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Distribution of serotonin in the brain of the Senegalese sole, *Solea senegalensis*: an immunohistochemical study

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Abstract

We report the distribution of serotonin immunoreactive (5-HT-ir) structures in the brain of the adult Senegalese sole, *Solea senegalensis*, using the streptavidin–biotin–peroxidase complex immunohistochemical method. We have found a wide distribution of immunoreactive fibers throughout the entire brain. 5-HT-ir cell bodies appeared restricted to some periventricular nuclei associated with the diencephalic recesses, and in the rhombencephalic reticular formation and inferior olivary region. Specifically, cerebrospinal fluid-contacting serotoninergic cells were found within the pars dorsalis and pars ventralis of the nucleus recessus lateralis, in the paraventricular organ and in the nucleus recessus posterioris. In the brainstem, 5-HT-ir perikarya appear within the superior and inferior raphe, the nucleus reticularis superioris, the nucleus interpeduncularis and the inferior olive. Although positive fibers were not found in the neurohypophysis, a few 5-HT-ir cells were identified in the adenohypophysis. This distribution is compared with those found in other fishes and discussed in the context of putative roles of 5-HT as a neuroendocrine factor and neurotransmitter in the Senegalese sole.

Keywords: Monoamines; Central nervous system; Pleuronectiformes; Teleost; Immunohistochemistry; Neuroanatomy

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an indoleamine that together with cathecolamines (dopamine, norepinephrine and epinephrine), constitutes the monoamine neurotransmitters group. The first evidence of the presence and distribution of 5-HT in CNS was reported in the mammalian brain using biochemical and histofluorescence techniques (Twarog and Page, 1953; Bogdansky et al., 1956; Falck, 1962; Dahlström and Fuxe, 1964; Fuxe, 1965). Subsequently, Steinbusch et al. (1978) used antibodies directed against 5-HT to locate the serotoninergic systems immunocytochemically. To date, mapping of 5-HT systems has been conducted in many vertebrate species, including elasmobranchs (Ritchie et al., 1983; Northcutt et al., 1988; Stuesse et al., 1990; Stuesse and Cruce, 1991; Stuesse et al., 1991a,b), primitive and teleost fishes (Kah and Chambolle, 1983; Yoshida et al., 1983; Ekström and Van Veen, 1984; Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Ekström et al., 1985; Margolis-Kazan et al., 1985; Ekström and Ebbesson, 1988, 1989a,b; Grant et al., 1989; Meek and Joosten, 1989; Bonn and Konig, 1990; Johnston et al., 1990; Reiner and Northcutt, 1992; Khan and Thomas, 1993), amphibians (Ueda et al., 1984), reptiles (Ueda et al., 1983; Wolters et al., 1985; Smeets, 1988; Smeets and Steinbusch, 1988), birds (Sano et al., 1983) and mammals (Steinbusch, 1984; Nieuwenhuys, 1985; Azmitia, 1987).

In fishes, 5-HT seems to be implicated in the control of several hypophysial functions such as the secretion of melanophore-stimulating hormone (MSH) (Olivereau, 1978a), prolactin (PRL) (Olivereau, 1978b), gonadotropin (Margolis-Kazan et al., 1985; Somoza et al., 1988; Somoza and Peter, 1991; Khan and Thomas, 1992; Trudeau, 1997), and growth hormone (Somoza

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and Peter, 1991; Wong, 1993; Peng and Peter, 1997). Also 5-HT seems to stimulate the gonadotropin-releasing hormone release from the hypothalamus and the hypophysis (Kiss and Halasz, 1985; Vitale et al., 1986; Yu et al., 1991) and putative interactions between both systems have been suggested (Khan and Thomas, 1993). In recent years, several studies have also suggested a role for brain 5-HT in stress responses, food intake and social interactions (Winberg et al., 1992, 1993, 1997; Overli et al., in press). Furthermore, 5-HT acting as a neurotransmitter has been implicated in the central regulation of different vertebrate neuroendocrine systems (Calas, 1977; Kordon et al., 1981; Holmes et al., 1982; Jennes et al., 1982; López et al., 1987; Shannon and Moore, 1987; Cohen et al., 1990).

The Senegalese sole, *Solea senegalensis* is a pleuronectiform fish characteristic of south Atlantic and Mediterranean coasts (Rodríguez and Rodríguez, 1980; Dinis, 1992). Recently, some studies have addressed different aspects of its development and reproductive biology (Rodríguez, 1984; Gutiérrez et al., 1985; Sarasquete et al., 1993a,b; Mourente and Vázquez, 1996; Sarasquete et al., 1996). However, less attention has been paid to endocrine studies (Pendón et al., 1994a,b; Rendón et al., 1997). Currently much effort is being directed towards the intensive aquaculture of commercially novel species such as the Senegalese sole, but many problems are being encountered in reproductive performance and early development (Dinis, 1992). At least in part, some of these problems could be the consequence of neuroendocrine disruptions under particular conditions of cultivation used. Unfortunately, in the Senegalese sole there is no published information on the form of action of those neuroendocrine factors that traditionally have been related to reproduction in fish (e.g. GnRH, catecholamines, neuropeptide Y and serotonin) nor on their distribution in the brain.

In this paper we examine the precise distribution of 5-HT immunoreactive structures in the brain of the Senegalese sole, with the help of a brain atlas recently developed for this species (Rodríguez-Gómez et al., in press; Rodríguez-Gómez et al., in preparation). The present article forms part of a series of studies on neuroendocrine systems potentially implicated in the regulation of reproduction in this important species for marine aquaculture. The main objective of this basic research is to provide useful information in order to improve reproduction and rearing of this species under practical conditions of cultivation.

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. Specimens were anesthetized with 2-phenoxiethanol (Sigma, St. Louis, MO) and perfused via the aortic bulb with 0.6% saline solution, followed by Bouin fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, 0.2% picric acid). The brain with the pituitary attached was then carefully removed and further postfixed in the same fixative for 6 h in darkness at 4°C. After fixation, tissues were cryoprotected in 15% sucrose in 0.1 M phosphate buffer for other 6 h, and finally embedded in 'tissue-tek' and kept at −80°C until processing. Serial transverse brain sections 16 μm-thick were obtained in a cryomicrotome and mounted on gelatin-coated glass slides.

Immunohistochemical staining was performed as it has been described previously (Rendón et al., 1997). Briefly, sections were incubated overnight in a humid chamber at room temperature with a rabbit anti-5-HT antiserum (kindly donated by Dr Tramu) at 1:5000 dilution. The specificity of the antiserum used in this study was previously demonstrated (Tramu et al., 1983). Sections were washed in buffer (0.01 M Veronal, 0.15 M NaCl, 0.1% Triton X-100) and incubated for 1 h at room temperature with biotinylated anti rabbit-IgG diluted 1:1000. After washing, sections were incubated for 1 h at room temperature with streptavidin–peroxidase complex diluted 1:1000. Peroxidase activity was detected in Tris–HCl 0.05 M, pH 7.6, containing 0.048% 4-chloro-1-naphthol (Sigma) and 0.05% hydrogen peroxide. To confirm the specificity of the immunostaining, controls were performed by replacement of primary antiserum with normal rabbit serum and omission of primary antiserum. The sections were mounted on an aqueous mounting media for microscopy (Aquatex, Merck). The precise localization of 5-HT-immunoreactive (5-HT-ir) fibers and cells was determined with the help of a recently developed brain atlas of *S. senegalensis* (Rodríguez-Gómez et al., in press; Rodríguez-Gómez et al., in preparation).

3. Results

In the Senegalese sole brain, two main 5-HT systems were found. The rostralmost system was composed of cerebrospinal fluid-contacting cells that are found around the medial ventricle and the lateral and posterior recesses. The 5-HT-ir neurons appeared in the paraventricular organ (PVO, Fig. 1E,F;Fig. 2A), the pars dorsalis (NRLd) and the pars ventralis (NRLv) of the nucleus recessus lateralis (Fig. 1E,F;Fig. 2A–D), and in the nucleus recessus posterioris (NRP, Fig. 1F;Fig. 3A). The 5-HT-ir cells of the paraventricular organ were small in size and appear densely packed along the medial ventricle, exhibiting manifest connections with the cerebrospinal fluid (Fig. 2A). Slightly

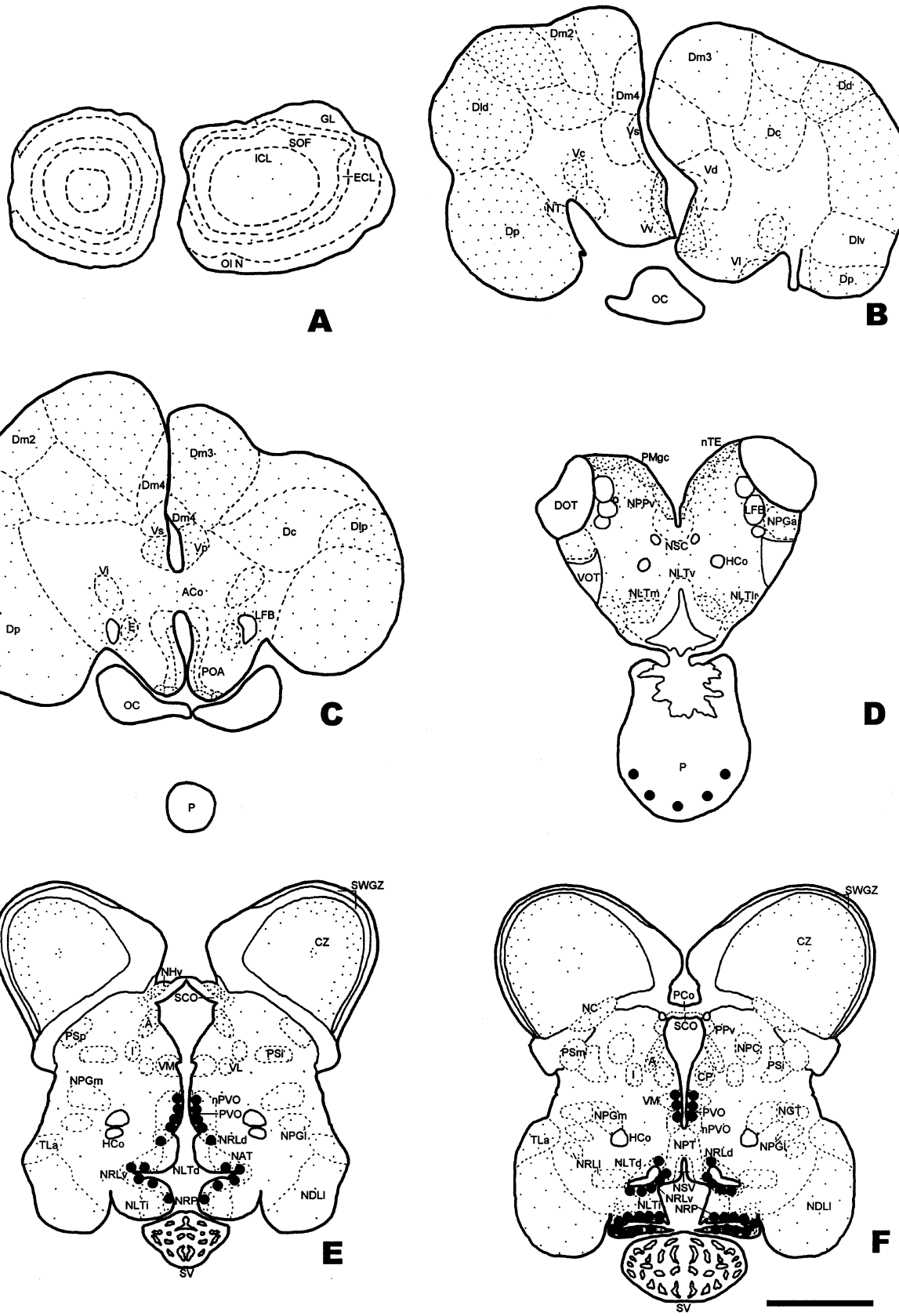

Fig. 1. Series of transverse sections through the brain of *Solea senegalensis* from rostral (A) to caudal (K) showing the distribution of 5-HT cells (large filled circles) and fibers (small dots). Bar scale = 1 mm. For abbreviations see Table 1.

ventral to the cells of the paraventricular organ were the 5-HT-ir neurons of the nucleus recessus lateralis pars dorsalis (Fig. 2A). Rostrally, positive cells of the nucleus recessus lateralis pars dorsalis were clearly distinguished from the cells of the paraventricular organ because they appeared more loosely packed and were polymorphic (round, ovoid, pyramidal and polygonal). The 5-HT-ir cell bodies of the nucleus recessus lateralis pars dorsalis started in a medial position (Fig. 2A,B). When the medial extensions of the lateral recess

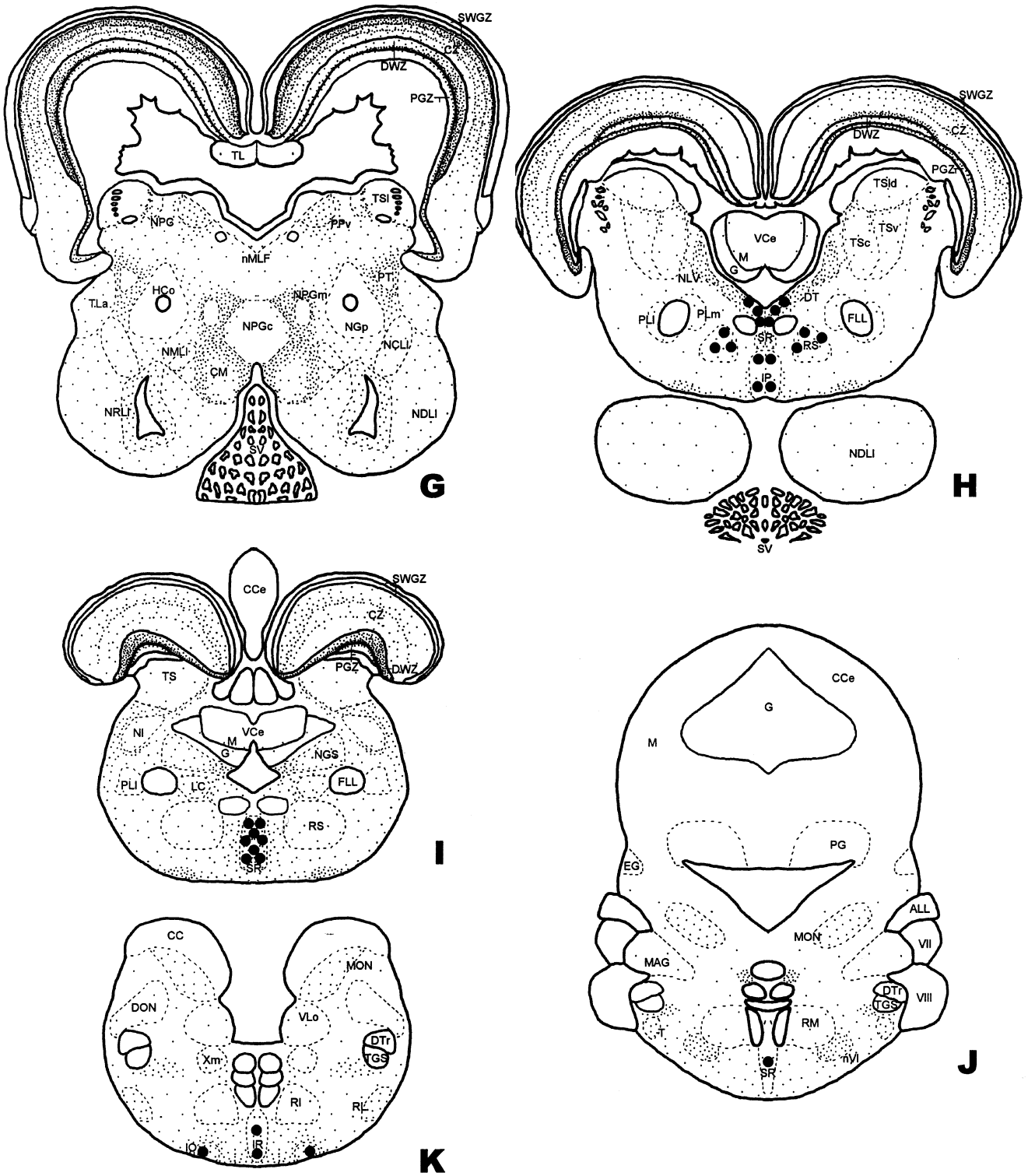

Fig. 1.

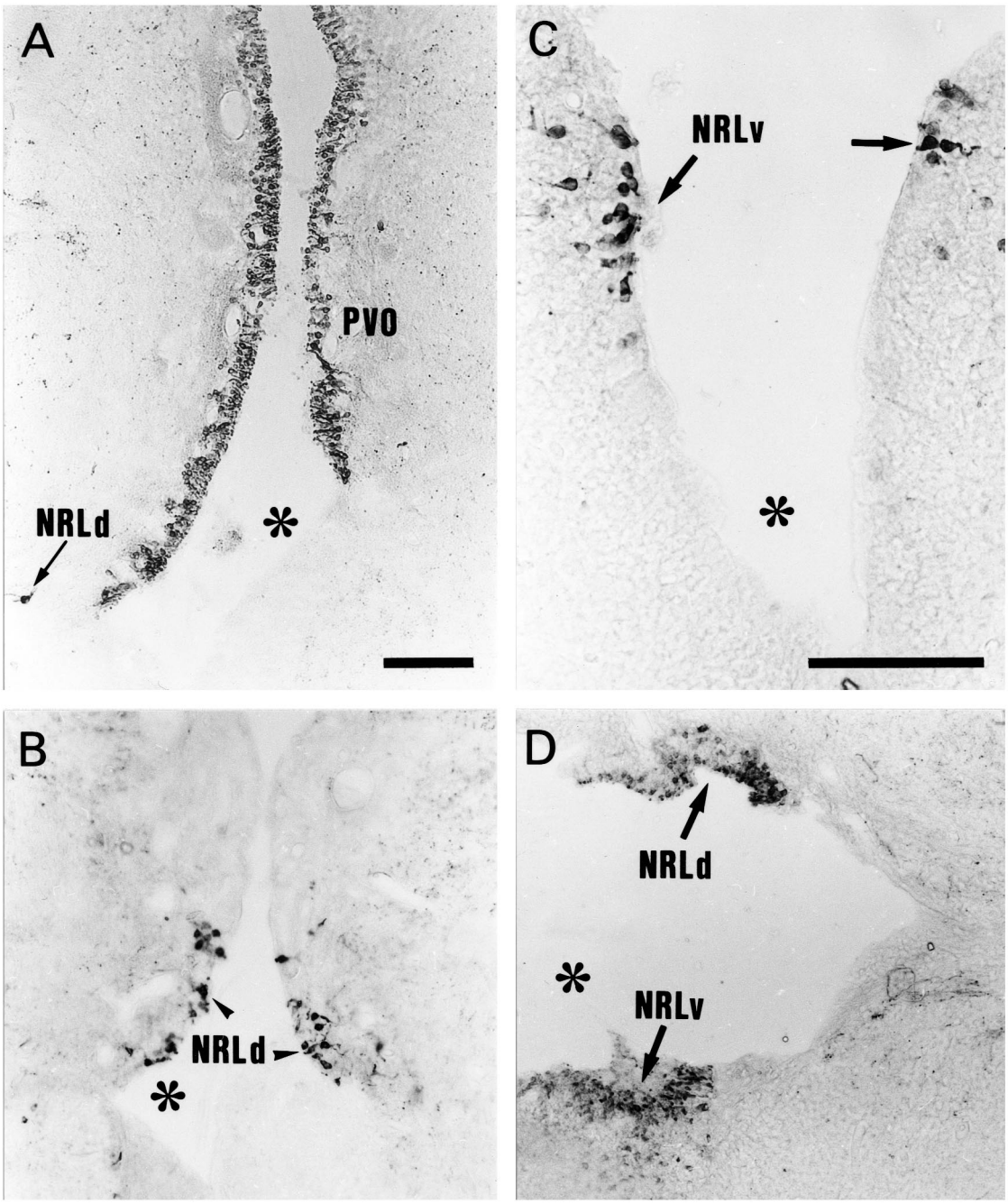

Fig. 2. Photomicrographs of transverse sections through the brain of *Solea senegalensis*, showing 5-HT-ir cells and fibers. (A) Posterior tubercular area. 5-HT-ir perikarya in the paraventricular organ (PVO) and nucleus recessus lateralis pars dorsalis (NRLd) in close contact with the ventricle. Bar represents 100 μm and is also valid for B and D. (B) Ventral hypothalamus. 5-HT-ir perikarya in the nucleus recessus lateralis pars dorsalis. Note the contact of some cells with the cerebrospinal fluid. (C). Ventral hypothalamus. 5-HT-ir perikarya in the nucleus recessus lateralis pars ventralis (NRLv). Cerebrospinal fluid-contacting cells are also evident. Bar represents 100 μm. (D). Ventral hypothalamus. Positive cells in the pars dorsalis and pars ventralis of the nucleus recessus lateralis bordering the dorsomedial and ventromedial expansions of the lateral recess, respectively. Asteriks represent the medial ventricle in A, B and C, and the lateral recess in D.

opened, they left the medial position and migrated laterally along the dorsal surface of the lateral recess (Fig. 2D). At this level, 5-HT-ir cell bodies of nucleus recessus lateralis pars dorsalis adopted a more compact organization than in the rostral pole. Further caudal, the lateral recess lost its connections with the medial ventricle and the cells of the nucleus recessus lateralis pars dorsalis appeared concentrated in the dorsomedial zone of the recess before disappearing. A similar morphology and rostrocaudal evolution was observed for 5-HT-ir cells of the nucleus recessus lateralis pars ventralis (Fig. 2C,D). As occurs with the cells of the

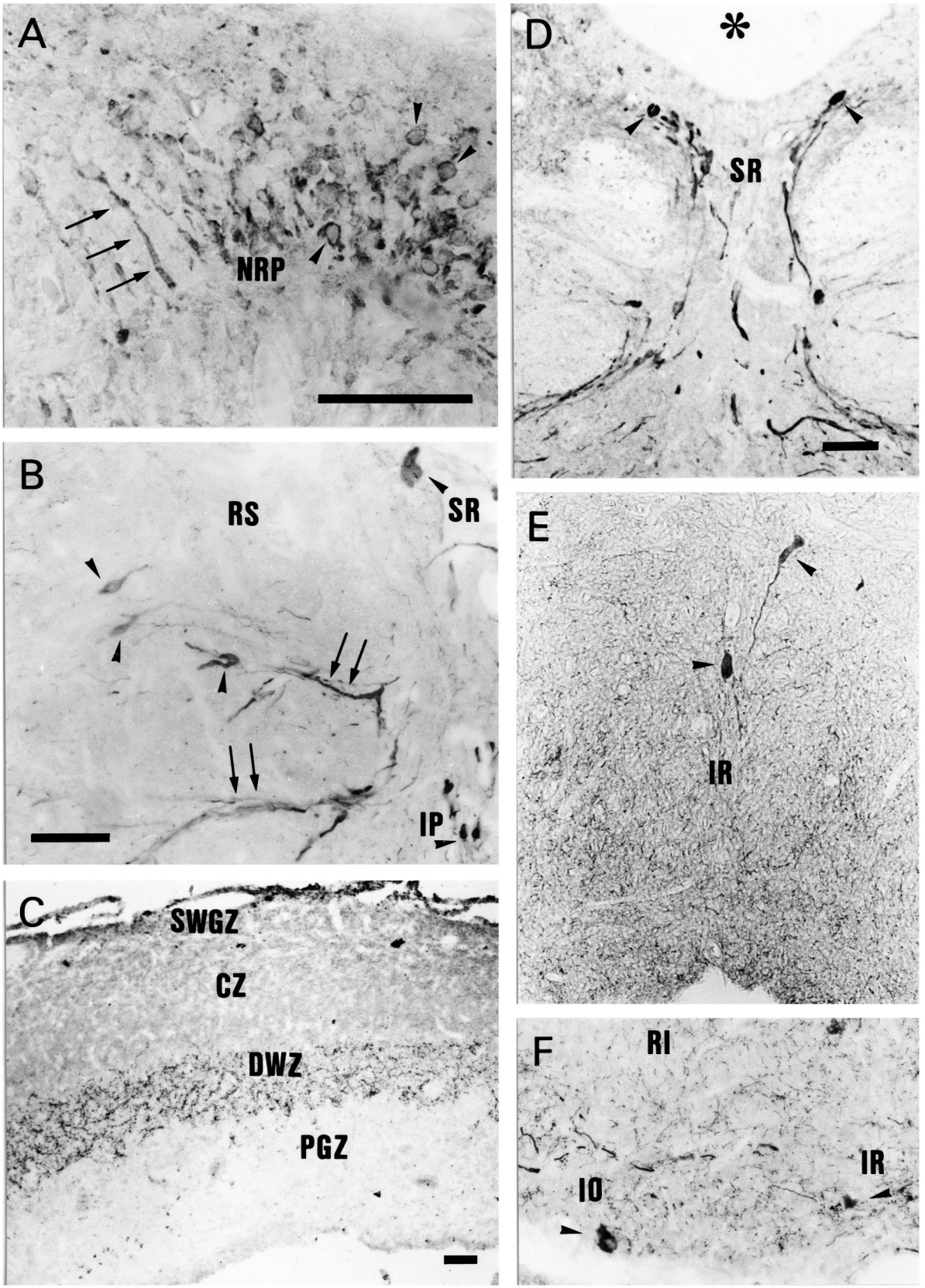

Fig. 3. Photomicrographs of transverse sections through the brain of *Solea senegalensis*, showing 5-HT-ir cells and fibers. (A) Caudal hypothalamus. 5-HT-ir cells (arrowheads) in the nucleus recessus posterioris (NRP). A thick process directed towards the posterior recess is shown by arrows. Bar represents 100 μm. (B) Rostral rhombencephalon. 5-HT-ir neurons (arrowheads) in the superior raphe (SR), nucleus reticularis superior (RS) and nucleus interpeduncularis (IP). Arrows mark thick immunostained processes. Bar is 100 μm and is also valid for E and F. (C). Optic tectum. 5-HT-ir fibers in DWZ. Bar represents 100 μm. (D) Rostral rhombencephalon. 5-HT-ir neurons and fibers in the superior raphe. Asterisk marks the ventricle. Bar represents 100 μm. (E). Caudal rhombencephalon. 5-HT-ir cells in the inferior raphe (IR). (F). Caudal rhombencephalon. 5-HT-ir cells in the inferior raphe and inferior olive (IO). An intense 5-HT-ir innervation is also observed. For other abbreviations see Table 1.

Table 1
Abbreviations

A: nucleus anterior thalami

ACo: anterior commissure
ALL: anterior lateral line nerve

CC: crista cerebellaris

CCe: corpus cerebelli

CM: corpus mammillare

CP: nucleus centralis posterior thalami
CZ: central zone of the optic tectum
Dc: area dorsalis telencephali pars centralis
Dd: area dorsalis telencephali pars dorsalis
Dld: area dorsalis telencephali pars lateralis dorsal
Dlp: area dorsalis telencephali pars lateralis posterior
Dlv: area dorsalis telencephali pars lateralis ventral
Dm1: area dorsalis telencephali pars medialis subdivision 1
Dm2: area dorsalis telencephali pars medialis subdivision 2
Dm3: area dorsalis telencephali pars medialis subdivision 3
Dm4: area dorsalis telencephali pars medialis subdivision 4
DON: nucleus octavus descendens
DOT: dorsal optic tract

Dp: area dorsalis telencephali pars posterioris
DT: nucleus tegmentalis dorsalis

DTr: descending trigeminal tract
DWZ: deep white zone of the optic tectum
E: nucleus entopeduncularis

ECL: external cellular layer

EG: eminentia granularis

FLL: fasciculus longitudinalis lateralis
G: granular layer of the cerebellum
GL: glomerular layer
HCo: horizontal commissure
I: nucleus intermedius thalami
ICL: internal cellular layer
IO: inferior olive
IP: nucleus interpeduncularis
IR: nucleus raphes inferior
LC: nucleus of the locus coeruleus
LFB: lateral forebrain

M: molecular layer of the cerebellum
MAG: nucleus magnocellularis

MON: nucleus octavolateralis medialis
NAT: nucleus anterior tuberis
NC: nucleus corticalis
NCLI: nucleus centralis lobi inferioris
NDLI: nucleus diffusus lobi inferioris
NGp: nucleus glomerulosus pars posterioris
NGS: nucleus gustatorius secundarius
NGT: nucleus gustatorius tertius
NHv: nucleus habenularis pars ventralis
NI: nucleus ithsmi

NLTd: nucleus lateralis tuberis pars dorsalis
NLTi: nucleus lateralis tuberis pars inferioris
NLTlr: nucleus lateralis tuberis pars lateralis rostralis
NLTm: nucleus lateralis tuberis pars medialis
NLTv: nucleus lateralis tuberis pars ventralis
NLV: nucleus lateralis valvulae

NMLF: nucleus of the medial longitudinal fasciculus
NMLI: nucleus medialis lobi inferioris
NPC: nucleus pretectalis centralis
NPGa: nucleus preglomerulosus anterioris
NPGc: nucleus preglomerulosus commissuralis
NPGl: nucleus preglomerulosus lateralis
NPGm: nucleus preglomerulosus medialis
NPPv: nucleus posterioris periventricularis
NPT: nucleus posterior tuberis
NPVO: nucleus of the paraventricular organ
NRLd: nucleus recessus lateralis pars dorsalis
NRLv: nucleus recessus lateralis pars ventralis
NRLl: nucleus recessus lateralis pars lateralis
NRP: nucleus recessus posterioris
NSC: nucleus suprachiasmaticus

Table 1 (Continued)

NSV: nucleus saccus vasculosus
NT: nucleus taenia

NTE: nucleus eminentia thalami

NVI: nucleus nervi abducentis

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: nucleus perilemniscularis pars lateralis
PLm: nucleus perilemniscularis pars medialis
PMgc: nucleus preopticus magnocellularis pars gigantocellularis
POA: preoptic area

PPv: nucleus pretectalis periventricularis pars ventralis
PSi: nucleus pretectalis superficialis pars intermedius
PSm: nucleus pretectalis superficialis pars magnocellularis
PSp: nucleus pretectalis superficialis pars parvocellularis
PT: nucleus posterior thalami

PVO: paraventricular organ

RI: nucleus reticularis inferioris
RL: nucleus reticularis lateralis
RM: nucleus reticularis medius
RS: nucleus reticularis superioris
SCO: subcommissural organ
SOF: secondary olfactory fibers
SR: nucleus raphes superior
SV: saccus vasculosus
SWGZ: superficial white and grey zone of the optic tectum

T: nucleus tangentialis

TGS: tractus gustatorius secundarius
TL: torus longitudinalis
TLa: nucleus tori lateralis
TS: torus semicircularis
TSc: torus semicircularis pars centralis
TSl: torus semicircularis pars lateralis
TSld: torus semicircularis pars lateralis dorsalis
TSv: torus semicircularis pars ventralis
Vc: area ventralis telencephali pars centralis
VCe: valvula cerebelli

Vd: area ventralis telencephali pars dorsalis
Vi: area ventralis telencephali pars intermedia
VII: nervus facialis

VIII: nervus octavus

Vl: area ventralis telencephali pars lateralis
VL: nucleus ventrolateralis thalami
VLo: vagal lobe

VM: nucleus ventromedialis thalami
VOT: ventral optic tract

Vp: area ventralis telencephali pars postcommissuralis
Vs: area ventralis telencephali pars supracommissuralis
Vv: area ventralis telencephali pars ventralis
Xm: nucleus motorius nervi vagi

paraventricular organ, the connections of the cells of the pars dorsalis and ventralis of the nucleus recessus lateralis with the cerebrospinal fluid were evident (Fig. 2B,C). The caudalmost cell group of 5-HT-ir cells in the forebrain of Senegalese sole was found in the nucleus recessus posterioris (NRP, Fig. 1F;Fig. 3A). These cells appeared unevenly immunostained and exhibited large nucleus, a thin cytoplasm and thick processes directed towards the recess (Fig. 3A, arrows).

A second population of discontinuous 5-HT-ir cells was observed in the rhombencephalon of the Senegalese sole (Fig. 1H–K; Fig. 3B,D–F). The midline positive cells appeared in the nucleus raphe superioris (SR, Fig. 3B,D), nucleus raphe inferioris (IR, Fig. 3E,F) and in the nucleus interpeduncularis (IP, Fig. 3B). The 5-HT-ir cells of superior raphe were the largest of the rhombencephalic division. They exhibited intensely stained cell bodies, ovoid and elongated in shape (Fig. 3D). Lateral to the midline, thick immunostained processes appeared in the nucleus reticularis superioris (RS), probably originating in the superior raphe cells (Fig. 3B). However, these processes could also belong to medium-sized to large ovoid 5-HT-ir cells of the nucleus reticularis superioris, which were much more lightly stained than the other rhombencephalic positive cells (Fig. 3B). The 5-HT-ir cells of the nucleus interpeduncularis displayed small and intensely stained perikarya, just ventral to the large cells of the superior raphe (Fig. 3B). The inferior raphe, in the caudal rhombencephalon, also contained 5-HT-ir cells (Fig. 3E,F). The cells were smaller in the rostral pole of the nucleus than in the caudal one, in which they adopted a more ventral position. In the ventral surface of the caudal rhombencephalon, the inferior olivary area also exhibited small and medium-sized cells, ovoid, elongated or polygonal in shape (Fig. 3F).

Serotoninergic fibers appeared widely distributed in the brain of *S. senegalensis*. In the olfactory bulbs, only a few labeled fibers were observed, especially at the caudal end, distributed in the internal and external cellular layers (Fig. 1A). The immunoreactive axons were very evident in telencephalic hemispheres, although with a major presence in its dorsal area. The greater 5-HT-ir innervation was observed in the pars lateralis dorsal (Dld), pars dorsalis (Dd), rostral zone of the pars lateralis ventral (Dlv) and posterior (Dp) subdivisions of the area dorsalis (Fig. 1B,C). Within the dorsomedial zone, only the subdivision 3 (Dm3) displayed a conspicuous fiber immunoreactivity (Fig. 1B,C) and a few projections were also identified in the pars centralis of the dorsal telencephalon (Dc, Fig. 1B,C). In the ventral telencephalon, 5HT-ir axons were mainly concentrated around the pars ventralis (Vv), pars centralis (Vc) and the pars lateralis (Vl), the nucleus entopeduncularis and around the lateral forebrain bundles. At this level, immunoreactive fibers in the anterior commissure, pars intermedia (Vi) and pars posterioris (Vp) of the ventral telencephalon were also prominent (Fig. 1B,C). In the diencephalon, the 5HT-ir projections were mainly distributed around the preoptic area, the medial tuberal zone of the hypothalamus, dorsal thalamus and the periventricular pretectum (Fig. 1C–G). Also, we found many positive fibers around the migrated nuclei of the posterior tuberculum. In the optic tectum, 5-HT-ir innervation was evident within the deep white zone, especially at the limits with the central zone and the periventricular zone, but also in the external border of the central zone (Fig. 1E–I; Fig. 3C). In the tegmentum, the positive axons were widely distributed around the mesencephalic ventricle, especially in the nucleus lateralis valvulae, the nucleus tegmentalis dorsalis and the medial zone of the nucleus gustatorius secundarius, with the lateral aspects being scarce (Fig. 1G,H). In the posterior brain, the cerebellum showed only a few 5HT-ir axons, mainly restricted to the transitional area between the granular and molecular layers of the valvula cerebelli, where the Purkinje cells were located (Fig. 1H,I). The rhombencephalon displayed an important serotoninergic innervation in the areas where 5-HT-ir cell bodies appeared, i.e. superior and inferior raphe, nucleus interpeduncularis, nucleus reticularis superioris and inferior olive (Fig. 1H–K;Fig. 3B,D–F). Furthermore, this innervation was also evident in zones devoid of positive cells, such as the locus coeruleus, the nucleus reticularis lateralis, the medial nucleus of the octavolateral area, the nucleus tangentialis, the neuropil surrounding the descending trigeminal tract and the tractus gustatorius secundarius, the vagal sensory and motor areas and the nucleus nervi abducentis (Fig. 1I–K).

Finally, the pituitary of *S. senegalensis* did not display 5-HT-ir axons entering through the neurohypophysis. However, it was possible to identify a few immunoreactive cells, rounded to ovoid in shape, in the adenohypophysis (not shown).

4. Discussion

We report here the distribution of serotonin immunoreactive (5-HT-ir) structures in the brain of the adult Senegalese sole, *S. senegalensis* using immunohistochemical methods. In this species, 5-HT-ir cell bodies appeared concentrated around the diencephalic recesses and in the rhombencephalic reticular formation and inferior olivary area. In turn, we have observed a wide distribution of immunoreactive fibers throughout the entire brain.

The absence of 5-HT-ir perikarya in the teleost olfactory bulbs has been reported in several studies (Kah and Chambolle, 1983; Yoshida et al., 1983; Ekström and Van Veen, 1984; Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Ekström et al., 1985; Margolis-Kazan et al., 1985; Ekström and Ebbesson, 1988, 1989a,b; Grant et al., 1989; Meek and Joosten, 1989; Bonn and Konig, 1990). However, in a marine teleost, *Micropogonias undulatus*, serotoninergic cells were detected in the peripheral layers of the olfactory bulbs (Khan and Thomas, 1993). In the Senegalese sole, these layers of the olfactory bulb only contained 5-HT-ir fibers.

The 5-HT-ir axons in the dorsal telencephalon of Senegalese sole adopted a similar arrangement to that

in *Apteronotus* (Johnston et al., 1990) and *M. undulatus* (Khan and Thomas, 1993). In these species, the higher concentration of 5-HT-ir axons was found in the pars laterodorsal of the area dorsalis, which is consistent with the presence of many 5-HT-ir axons in Dld of the Senegalese sole. Also, in *Polypterus senegalus*, profuse labeled axons were found in P2 and P3 pallial subdivisions (Reiner and Northcutt, 1992), which seem to correspond to our Dd and Dld. There is a notable presence of substantial 5-HT-ir innervation throughout forebrain ventral areas such as telencephalic Vv,Vc and Vl or the preoptic area. These areas represent identified neurendocrine regions in most teleosts, including the Senegalese sole (Kah et al., 1993; Rodríguez-Gómez et al., in preparation). Thus, a possible role of 5-HT in modulating the secretions of some neurohormones and/or releasing factors in neurosecretory cells might also be expected.

The 5-HT-ir cells in the diencephalon of *S. senegalensis* appear restricted to the areas that surround the third ventricle, the paraventricular organ, the pars dorsalis and ventralis of the nucleus recessus lateralis and the nucleus recessus posterioris. All these nuclei represent characteristic 5-HT-ir brain areas in different groups of fishes (Hafeez and Zerihun, 1976; Bonn and Konig, 1990; Johnston et al., 1990; Khan and Thomas, 1993). Furthermore, serotoninergic cell masses have been described in the preoptic area of teleosts (Johnston et al., 1990), elasmobranchs (Ritchie et al., 1983) and amphibians (Ueda et al., 1984). Thalamic 5-HT-ir cells are also present in the diencephalon of teleosts and elasmobranchs (Kah and Chambolle, 1983; Ritchie et al., 1983; Yoshida et al., 1983; Ekström and Van Veen, 1984; Ekström and Ebbesson, 1988; Johnston et al., 1990). However, preoptic 5-HT-ir cells were not described in reptiles and thalamic 5-HT-ir neurons were absent in both amphibians and reptiles (Steinbusch, 1984; Smeets and Steinbusch, 1988). Smeets and Steinbusch (1988) suggested the existence of a gradual decrease of 5-HT diencephalic cell masses and an increase of 5-HT rhombencephalic nuclei during evolution. In Senegalese sole, neither preoptic nor thalamic 5-HT-ir cell masses were observed in none of the specimens studied. According to our results, preoptic 5-HT-ir cells were not detected in the brain of goldfish (Kah and Chambolle, 1983; Yoshida et al., 1983), three-spined stickleback (Ekström and Van Veen, 1984), coho salmon (Ekström and Ebbesson, 1988, 1989a,b), trout (Frankenhuis-van den Heuvel and Nieuwenhuys, 1984) and platyfish (Margolis-Kazan et al., 1985). Nevertheless, some inconsistencies in the comparison of 5-HT cell masses among teleosts could be the consequence of differences in neuroanatomical nomenclatures more than real specific differences. In fact, the thalamic 5-HT-ir nPPV cells of *Apteronotus* (Johnston et al., 1990) seem to represent the paraventricular organ 5-HT-ir cells of Senegalese sole, which we have ascribed to the posterior tuberculum.

As in the case of sea bream and sea bass (Muñoz-Cueto et al., in press; J.M. Cerdá-Reverter, S. Zanuy and J.A. Muñoz-Cueto, submitted), three cell masses were observed around the lateral recess of the Senegalese sole: two medial populations, the ventral and the dorsal parts of the nucleus recessus lateralis, and a lateral one. We have assigned such nomenclature to the ventral and dorsal components of this nucleus because they lie along the ventromedial and dorsomedial surface of the rostral lateral recess, respectively. Both nuclei exhibit serotonin-immunoreactive neurons in the Senegalese sole, suggesting that they could have the same embryonic origin. However, in the Senegalese sole, the pars ventralis of the nucleus recessus lateralis displayed galanin-like immunoreactive cells whereas the pars dorsalis did not (Rodríguez-Gómez et al., in press). Maler et al. (1991), in their brain atlas of *Apteronotus lepthorynchus*, recognized medial and inferior subdivisions of the nucleus of the lateral recess, that seem to correlate anatomically with our pars dorsalis and pars ventralis of the nucleus recessus lateralis, respectively. This assumption is reinforced by immunohistochemical data in *Apteronotus* indicating the presence of 5-HT-ir cells in both cells masses (Johnston et al., 1990). The ventral part of the nucleus recessus lateralis described in this study seems to represent the nucleus lateralis hypothalami described in other teleosts (Braford and Northcutt, 1983; Northcutt and Wullimann, 1988; Striedter, 1990; Butler and Northcutt, 1993) although the periventricular region could correspond to the caudal pole of the ventral hypothalamus described by Braford and Northcutt (1983). In a holocephalian, *Hidrolagus colliei*, a similar 5-HT-ir cell population has been considered in the lateral tuberal nucleus (Stuesse and Cruce, 1991). The dorsal part of the nucleus recessus lateralis described in this study was considered a migrated population of the paraventricular organ in *Carassius auratus* (Braford and Northcutt, 1983), *Haplochromis burtoni* (Fernald and Shelton, 1985), *Ictalurus punctatus* (Striedter, 1990), *Lepisosteus osseus* (Northcutt and Butler, 1993), holocephalian (Stuesse and Cruce, 1991) and elasmobranchs (Stuesse et al., 1990). In the Senegalese sole, the dorsal part of the nucleus recessus lateralis exhibits slightly larger and less densely packed cells than those of the paraventricular organ. However, a common origin of both cell masses cannot be excluded because, at least in *S. senegalensis* and *Sparus aurata* (unpublished data), the paraventricular organ also exhibits serotonin-immunoreactive cells. In *Polypterus senegalus*, the dorsal hypothalamus (Hd) and the periventricular nucleus of the posterior tuberculum displayed 5-HT-ir cells (Reiner and Northcutt, 1992). These cell masses appear to be homologous to 5-HT-ir cells found

in the nucleus recessus lateralis and paraventricular organ, respectively, of the Senegalese sole.

The serotoninergic cells of nucleus recessus lateralis, paraventricular organ and nucleus recessus posterioris are characteristic cerebrospinal fluid (CSF)-contacting neurons. Serotonin-containing CSF-contacting neurons have been reported in different phylogenetic groups (Parent, 1981; Johnston et al., 1990). To date, the physiological mechanisms linking the CSF and periventricular neurons are not well understood. It has been proposed that these cells are implicated in regulatory hypothalamic processes, taking up 5-HT from CSF or secreting 5-HT to it. It is also known that these hypothalamic nuclei constitute a target for gonadal steroids (Kim et al., 1978; Linard et al., 1996). Remarkable differences in the distribution of catecholaminergic and serotoninergic systems has been observed in Senegalese sole (Rodríguez-Gómez et al., in preparation) and other teleosts (Meek, 1994). However, an overlapping of 5-HT and catecholamine innervation is observed in the paraventricular organ region, the ventral hypothalamus and ventral rhombencephalon. In some teleosts, many CSF-contacting neurons of the paraventricular organ that lies along the infundibulum of the third ventricle, are dopamine immunopositive or noradrenaline immunopositive but tyrosine hydroxylase immunonegative (Meek, 1994). This fact suggests that they do not synthesize dopamine and noradrenaline acquiring it from external sources. It remains to be elucidated whether 5-HT is synthesized in the paraventricular cells of the Senegalese sole or just taken up from neighboring territories.

A direct effect of 5-HT in stimulating GTH release has been demonstrated in goldfish (Somoza and Peter, 1991) and in Atlantic croaker (Khan and Thomas, 1992). Wong (1993) also reported in goldfish direct actions of 5-HT inhibiting GH release on somatotrophs. Furthermore, 5-HT immunoreactivity has been located in the proximal pars distalis (PPD) of goldfish (Kah and Chambolle, 1983), and in PPD and pars intermedia of the platyfish (Margolis-Kazan et al., 1985) and the Atlantic croaker (Khan and Thomas, 1993). In *S. senegalensis*, the absence of serotoninergic immunoreactive fibers in the neurohypophysis seems to discount a direct effect of brain 5-HT in the regulation of adenohypophyseal functions. However, a few 5-HT-ir cells could be found in the adenohypophysis of the Senegalese sole. It needs to be determined whether 5-HT has a direct effect on pituitary functions in the sole and whether this monoamine is really synthesized in adenohypophyseal cells or is taken up from the blood, as has been suggested in some species (Kah et al., 1993). As has been reported above, an indirect effect of 5-HT on gonadotropin secretion through the mediation of other neuroendocrine/neuromodulator factors (e.g. GnRH, catecholamines, etc.) can also be postulated for the Senegalese sole.

The mesencephalon of Senegalese sole exhibits serotoninergic fibers but lacks positive cell bodies. This observation agrees with results obtained in *A. leptorhynchus* (Johnston et al., 1990), carp (Cuadrado et al., 1992) and *P. senegalus* (Reiner and Northcutt, 1992). However, both positive fibers and cells were identified in *Eigenmannia lineata* (Bonn and Konig, 1990), *M. undulatus* (Khan and Thomas, 1993), holocephalian (Stuesse and Cruce, 1991) and elasmobranchs (Stuesse et al., 1990; Stuesse et al., 1991a,b; Stuesse and Cruce, 1992). The absence of 5-HT-ir cells and fibers in the cerebellum (only some positive axons in the lobus vestibulolateralis) and the extensive innervation of labeled projections in the reticular formation, octavolateral area and olivary region are also consistent with observations in other fishes (Bonn and Konig, 1990; Johnston et al., 1990; Stuesse and Cruce, 1991; Stuesse et al., 1991a,b; Khan and Thomas, 1993).

In the rhombencephalon of Senegalese sole, the reticular formation displayed a notable number of ir-perikarya and projections. Similar observations were described in differents groups of fishes (Ekström and Van Veen, 1984; Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Meek and Joosten, 1989; Bonn and Konig, 1990; Johnston et al., 1990; Stuesse and Cruce, 1991; Stuesse et al., 1991a,b; Stuesse and Cruce, 1992; Khan and Thomas, 1993). The reticular formation constitutes a phylogenetically ancient group of neurons reported in the mesencephalon and rhombencephalon of all vertebrates (Parent, 1983; Steinbusch, 1984; Cruce et al., 1988). Based on cytoarchitectonical studies we have considered a nucleus raphes superior, nucleus raphes inferior, nucleus interpeduncularis, nucleus reticularis superior, nucleus reticularis medius, nucleus reticularis inferior and nucleus reticularis lateralis in the reticular formation of Senegalese sole (Rodríguez-Gómez et al., preparation). However, a much more complex organization, with up to six raphe nuclei and 16 reticular cell masses, has been considered in other fishes (Stuesse et al., 1990, 1991a,b; Stuesse and Cruce, 1991, 1992; Johnston et al., 1990). The raphe dorsalis and medialis identified in the Atlantic croaker (Khan and Thomas, 1993), as well as the raphe dorsalis, medialis and centralis of *A. leptorhynchus* (Johnston et al., 1990) and the raphe dorsalis, linearis, centralis superior and part of the raphe magnus of cartilaginous fish (Stuesse et al., 1990, 1991a,b; Stuesse and Cruce, 1991, 1992) appear to be equivalent to the superior raphe of the Senegalese sole. In turn, the raphe posterioris described in *Apteronotus* (Johnston et al., 1990), and the raphe obscurus and raphe pallidus of cartilaginous fish (Stuesse et al., 1990, 1991a,b; Stuesse and Cruce, 1991, 1992) seem to be homologous to the inferior raphe of the Senegalese sole and other teleosts (Kah and Chambolle, 1983; Ueda et al., 1983; Ekström and Van Veen, 1984; Wolters et al., 1985; Meek et al.,

1989). In the Senegalese sole, raphe neurons emit large processes laterally to enter the nucleus reticularis, in which some 5-HT-ir cells can also be observed. Similar immunolabeled processes and cells were observed in the reticular formation of fishes (Johnston et al., 1990; Stuesse et al., 1990, 1991a,b; Stuesse and Cruce, 1991, 1992). On the other hand, positive cells were also identified in the nucleus interpeduncularis and inferior olivary region of the Senegalese sole. To our knowledge such serotoninergic cell masses have not been described previously in teleosts. However, some 5-HT-ir cells were observed in the nucleus interpeduncularis of two elasmobranchs, the thornback guitarfish (Stuesse et al., 1990) and the bat ray (Stuesse et al., 1991b). Probably, 5-HT neurons reported in other fishes within rostroventral raphe nuclei as the raphe linearis and raphe centralis (Stuesse et al., 1991a,b; Stuesse and Cruce, 1991) could correlate with the labeled perikarya presented in the nucleus interpeduncularis of *S. senegalensis*. Moreover, lateral expansions of the raphe posterior of *Apteronotus* contained 5-HT-ir cells in the proximity of the inferior olive (Johnston et al., 1990) but these serotoninergic cells did not attain the ventral surface of the rhombencephalon, as they do in the Senegalese sole. Some 5-HT-ir cells were also present in the inferior olive of holocephalian (see Fig. 2C of Stuesse and Cruce, 1991) and elasmobranchs (see Fig. 3K of Stuesse et al., 1991a). However, an homology of inferior olivary cells of Senegalese sole with 5-HT-ir cells presented in the nucleus reticularis paragigantocellularis, the nucleus reticularis magnocellularis and the nucleus reticularis ventralis of other fish (Stuesse et al., 1990, 1991a,b) cannot be discarded.

In conclusion, the distribution of serotoninergic cells in the brain of the Senegalese sole differs from other fish in that 5-HT-ir neurons are absent in preoptic and thalamic nuclei, as well as by the presence of positive cells in the nucleus interpeduncularis and inferior olive. Similarities found concerned the presence of CSF-contacting 5-HT-ir cells in the paraventricular organ and around the lateral and posterior recesses, as well as the identification of serotoninergic cells in the reticular formation. The absence of immunoreactive fibers in the neurohypophysis makes it necessary to undertake further physiological and morphological studies, in order to elucidate the form of action of this monoamine in the regulation of adenohypophyseal functions and putative interactions with other neuroendocrine systems.

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