequence,

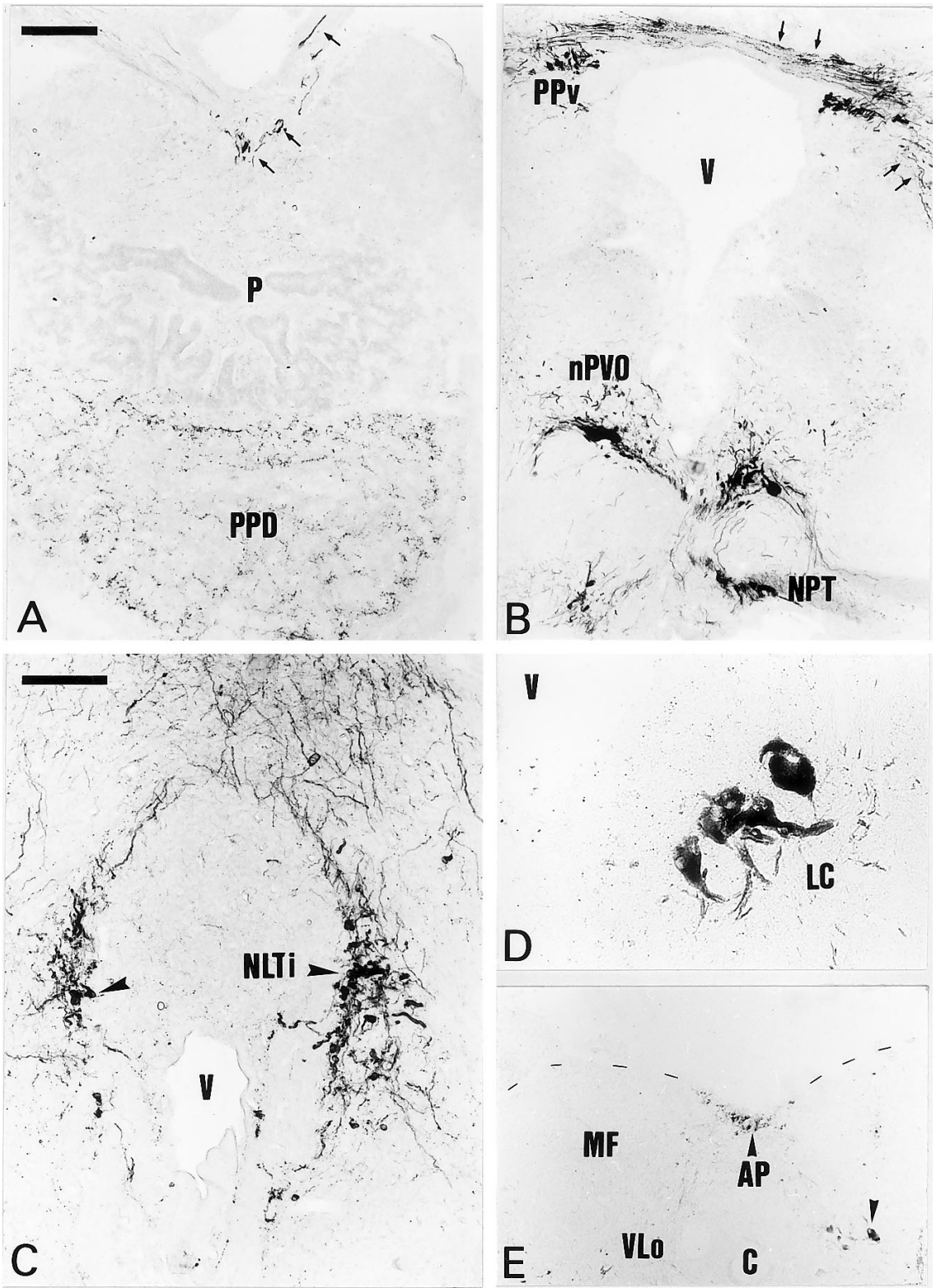

Fig. 4. Photomicrographs of transverse sections through the brain and pituitary of *S. senegalensis*, showing TH-ir cells and fibers. (A) Pituitary. TH-ir fibers in the pituitary stalk (arrows) and the proximal pars distalis (PPD). Bar scale is 200 μm and magnification is the same in B and E. (B) TH-ir cells and fibers in the periventricular pretectum (PPv) and two nuclei of the posterior tuberculum. Arrows mark TH-ir axons running through the posterior commissure and running laterally from pretectal TH-ir cells. (C) Caudomedial hypothalamus. TH-ir neurons and fibers in the inferior part of the lateral tuberal nucleus (NLTi). Bar scale represents 100 μm and magnification is the same in D. (D) Isthmic region. Large TH-ir cells in the locus coeruleus. (E) Caudal rhombencephalon. TH-ir cells in the area postrema (AP) and vagal lobe (VLo). For other abbreviations, see Table 1.

Table 1
Abbreviations

A, anterior thalamic nucleus
AP, area postrema
ACo, anterior commissure
ALL, anterior lateral line nerve
C, spinal cord canal
CC, crista cerebellaris
CCe, corpus of the cerebellum
CM, mammillary body
CP, central posterior thalamic nucleus
CZ, central zone of the optic tectum
Dc, central part of the dorsal telencephalon
Dd, dorsal part of the dorsal telencephalon
Dld, lateral dorsal part of the dorsal telencephalon
Dlp, lateral posterior part of the dorsal telencephalon
Dlv, lateral ventral part of the dorsal telencephalon
Dm2, subdivision 2 of the medial part of the dorsal telencephalon
Dm3, subdivision 3 of the medial part of the dorsal telencephalon
Dm4, subdivision 4 of the medial part of the dorsal telencephalon
DON, descendens octaval nucleus
DOT, dorsal optic tract
Dp, posterior part of the dorsal telencephalon
DT, dorsal tegmental nucleus
DTr, descending trigeminal tract
DWZ, deep white zone of the optic tectum
E, entopeduncular nucleus
ECL, external cellular layer of the olfactory bulbs
EG, eminentia granularis
FLL, lateral longitudinal fascicle
G, granular layer of the cerebellum
GL, glomerular layer of the olfactory bulbs
HCo, horizontal commissure
I, intermediate thalamic nucleus
ICL, internal cellular layer of the olfactory bulbs
IO, inferior olive
IP, interpeduncular nucleus
IR, inferior raphe
LC, nucleus of the locus coeruleus
LF, lateral funicular nucleus
LFB, lateral forebrain bundle
lt, lateral olfactory tract
M, molecular layer of the cerebellum
MAG, magnocellular nucleus
MF, medial funicular nucleus
MLF, medial longitudinal fascicle
MON, medial octavolateral nucleus
mt, medial olfactory tract
NAPv, anterior periventricular nucleus
NAT, anterior tuberal nucleus
NC, cortical nucleus
NCLI, central nucleus of the inferior lobe
NDLI, diffuse nucleus of the inferior lobe
NGp, posterior part of the glomerular nucleus
NGS, secondary gustatory nucleus
NGT, tertiary gustatory nucleus
NI, ithsmic nucleus
NLTd, dorsal part of the lateral tuberal nucleus
NLTi, inferior part of the lateral tuberal nucleus
NLTlc, lateral caudal part of the lateral tuberal nucleus
NLTm, medial part of the lateral tuberal nucleus
NLV, lateral nucleus of the valvula
nMLF, nucleus of the medial longitudinal fascicle
NMLI, medial nucleus of the inferior lobe
NPC, central pretectal nucleus
NPGa, anterior preglomerular nucleus
NPGc, commissural preglomerular nucleus
NPGl, lateral preglomerular nucleus
NPGm, medial preglomerular nucleus
NPOav, anteroventral part of the parvocellular preoptic nucleus
NPOpc, parvocellular part of the parvocellular preoptic nucleus
NPPv, posterior periventricular nucleus
NPT, posterior tuberal nucleus
nPVO, nucleus of the paraventricular organ
NRLd, dorsal part of the nucleus of the lateral recess
NRLv, ventral part of the nucleus of the lateral recess
NRLl, lateral part of the nucleus of the lateral recess
NSC, suprachiasmatic nucleus
NT, nucleus taenia
nTE, nucleus of the thalamic eminentia
nVI, abducens nucleus
OC, optic chiasm
OlN, olfactory nerve fibers
P, pituitary
PCo, posterior commissure
PG, periventricular granular cell mass
PGZ, periventricular grey zone of the optic tectum
PLl, lateral part of the perilemniscular nucleus
PLL, posterior lateral line nerve
PLm, medial part of the perilemniscular nucleus
PMgc, gigantocellular part of the magnocellular preoptic nucleus
PPD, proximal pars distalis
PPv, ventral part of the periventricular pretectal nucleus
PSi, intermediate part of the superficial pretectal nucleus
PSm, magnocellular part of the superficial pretectal nucleus
PT, posterior thalamic nucleus
PVO, paraventricular organ
RI, inferior reticular nucleus
RL, lateral reticular nucleus
RM, medial reticular nucleus
RP, posterior recess
RS, superior reticular nucleus
SCO, subcommissural organ
SOF, secondary olfactory fibers
SR, superior raphe
SV, saccus vasculosus
SWGZ, superficial white and grey zone of the optic tectum
T, tangential nucleus
TGS, secondary gustatory tract
TL, torus longitudinalis
TLa, nucleus of the torus lateralis
TS, torus semicircularis
TSc, central part of the torus semicircularis
TSl, lateral part of the torus semicircularis
TSld, lateral dorsal part of the torus semicircularis
TSv, ventral part of the torus semicircularis
V, ventricle
Vc, central part of the ventral telencephalon
VCe, valvula of the cerebellum
Vd, dorsal part of the ventral telencephalon
Vi, intermediate part of the ventral telencephalon
VII, facial nerve
VIII, octaval nerve
Vl, lateral part of the ventral telencephalon
VL, ventrolateral thalamic nucleus
VLo, vagal lobe
VM, ventromedial thalamic nucleus
VOT, ventral optic tract
Vp, postcommissural part of the ventral telencephalon
Vs, supracommissural part of the ventral telencephalon
Vv, ventral part of the ventral telencephalon
Xm, vagal motor nucleus

the right olfactory nerve and bulb are thicker than the contralateral ones. A similar asymmetry was observed in the winter flounder, *Pseudopleuronectes americanus*, in which the right telencephalon was also 8% larger than the left (Prasada Rao and Finger, 1984). In the Senegalese sole, this asymmetry seems to apply also to the localization and number of catecholaminergic cells. The presence of TH-/DA-ir neurons and fibers in the olfactory bulbs has been previously reported in all vertebrates (see Smeets and Reiner, 1994 for a comparative review). Small round TH-ir and/or DA-ir cells display a periglomerular position in the olfactory bulbs of elasmobranchs (Meredith and Smeets, 1987), reptiles (Smeets and Steinbusch, 1990), birds (Reiner et al., 1994) and mammals (Hökfelt et al., 1984). A similar periglomerular location was observed in the catecholaminergic cells of *Carassius auratus* (Hornby et al., 1987) or *Apteronotus leptorhynchus* (Sas et al., 1990). However, in lampreys (Pierre et al., 1994) and other teleost species such as *Tinca tinca*, *Barbus meridionalis*, *Salmo gairdnieri* (Alonso et al., 1989), *Gasterosteus aculeatus* (Ekström et al., 1990), *Dicentrarchus labrax* (Batten et al., 1993) or *Sparus aurata* (Muñoz-Cueto et al., 1997) most TH-ir cells appear in the interne layers of the olfactory bulbs.

The amount of TH-ir fibers was also higher in the right olfactory bulb of the Senegalese sole, especially in the glomerular layer and internal cell layer. These fibers seem to be, at least in part, intrinsic olfactory bulb plexuses originated from TH-ir cells of the olfactory bulb, but could also represent extrinsic fibers from TH-ir cells located in other areas. Efferent and afferent projections of the olfactory bulbs have been determined in various fish species (Finger, 1975; Bass, 1981a,b; Von Bartheld et al., 1984; Levine and Dethier, 1985; Rooney et al., 1989; Sas et al., 1993). Fascicles of the medial and lateral olfactory tracts project to different nuclei of the ventral and dorsal telencephalon, as well as to the diencephalic habenula, preoptic region (parvocellular and magnocellular areas) and caudal hypothalamus. Bulbopetal cells have been found in the contralateral olfactory bulb, the transitional zone between the olfactory bulb and the telencephalon, some nuclei of the ventral and dorsal telencephalon, the basal preoptic area, the caudal hypothalamus, and some mesencephalic and isthmal nuclei such as the nucleus raphe or the locus coeruleus. In another pleuronectiform species, the winter flounder, an asymmetry in the projections of the right and left bulbs has been observed, the right bulb having more extensive projections (Prasada Rao and Finger, 1984). In this species, neurons afferent to the olfactory bulbs are found in some nuclei of the telencephalon as Vd or Vv, in the ventral region of the preoptic area and in the locus coeruleus, among others (Prasada Rao and Finger, 1984), which at least in the Senegalese sole, contained TH-ir cells. This asymmetry in the number and distribution of rostral catecholaminergic cells in Senegalese sole could be a consequence of developmental mechanisms, being possibly related to the establishment of differences in the connections of the right and left bulbs in this species. Further studies are being directed towards an examination of the connections of the right and left bulbs and the ontogeny of catecholaminergic systems in Senegalese sole. These studies could clarify if there are also asymmetries in the connectivity of the bulbar catecholaminergic cells during symmetric larval period and/or adult asymmetric stages. It will also be interesting to know if these asymmetries could apply to other central nervous system structures (e.g. extrabulbar catecholaminergic systems). In teleosts, the existence of structural asymmetries in the epithalamus of several species and in dorsal/ventral crossing at the optic chiasm of flatfishes (Bisazza et al., 1998) has been described. Furthermore, NADPH diaphorase activity in the optic tectum of metamorphic turbots (Jansen and Enger, 1996) and [^{14}C]2-deoxyglucose uptake by neurons of the octavolateralis complexes of adult flatfishes (Meyer et al., 1981) also exhibit a distinct bilateral asymmetry.

The presence of TH-ir cells in the ventral telencephalon has been described extensively in teleosts but some discrepancies are observed, which might reflect differences in neuroanatomical nomenclature rather than true species differences (Hornby et al., 1987; Ekström et al., 1990; Sas et al., 1990; Meek and Joosten, 1993; Batten et al., 1993; Manso et al., 1993; Muñoz-Cueto et al., 1997; Briñón et al., 1998). A large majority of telencephalic TH-ir cells represent dopaminergic cells, but the presence of putative L-DOPA-producing cells cannot be discarded, at least in some teleosts (Ekström et al., 1990; Meek, 1994; Meek and Joosten, 1993).

The distribution of TH-ir cells and fibers in the diencephalon of the Senegalese sole agrees with that observed in most of the other teleost species (Meek, 1994). Nevertheless, no immunostaining was seen in the ventromedial thalamic nucleus, which has been considered to contain catecholamines in most of the teleosts studied (Meek, 1994). In some teleosts, the ventral thalamus is reciprocally connected with the retina and the optic tectum and has been implicated in visual integration (Presson et al., 1985; Striedter, 1990; Northcutt and Butler, 1991). Catecholaminergic cells and/or fibers have also been detected in retina and optic tectum of teleosts and other vertebrate species (Jaffe et al., 1991; Meek, 1994; Smeets and Reiner, 1994). It should be noted that the Senegalese sole is a benthonic fish in which visual sense and structures are reduced in comparison to other marine pelagic species (personal observations). However, to date, the role of catecholamines in visual processing is not well understood.

Catecholaminergic cells groups are present in the preoptic region of *Polypterus* (Reiner and Northcutt, 1992; Piñuela and Northcutt, 1995), myxinoids (Wicht and Northcutt, 1994), cartilaginous fish (Meredith and Smeets, 1987; Molist et al., 1993), teleosts (Bonn and Kramer, 1987; Hornby et al., 1987; Sas et al., 1990; Meek and Joosten, 1993; Muñoz-Cueto et al., 1997), amphibians (Franzoni et al., 1986), reptiles (Smeets et al., 1986, 1987), and mammals (Hökfelt et al., 1984). In the Senegalese sole, TH-ir fibers from preoptic cells running ventrolaterally reach the mediobasal hypothalamus and appear to enter the hypophysis. The presence of hypophysiotrophic neurons in the preoptic region has been identified in several species such as goldfish (Fryer and Maler, 1981; Anglade et al., 1993), *Apteronotus leptorhynchus* (Johnston and Maler, 1992), *Clarias batrachus* (Prasada Rao et al., 1993) and rainbow trout (Anglade, 1994). Some of these hypophysiotrophic nuclei (e.g. the parvocellular preoptic nucleus, anterior periventricular nucleus and suprachiasmatic nucleus) contain TH-/DA-ir neurons in *Apteronotus leptorhynchus* (Sas et al., 1990) and rainbow trout (Linard et al., 1996b), as we have found in the Senegalese sole. Moreover, using carbocyanine tract tracing in combination with TH immunohistochemistry, it has been shown that catecholaminergic cells in the anterior periventricular nucleus innervate the pituitary in the Atlantic salmon (Holmqvist and Ekström, 1995).

Several studies have demonstrated that catecholamines can regulate the release of pituitary homones in fishes (Chang et al., 1985; Wong et al., 1992; Kah et al., 1993; Trudeau, 1997). In some teleosts, noradrenaline seems to stimulate the release of gonadotrophins (GTHs) (Kah et al., 1993; Trudeau, 1997) whereas numerous data provide evidence of a powerful inhibitory effect of DA on the release of GTHs (Billard et al., 1984; Peter et al., 1986; Van der Kraak et al., 1986; Goos et al., 1987; Linard et al., 1995). In goldfish, histofluorescence, radioautography and immunohistochemistry reveal the presence of catecholaminergic fibers in the neurohypophysis and in the proximal pars distalis of the adenohypophysis, in direct contact with gonadotrophic cells (Kah et al., 1984a,b, 1986, 1987). However, evidence of such inhibition has not been found in some marine fishes such as the Atlantic croacker (Copeland and Thomas, 1989) and the gilthead sea bream (Zohar et al., 1995). Furthermore, DA can stimulate the release of growth hormone (GH) in cyprinids by acting on pituitary D1 receptors (Chang et al., 1990; Wong et al., 1992). In the Senegalese sole, TH-ir fibers can be observed in the proximal pars distalis of the adenohypophysis, where GTH and GH cells are located (Rendón et al., 1997). Further studies should be directed at elucidating whether catecholamines modulate the GTH/GH secretion in the Senegalese sole and at determining which TH-/DA-ir nuclei represent hypophysiotrophic cell masses in this species.

In the synencephalon of the Senegalese sole, TH-ir axons originating in the ventral part of the periventricular pretectal nucleus run laterally to enter the pretectal area and the mesencephalic tegmentum. These cells seem to be the origin of the intense catecholaminergic innervation observed in the optic tectum of the Senegalese sole, as has been suggested in other teleosts (Hornby et al., 1987). The presence of catecholaminergic cells in the periventricular pretectum appears to be a conservative feature of teleosts (Hornby and Piekut, 1990; Hornby et al., 1987; Roberts et al., 1989; Ekström et al., 1990; Sas et al., 1990; Batten et al., 1993; Manso et al., 1993; Meek and Joosten, 1993; Muñoz-Cueto et al., 1997). Putative L-DOPA-synthesizing neurons are also observed in this area in sea bass (Batten et al., 1993) and *Gnathonemus* (Meek, 1994).

Two TH-ir cell populations can be observed in the posterior tuberculum of the Senegalese sole. The TH-ir cells that we have ascribed to the nucleus of the paraventricular organ seem to represent the large TH-/DA-ir cells of the periventricular nucleus of the posterior tuberculum (TPp) or the magnocellular hypothalamic nucleus (nmh) described in *Apteronotus* (Sas et al., 1990) and *Gnathonemus* (Meek and Joosten, 1993; Meek et al., 1989), respectively. Similar catecholaminergic cells were also described in the posterior tuberculum of *Carassius* (Hornby and Piekut, 1989), *Anguilla* (Roberts et al., 1989) and *Gasterosteus* (Ekström et al., 1990). In turn, the localization of catecholaminergic cells in the posterior tuberal nucleus appear to be evolutionarily conservative, being present not only in teleosts (Meek, 1994), but also in cartilaginous fishes (Molist et al., 1993; Stuesse et al., 1994), in amphibians (González and Smeets, 1994) and probably in mammals (Tillet, 1994). Contrary to that described in some teleosts as *Gnathonemus*, *Carassius*, and *Apteronotus* (see Meek, 1994 for a revision), no cerebrospinal fluid (CSF)-contacting TH-ir neurons were observed in the posterior tuberculum of the Senegalese sole. This absence is consistent with that reported in *Gasterosteus* (Ekström et al., 1990) or *Dicentrarchus* (Batten et al., 1993) and might represent a derived character of highly evolved teleosts. In teleosts, the majority of CSF-contacting neurons of the posterior tuberculum are DA-ir but not TH-ir cells (Ekström et al., 1990). These cells have been considered to be DA-acquiring cells because it seems that they cannot syntesize DA themselves and have to acquire it from external sources (Meek, 1999).

The TH-ir cells located in the ventromedial zone of the caudal hypothalamus of the Senegalese sole belong to the inferior part of the lateral tuberal nucleus. The lateral tuberal nucleus has been reported to be one of the major hypophysiotropic areas (Fryer and Maler, 1981), and retrogradely labeled neurons have been de-

scribed in the inferior subdivision of this nucleus after DiI implantions in the goldfish pituitary (Anglade et al., 1993). Thus, TH-ir neurons of the inferior part of the lateral tuberal nucleus could represent another source of catecholaminergic fibers projecting to the pituitary. Distinct TH-/DA-ir cell groups are also present in the caudoventral hypothalamus of hagfishes (Wicht and Northcutt, 1994), cartilaginous fishes (Molist et al., 1993; Stuesse et al., 1994) and bony fishes (Sas et al., 1990; Hornby and Piekut, 1990; Meek and Joosten, 1993; Manso et al., 1993), but different nomenclatures have been used to name these cell populations.

The absence of TH-ir cell groups in the mesencephalon represents a major difference between the catecholaminergic systems of teleosts and other vertebrates (Smeets and Reiner, 1994). However, many TH-ir axons are observed in the optic tectum of Senegalese sole, especially in the central zone, and a moderate TH-ir innervation is also present in the torus semicircularis. A similar observation was reported in *Apteronotus* (Sas et al., 1990) and carp (Cuadrado et al., 1992). The isthmus region of the Senegalese sole, at the transition between the mesencephalon and the rhombencephalon, also contains TH-ir fibers. This catecholaminergic innervation seems to originate in TH-ir cells of the locus coeruleus. This nucleus also contains neuropeptide Y-immunoreactive cells in the Senegalese sole (Rodríguez-Gómez et al., in preparation) and has been reported as a noradrenergic nucleus, not only in teleosts but also in most vertebrates (Ma, 1994a,b, 1997; Smeets and Reiner, 1994). Thus, the presence of TH and DA immunoreactivity in this nucleus should be interpreted as intermediate steps in the synthesis of noradrenaline, although the presence of dopaminergic cells cannot be excluded (Hökfelt et al., 1984; Hornby and Piekut, 1990). Immunoreactive neurons described in the vagal sensory lobe of Senegalese sole were also identified from early to more recently developed teleosts (Roberts et al., 1989; Ekström et al., 1990; Sas et al., 1990; Manso et al., 1993; Meek and Joosten, 1993; Muñoz-Cueto et al., 1997). The presence of noradrenergic cells (i.e. dopamine β-hydroxylase or noradrenaline immunoreactive cells) have also been reported in this area in *Apteronotus* and *Gasterosteus* (Sas et al., 1990; Ekström et al., 1990). The most caudal TH-ir cell population in the brain of the Senegalese sole is a dorsal midline structure that appears in the caudal medulla. This catecholaminergic cell group was described in detail by Morita and Finger (1987) in the goldfish and represents the area postrema described in many other teleosts (Meek, 1994) and tetrapods (Moore and Card, 1984; González and Smeets, 1994; Smeets and Reiner, 1994; Reiner et al., 1994). Although catecholaminergic cells were also observed in the rhombencephalic reticular formation of most teleosts studied (Meek, 1994), similar TH-ir cells were not observed in Senegalese sole. The absence of a reticular TH-ir group could be a derived condition of highly evolved teleosts because such as caudal rhombencephalic cell population was neither described in gasterosteiforms (Ekström et al., 1990) nor perciforms (Batten et al., 1993; Muñoz-Cueto et al., 1997).

In conclusion, this study provides a detailed description of the organization of catecholaminergic systems in the brain of a pleuronectiform species, the Senegalese sole and it extends the series of teleostean species in which TH immunohistochemistry is applied to the most advanced and recently developed percomorpha. To our knowledge, this analysis represents the first report of an interhemispheric asymmetry in the catecholaminergic systems of a vertebrate species, which seems to be correlated with an asymmetry in the size of the olfactory organs, nerves and bulbs. Interestingly, Smeets and Reiner (1994) speculated that a predominantly periglomerular location of catecholaminergic neurons in the olfactory bulbs is a shared derived characteristic of amniotes, whereas a location in deeper bulbar layers is the primitive condition. It remains to be determined whether the distribution of catecholaminergic cells in the olfactory bulbs of the Senegalese sole represents an intermediate stage or a derived condition. Furthermore, striking differences were observed in the distribution of catecholaminergic systems in Senegalese sole, including the absence of TH-ir cells in the ventromedial thalamic nucleus, the CSF-contacting cells of the paraventricular organ or the reticular formation. This study will also constitute a basis for investigating the role of catecholamines in the neuroendocrine regulation of the gonadotrophic functions and reproduction in this species, which is of increasing commercial importance for Mediterranean and South Atlantic aquaculture.

Acknowledgements

This work was supported by a grant from the Spanish Ministry of Education and Science (DGICYT PB93-1209). We thank Dr C. Piñuela and Royston Snart for checking the English and D. González for his helpful assistance.

References

Alonso, J.R., Coveñas, R., Lara, J., Arévalo, R., De León, M., Aijón, J., 1989. Tyrosine hydroxylase immunoreactivity in a subpopulation of granule cells in the olfactory bulbs of teleost fish. Brain Behav. Evol. 34, 318–324.

Anglade, I., 1994. Étude morphofonctionnelle des systèmes neuroendocriniens impliqués dans le contróle de la fonction gonadotrope chez les poissons osseux. Ph.D. Dissertation, Université de Bourdeaux, France.

Anglade, I., Zandbergen, H.A., Kah, O., 1993. Origin of the pituitary innervation in the goldfish. Cell Tissue Res. 273, 345–355.

Bass, A.H., 1981a. Olfactory bulb efferents in the channel catfish, *Ictalurus punctatus*. J. Morphol. 169, 91–111.

Bass, A.H., 1981b. Telencephalic efferents in the channel catfish, *Ictalurus punctatus*: projections to the olfactory bulb and optic tectum. Brain Behav. Evol. 19, 1–16.

Batten, T.F.C., Berry, P.A., Maqbool, A., Moons, L., Vandesande, F., 1993. Immunolocalization of catecholamine enzimes, serotonin, dopamine and L-dopa in the brain of *Dicentrarchus labrax* (Teleostei). Brain Res. Bull. 31, 233–252.

Billard, R., Reinaud, P., Hollenbecq, M.G., Breton, B., 1984. Advancement and synchronization of spawning in *Salmo gairdneri* and *Salmo trutta* following administration of LHRHa combined or not with pimozide. Aquaculture 43, 57–66.

Bisazza, A., Rogers, L.J., Vallortigara, G., 1998. The origins of cerebral asymmetry a review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. Neurosci. Biobehav. Rev. 22, 411–426.

Björklund, A., Hökfelt, T., 1984. Central catecholamine neurons as revealed by immunohistochemistry with special reference to adrenaline neurons. In: Björklund, A., Hökfelt, T. Jr (Eds.), Handbook of Chemical Neuroanatomy. Classical Transmitters in the CNS, Part I, vol. 2. Elsevier, Amsterdam, pp. 157–276.

Bonn, V., Kramer, B., 1987. Distribution of monoamine-containing neurons in the brain of the weakly electric teleost, *Eigenmannia lineata* (Gymnotiformes: Rhamphichtyidae). Z. Mikrosk. Anat. Forsch. 101, 339–362.

Boularand, S., Biguet, N.F., Vidal, B., Veron, M., Mallet, J., Vincent, J.D., Dufour, S., Vernier, P., 1998. Tyrosine hydroxylase in the European eel (*Anguilla anguilla*) cDNA cloning, brain distribution and phylogenetic analysis. J. Neurochem. 71, 460–470.

Braford, M.R. Jr, Northcutt, R.G., 1983. Organization of the diencephalon and pretectum of the ray-finned fishes. In: Northcutt, R.G., Davis, R.E. (Eds.), Fish Neurobiology, vol. 2. University of Michigan Press, Ann Arbor, pp. 117–163.

Briñón, J.G., Medina, M., Arévalo, R., Alonso, J.R., Lara, J.M., Aijón, J., 1993. Volumetric analysis of the telencephalon and tectum during metamorphosis in a flatfish, the turbot *Scopthalmus maximus*. Brain Behav. Evol. 41, 1–5.

Briñón, J.G., Arévalo, R., Weruaga, E., Crespo, C., Alonso, J.R., Aijón, J., 1998. Tyrosine hydroxylase-like immunoreactivity in the brain of the teleost fish *Tinca tinca*. Arch. Ital. Biol. 136, 17–44.

Caroff, J., Barthelemy, L., Sebert, P., 1986. Brain and plasma biogenic amines analysis by the EC-HPLC technique: application to fish. Comp. Biochem. Physiol. 84C, 151–153.

Chang, J.P., Marchant, T.A., Cook, A.F., Nahorniak, C.S., Peter, R.E., 1985. Influences of catecholamines on growth hormone release in female goldfish, *Carassius auratus*. Neuroendocrinology 40, 463–470.

Chang, J.P., Yu, K.L., Wong, A.O.L., Peter, R.E., 1990. Differential actions of dopamine receptor subtypes on gonadotropin and growth hormone release in vitro in goldfish. Neuroendocrinology 51, 661–674.

Copeland, P.A., Thomas, T., 1989. Control of gonadotropin release in the Atlantic croaker (*Micropogonias undulatus*): evidence for lack of dopaminergic inhibition. Gen. Comp. Endocrinol. 74, 474–483.

Cuadrado, M.I., Coveñas, R., Tramu, G., 1992. Neuropeptides and monamines in the torus semicircularis of the carp (*Cyprinus carpio*). Brain Res. Bull. 29, 529–539.

Dinis, M.T., 1992. Aspects of the potential of *Solea senegalensis* Kaup for aquaculture larval rearing and weaning to an artificial diet. Aquacult. Fish. Manage. 23, 515–520.

Drake, P., Arias, A.M., Rodríguez, R.B., 1984. Cultivo extensivo de peces marinos en los esteros de las salinas de San Fernando (Cádiz). II. Características de la producción de peces. Inf. Tec. Inst. Inv. Pesq. 116, 1–23.

Ekström, P., Honkanen, T., Steinbusch, H.W.M., 1990. Distribution of the dopamine-immunoreactive neuronal perikarya and fibers in the brain of a teleost, *Gasterosteus aculeatus* L. Comparison with tyrosine hydroxylase- and dopamine-β-hydroxylase-immunoreactive neurons. J. Chem. Neuroanat. 3, 233–260.

Fehri-Bedoui, R., 1997. *Solea senegalensis* (Kaup, 1858). Elevage, comportement trophique et morpho-histologie, Ph.D. Dissertation, University of Tunis, Tunis, p. 223.

Finger, T.E., 1975. The distribution of the olfactory tracts in the bullhead catfish, *Ictalurus nebulosus*. J. Comp. Neurol. 161, 125–142.

Finger, T.E., 1983. Organization of the teleost cerebellum. In: Northcutt, R.G., Davis, R.E. (Eds.), Fish Neurobiology, vol. 1. University of Michigan Press, Ann Arbor, pp. 261–284.

Franzoni, M.F., Thibault, J., Fasolo, A., Martinoli, M.G., Scarani, F., Calas, A., 1986. Organization of tyrosine-hydroxylase immunopositive neurons in the brain of the crested newt, *Triturus cristatus carnifex*. J. Comp. Neurol. 251, 121–134.

Fryer, J.N., Maler, L., 1981. Hypophysiotropic neurons in the goldfish hypothalamus demonstrated by retrogade transport of horseradish peroxidase. Cell Tissue Res. 218, 93–102.

González, A., Smeets, W.J.A.J., 1994. Catecholamine systems in the CNS of amphibians. In: Smeets, W.J.A.J., Reiner, A. (Eds.), Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates, Part I. Cambridge University Press, Cambridge, pp. 77–102.

Goos, H.J.T., Joy, K.P., DeLeeuw, R., Va Oordt, P.G.W.J., Van Delft, A.M.L., Gielen, J.T., 1987. The effect of luteinizing hormone-releasing hormone analogue (LHRHa) in combination with different drugs with anti-dopamine and anti-serotonin properties on gonadotropin release and ovulation in the African catfish, *Clarias gariepinus*. Aquaculture 63, 143–156.

Halpern-Sebold, L.R., Margolis-Kazan, H., Schreibman, M.P., Joh, T.H., 1985. Immunoreactive tyrosine hydroxylase in the brain and pituitary gland of the platyfish. Proc. Soc. Exp. Biol. Med. 178, 486–489.

Hökfelt, T., Martensson, R., Björklund, A., Kleinau, S., Goldstein, M., 1984. Distributional maps of tyrosine hydroxylase-immunoreactive neurons in the rat brain. In: Björklund, A., Hökfelt, T. (Eds.), Classical Transmitters in the CNS. Handbook of Chemical Neuroanatomy, Part I, vol. 2. Elsevier, Amsterdam, pp. 277–379.

Holmqvist, B.I., Ekström, P., 1995. Hypophysiotrophic systems in the brain of the Atlanctic salmon. Neuronal innervation of the pituitary and the origin of pituitary dopamine and nonapeptides identified by means of combined carbocyanine tract tracing and immunocytochemistry. J. Chem. Neuroanat. 8, 125–145.

Hornby, P.J., Piekut, D.T., 1989. Immunoreactive dopamine hydroxylase in neuronal groups of the goldfish brain. Brain Behav. Evol. 32, 252–256.

Hornby, P.J., Piekut, D.T., 1990. Distribution of catecholamine-synthesizing enzymes in goldfish brain. Presumptive dopamine and norepinephrine neural organization. Brain Behav. Evol. 35, 49–64.

Hornby, P.J., Piekut, D.T., Demski, L.S., 1987. Localization of immunoreactive tyrosine hydroxylase in the goldfish brain. J. Comp. Neurol. 261, 1–14.

Jaffe, E.H., Urbina, M., Drujan, B.D., 1991. Possible neurotransmitter role of noradrenaline in the teleost retina. J. Neurosci. Res. 29, 190–195.

Jansen, J.K., Enger, P.S., 1996. NADPH diaphorase activity is asymmetrically distributed in the optic tectum during the period of eye migration in turbots. Acta Physiol. Scand. 157, 515–517.

Johnston, S.A., Maler, L., 1992. Anatomical organization of the hypophysiotropic systems in the electric fish, *Apteronotus leptorhynchus*. J. Comp. Neurol. 317, 421–437.

Kah, O., Chambolle, P., Thibault, J., Geffard, M., 1984a. Existence of dopaminergic neurons in the preoptic region of the goldfish. Neurosci. Lett. 48, 293–298.

Kah, O., Dubourg, P., Chambolle, P., Calas, A., 1984b. Ultrastructural identification of catecholaminergic fibers in the goldfish pituitary. Cell Tissue Res. 238, 621–626.

Kah, O., Dubourg, P., Onteniente, B., Geffard, M., Calas, A., 1986. The dopaminergic innervation of the goldfish pituitary: an immunocytochemical study at the electron-microscope level using antibodies against dopamine. Cell Tissue Res. 244, 577–582.

Kah, O., Dulka, J.G., Dubourg, P., Thibault, G., Peter, R.E., 1987. Neuroanatomical substrate for the inhibition of gonadotropin secretion in goldfish existence of a dopaminergic preoptico-hypophyseal pathway. Neuroendocrinology 45, 451–458.

Kah, O., Anglade, I., Leprêtre, E., Dubourg, P., de Monbrison, D., 1993. The reproductive brain in fish. Fish Physiol. Biochem. 11, 85–98.

Levine, R.L., Dethier, S., 1985. The connections between the olfactory bulb and the brain in the goldfish. J. Comp. Neurol. 237, 427–444.

Linard, B., Bennani, S., Saligaut, C., 1995. Catecholamine metabolism of the rainbow trout (*Oncorhynchus mykiss*) brain and pituitary: catecholamine inactivation and control of gonadotrophin release. Gen. Comp. Endocrinol. 99, 192–196.

Linard, B., Bennani, S., Jego, P., Saligaut, C., 1996a. Tyrosine hydroxylase activity and dopamine turnover of rainbow trout (*Oncorhynchus mykiss*) brain the special status of the hypothalamus. Fish Physiol. Biochem. 15, 41–48.

Linard, B., Anglade, I., Corio, M., Navas, J.M., Pakdel, F., Saligaut, C., Kah, O., 1996b. Estrogen receptors are expressed in a subset of tyrosine hydroxylase-positive neurons of the anterior preoptic region in the rainbow trout. Neuroendocrinology 63, 156–165.

Ma, P.M., 1994a. Catecholaminergic systems in the zebrafish. I. Number, morphology, and histochemical characteristics of neurons in the locus coeruleus. J. Comp. Neurol. 344, 242–255.

Ma, P.M., 1994b. Catecholaminergic systems in the zebrafish. II. Projection pathways andpattern of termination of the locus coeruleus. J. Comp. Neurol. 344, 256–269.

Ma, P.M., 1997. Catecholaminergic systems in the zebrafish. III. Organization and projection pattern of medullary dopaminergic and noradrenergic neurons. J. Comp. Neurol. 381, 411–427.

Manso, M.J., Becerra, M., Molist, P., Rodríguez-Moldes, I., Anadón, R., 1993. Distribution and development of catecholaminergic neurons in the brain of the brown trout A tyrosine hydroxylase immunohistochemical study. J. Hirnforsch. 34, 239–260.

McCormick, C.A., 1982. The organization of the octavolateralis area in actinopterygian fishes: a new interpretation. J. Morphol. 171, 159–181.

Meek, J., 1994. Catecholamines in the brains of Osteichthyes bony fishes. In: Smeets, W.J.A.J., Reiner, A. (Eds.), Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates, Part I. Cambridge University Press, Cambridge, pp. 49–76.

Meek, J., 1999. The paraventricular organ of mormyrid fish uptake or release of intraventricular biogenic amines? Eur. J. Morphol. 37, 107–111.

Meek, J., Joosten, H.W.J., 1993. Tyrosine hydroxylase-immunoreactive cell groups in the brain of the teleost fish, *Gnathonemus petersii*. J. Chem. Neuroanat. 6, 431–446.

Meek, J., Joosten, H.W.J., Steinbusch, H.W.M., 1989. Distribution of dopamine immunoreactivity in the brain of the mormyrid teleost *Gnathonemus petersii*. J. Comp. Neurol. 281, 362–383.

Meredith, G.E., Smeets, W.J.A.J., 1987. Immunocytochemical analysis of the dopamine systems in the forebrain and midbrain of *Raja clavata* Evidence for a substantia nigra and ventral tegmental area in a cartilaginous fish. J. Comp. Neurol. 265, 530–548.

Meyer, D.L., von Seydlitz-Kurzbach, U., Fiebig, E., 1981. Bilaterally asymmetrical uptake of [^{14}C]2-deoxyglucose by the octavo-lateralis complexes in flatfish. Cell Tissue Res. 214, 659–662.

Molist, P., Rodríguez-Moldes, I., Anadón, R., 1993. Organization of catecholaminergic systems in the hypothalamus of two elasmobranch species, *Raja undulata* and *Scyliorhinus canicula*. Histofluorescence and immunohistochemical study. Brain Behav. Evol. 41, 290–302.

Moore, R.Y., Card, J.P., 1984. Noradrenaline-containing neuron systems. In: Björklund, A., Hökfelt, T. (Eds.), Handbook of Chemical Neuroanatomy. Classical Transmitters in the CNS, Part I, vol. 2. Elsevier, Amsterdam, pp. 123–156.

Morita, Y., Finger, T.E., 1987. Area postrema of the goldfish, *Carassius auratus*: ultrastructure, fiber connections and immunocytochemistry. J. Comp. Neurol. 256, 104–116.

Muñoz-Cueto, J.A., González, D., Piñuela, C., Rendón, C., Rodríguez-Gómez, F.J., González de Canales, M.L., Kah, O., Sarasquete C., 1997. Immunohistochemical localization of tyrosine hydroxylase in the brain of *Sparus aurata*. Proceedings of the 18th Annual Conference of the European Society for Comparative Physiology and Biochemistry, pp. 28.

Muñoz-Cueto, J.A., Sarasquete, C., Zohar, Y., Kah, O. An atlas of the brain of the gilthead seabream, *Sparus aurata*. Sea Grant University of Maryland, Baltimore, in press.

Nilsson, G.E., 1989. Regional distribution of monoamines and monoamine metabolites in the brain of the crucian carp, *Carassius carassius* L. Comp. Biochem. Physiol. 94C, 223–228.

Northcutt, R.G., 1983. Evolution of the optic tectum in ray-finned fishes. In: Northcutt, R.G., Davis, R.E. (Eds.), Fish Neurobiology, vol. 2. University of Michigan Press, Ann Arbor, pp. 1–42.

Northcutt, R.G., Davis, R.E., 1983. Telencephalic organization in ray-finned fishes. In: Davis, R.E., Northcutt, R.G. (Eds.), Fish Neurobiology, vol. 2. University of Michigan Press, Ann Arbor, pp. 203–236.

Northcutt, R.G., Butler, A.B., 1991. Retinofugal and retinotectal projections in the green sunfish, *Lepomis cyanellus*. Brain Behav. Evol. 37, 333–354.

Parent, A., 1983. The monoamine-containing neuronal systems in the teleostean brain. In: Davis, R.F., Northcutt, R.G. (Eds.), Fish Neurobiology, vol. 2. University of Michigan Press, Ann Arbor, pp. 285–316.

Parent, A., Dube, L., Braford, M.R., Northcutt, R.G., 1978. The organization of the monoamine-containing neurons in the brain of the sunfish (*Lepomis gibosus*) as revealed by fluorescence microscopy. J. Comp. Neurol. 189, 496–516.

Peter, R.E., Gill, V.E., 1975. A stereotaxic atlas and technique for forebrain nuclei in the goldfish, *Carassius auratus*. J. Comp. Neurol. 159, 69–102.

Peter, R.E., Chang, J.P., Nahorniak, C.S., Omeljaniuk, R.J., Sokolowska, M., Shih, S.H., Billard, R., 1986. Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. Recent Prog. Horm. Res. 42, 513–548.

Pierre, J., Rio, J.P., Mahouche, M., Repérant, J., 1994. Catecholamine systems in the brain of cyclostomes, the Lamprey, *Lampetra fluvialis*. In: Smeets, W.J.A.J., Reiner, A. (Eds.), Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates, Part I. Cambridge University Press, Cambridge, pp. 7–19.

Piñuela, C., Northcutt, R.G., 1995. Dopamine distribution in the forebrain of the Senegal bichir. Soc. Neurosci. Abstr. 21, 432.

Prasada Rao, P.D., Finger, T.E., 1984. Asymmetry of the olfactory system in the brain of the winter flounder, *Pseudopleuronectes americanus*. J. Comp. Neurol. 225, 492–510.

Prasada Rao, P.D., Job, T.C., Schreibman, M.P., 1993. Hypophysiotrophic neurons in the hypothalamus of the catfish *Clarias batrachus*: a cobaltous lysine and HRP study. Brain Behav. Evol. 42, 24–38.

Prasada Rao, P.D., Jadhao, A.G., Sharma, S.C., 1987. Descending projection neurons to the spinal cord of the goldfish, *Carassius auratus*. J. Comp. Neurol. 265, 96–108.

Presson, J., Fernald, R.D., Max, M., 1985. The organization of retinal projections to the diencephalon and pretectum in the cichlid fish, *Haplochromis burtoni*. J. Comp. Neurol. 235, 360–374.

Reiner, A., Northcutt, R.G., 1992. An immunohistochemical srudy of the telencephalon of the Senegal bichir (*Polypterus senegalus*). J. Comp. Neurol. 31, 359–386.

Reiner, A., Karle, E.J., Anderson, K.D., Medina, L., 1994. Catecholaminergic perikarya and fibers in the avian nervous system. In: Smeets, W.J.A.J., Reiner, A. (Eds.), Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates, Part I. Cambridge University Press, Cambridge, pp. 135–181.

Rendón, C., Rodríguez-Gómez, F.J., Muñoz-Cueto, J.A., Piñuela, C., Sarasquete, C., 1997. An immunocytochemical study of pituitary cells of the Senegalese sole, *Solea senegalensis* (Kaup, 1858). Histochem. J. 29, 813–822.

Roberts, B.L., Meredith, G.E., Maslam, S., 1989. Immunocytochemical analysis of the dopamine system in the brain and spinal cord of the European eel, *Anguilla anguilla*. Anat. Embryol. 180, 401–412.

Rodríguez-Gómez, F.J., Sarasquete, C., Muñoz-Cueto, J.A., 2000. A morphological study of the brain of *Solea senegalensis*. I The telencephalon. Histol. Histopathol., 15, 355–364.

Rooney, D.J., New, J.G., Szabo, T., Ravaille-Veron, M., 1989. Central connections of the olfactory bulb in the weakly electric fish, *Gnathonemus petersii*. Cell Tissue Res. 257, 423–436.

Saligaut, C., Salbert, G., Bailhache, T., Bennani, S., Jégo, P., 1992. Serotonin and dopamine turnover in the female rainbow trout (*Oncorhynchus mykiss*) brain and pituitary changes during the annual reproductive cycle. Gen. Comp. Endocrinol. 85, 261–268.

Saligaut, C., Bennani, S., Bailhache, T., 1993. Catecholamine synthesis in the rainbow trout (*Oncorhynchus mykiss*) brain modulation of tyrosine hydroxylase activity. Fish Physiol. Biochem. 11, 1–6.

Sas, E., Maler, L., Tinner, B., 1990. Catecholaminergic systems in the brain of a gymnotiform teleost fish: an immunohistochemical study. J. Comp. Neurol. 292, 127–162.

Sas, E., Maler, L., Weld, M., 1993. Connections of the olfactory bulb in the gymnotiform fish, *Apteronotus leptorhynchus*. J. Comp. Neurol. 335, 486–507.

Senthilkumaran, B., Joy, K.P., 1995. Changes in hypothalamic catecholamines, dopamine-β-hydroxylase and phenylethanolamine-*N*-methyl transferase in the catfish, *Heteropneustes fossilis*, in relation to season, raised photoperiod and temperature, ovariectomy and estradiol-17-β replacement. Gen. Comp. Endocrinol. 97, 121–134.

Smeets, W.J.A.J., Steinbusch, H.W.M., 1990. New insights into the reptilian catecholaminergic systems as revealed by antibodies against the neurotransmiters and their enzymes. J. Chem. Neuroanat. 3, 25–44.

Smeets, W.J.A.J., Reiner, A., 1994. Catecholamines in the CNS of vertebrates: current concepts of evolution and functional significance. In: Smeets, W.J.A.J., Reiner, A. (Eds.), Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates, Part III. Cambridge University Press, Cambridge, pp. 463–481.

Smeets, W.J.A.J., Hoogland, P.V., Voorn, P., 1986. The distribution of dopamine immunoreactivity in the forebrain and midbrain of the lizard *Gekko gecko*. An immunohistochemical study with antibodies against dopamine. J. Comp. Neurol. 253, 46–60.

Smeets, W.J.A.J., Jonker, A.J., Hoogland, P.V., 1987. Distribution of the dopamine in the forebrain and midbrain of the red-eared turtle, *Pseudemys scripta elegans*, reinvestigated using antibodies against dopamine. Brain Behav. Evol. 30, 121–142.

Striedter, G.F., 1990. The diencephalon of the channel catfish, *Ictalurus punctatus*. II. Retinal, tectal, cerebellar and telencephalic connections. Brain Behav. Evol. 36, 355–377.

Stuesse, S.L., Cruce, W.L.R., Northcutt, R.G., 1994. Localization of catecholamines in the brain of Chondrichthyes (cartilaginous fishes). In: Smeets, W.J.A.J., Reiner, A. (Eds.), Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates, Part I. Cambridge University Press, Cambridge, pp. 21–47.

Tillet, Y., 1994. Catecholaminergic neuronal systems in the diencephalon of mammals. In: Smeets, W.J.A.J., Reiner, A. (Eds.), Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates, Part I. Cambridge University Press, Cambridge, pp. 207–246.

Trudeau, V.L., 1997. Neuroendocrine regulation of gonadotrophin-II release and gonadal growth on the goldfish, *Carassius auratus*. Rev. Reprod. 2, 55–68.

Van der Kraak, G., Donaldson, E.M., Chang, J.P., 1986. Dopamine involvement in the regulation of gonadotrophin secretion in Coho salmon. Can. J. Zool. 64, 1245–1248.

Von Bartheld, C.S., Meyer, D.L., Fiebing, E., Ebbesson, S.O.E., 1984. Central connections of the olfactory bulb in the goldfish, *Carassius auratus*. Cell Tissue Res. 238, 475–487.

Watson, A.D.H., 1980. The distribution of aminergic neurons and their projections in the brain of the teleost *Myoxocephalus scorpius*. Cell Tissue Res. 202, 299–312.

Wicht, H., Northcutt, R.G., 1994. An immunohistochemical study of the telencephalon and the diencephalon in a myxinoid jawless fish, the Pacific hagfish, *Eptatretus stouti*. Brain Behav. Evol. 43, 140–161.

Wong, A.O.L., Chang, J.P., Peter, R.E., 1992. Dopamine stimulates growth hormone release from the pituitary of goldfish, *Carassius auratus*, through the dopamine D1 receptors. Endocrinology 130, 1201–1210.

Wullimann, M.F., Northcutt, R.G., 1988. Connections of the corpus cerebelli in the green sunfish and the common goldfish: a comparison of perciform and cypriniform teleosts. Brain Behav. Evol. 32, 293–316.

Wullimann, M.F., Meyer, D.L., 1990. Phylogeny of putative cholinergic visual pathways through the pretectum to the hypothalamus in teleost fish. Brain Behav. Evol. 36, 14–29.

Yoshida, M., Nagatsu, I., Kawakami-Kondo, Y., Karasawa, N., Spatz, M., Nagatsu, E., 1983. Monoaminergic neurons in the brain of goldfish as observed by immunohistochemical techniques. Experientia 39, 1171–1174.

Zohar, Y., Harel, M., Hassin, S., Tandler, A., 1995. Broodstock management and manipulation of spawning in the gilthead seabream *Sparus aurata*. In: Bromage, N., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science Press, London, pp. 94–117.