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Localization of galanin-like immunoreactive structures in the brain of the Senegalese sole, *Solea senegalensis*

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Summary

The distribution of galanin-like immunoreactive structures was studied in the brain of the Senegalese sole, *Solea senegalensis*, using immunohistochemical methods. Periventricular immunoreactive cell bodies were observed in the rostral pole of the preoptic recess, within the pars parvocellularis of the nucleus preopticus parvocellularis. Another galanin-immunoreactive cell population was observed more caudal in the ventromedial hypothalamus, along the medial evaginations of the lateral recess. These cells appear within the cytoarchitectonic limits of the nucleus recessus lateralis pars ventralis. We found an extensive presence of galanin-immunoreactive fibres throughout the entire brain, although the most massive network of fibres was observed in the caudal olfactory bulbs, ventral telencephalon, preoptic area and around diencephalic ventricular recesses. Also, the hypophysis, ventricular mesencephalic area, median reticular formation and viscerosensory rhombencephalon displayed important plexuses of galanin-immunoreactive axons.The widespread distribution of these immunoreactive structures in the brain and pituitary of the Senegalese sole suggests an important role for galanin in neuroendocrine regulation of brain and adenohypophyseal functions.

Introduction

Galanin (GAL) is a brain-gut peptide of 29 residues present in the central nervous system (CNS) of vertebrates and invertebrates. It was firstly isolated by Tatemoto *et al*. (1983) from the porcine intestine. The presence of GAL-like immunoreactive structures has been extensively reported in the CNS of mammals (Skofitsch & Jacabowitz 1985, Melander *et al*. 1986, Levin *et al*. 1987, Palkovits *et al*. 1987, Blasco *et al*. 1989, Gaymann & Martin 1989, Gentleman *et al*. 1989, Walker *et al*. 1989, Kordower & Mufson 1990, Meister *et al*. 1990, Elmquist *et al*. 1992, Kordower *et al*. 1992), amphibians (Wolfbauer & Skofitsch 1989, Lázár *et al*. 1991, Olivereau & Olivereau 1992), reptiles (Jiménez *et al*. 1994), birds (Józsa & Mess 1993), lampreys (Jiménez *et al*. 1996), elasmobranchs (Vallarino *et al*. 1991) and teleosts (Batten *et al*. 1990a,b, Cornbrooks & Parsons 1991a,b, Holmqvist & Ekström 1991, Olivereau & Olivereau 1991a, Yamamoto *et al*. 1992, Magliulo-Cepriano *et al*. 1993, Anglade *et al*. 1994). GAL receptors have also been detected in the brain and pituitary of the sea bass (Moons *et al*. 1991) and Atlantic salmon (Holmqvist & Carlberg 1992).

In fishes, the widespread distribution of GAL and GAL-binding sites in brain and pituitary, suggests many functional implications of this peptide, acting as a neurohormone, neuromodulator or neurotransmitter. Specifically, a role has been proposed for GAL in olfactory, gustatory and visual processing, somatosensory transmission, osmoregulation, sex-specific behaviours, and reproduction (Batten 1990b, Olivereau & Olivereau 1991a, Cornbrooks & Parsons 1991b, Holmqvist & Carlberg 1992). In eels, GAL immunoreactivity is affected by steroids, also suggesting the existence of a seasonality in GAL levels according to the reproductive stage (Olivereau & Olivereau 1991b).

Although most studies of GAL systems in the brain of teleosts have recognized two main immunoreactive cell groups in the basal forebrain, there is disagreement on the precise identification of these cell masses (Batten *et al*. 1990b, Olivereau & Olivereau 1991a, Anglade *et al*. 1994, Power *et al*. 1996). This disagreement could represent real species-related differences, but also discrepancies in neuroanatomical nomenclatures. Thus, species-specific studies using reliable neuroanatomical tools could enable more certain information to be obtained. In this paper we present the results of an immunohistochemical study on the location of GAL-like peptide in the brain and pituitary of the Senegalese sole, *Solea senegalensis*, using a brain atlas recently developed in our group (Rodríguez-Gómez *et al*. in press, F.J. Rodríguez-Gómez, C. Sarasquete & J.A. Muñoz-Cueto, in preparation). The aim of the present study is to obtain basic information on the possible role of GAL in the neuroendocrine regulation of adenohypophyseal cell functions in this species.

Materials and methods

Adult specimens of Senegalese sole, *S. senegalensis*, were purchased from a local fishery (Cupimar, S.A. San Fernando, Spain) and kept in the laboratory in running sea-water. Specimens were deeply anaesthetized 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). Brains with the pituitary attached were then carefully removed and further postfixed in the same fixative for 6 h in the dark at 4 °C. After fixation, tissues were cryoprotected in 15% sucrose in 0.1 M phosphate buffer for 6 h, and finally, embedded in Tissue-tek and kept at −80 °C until processing. Serial transverse brains sections, 16 μm-thick, were obtained with a cryomicrotome and mounted on gelatin-coated glass slides.

Immunohistochemical staining was performed using a streptavidin–biotin–peroxidase complex method. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide in Coons buffer (0.01 M veronal, 0.15 M NaCl) containing 0.1% Triton X-100 (CBT) for 30 min. Before immunostaining, sections were transferred for 5 min to CBT and then to CBT containing 0.5% casein for 30 min. Sections were incubated overnight in a humid chamber at room temperature with a rabbit antiserum against porcine GAL (Anglade *et al*. 1994) diluted 1 : 1000. Sections were washed in CBT and incubated for 1 h at room temperature with biotinylated anti rabbit IgG diluted 1 : 1000 in CBT. After washing in CBT, sections were incubated for 1 h at room temperature with streptavidin–peroxidase complex diluted 1 : 1000 in CBT. Finally, sections were washed with CBT followed by 0.05 M Tris–HCl buffer (pH 7.4). Peroxidase activity was detected by immersion in 0.05 M Tris–HCl, pH 7.6, containing 0.048% 4-chloro-1-naphthol (Sigma, St Louis, MO), previously diluted in 20 ml 100% ethanol, and 0.05% hydrogen peroxide. To confirm the specificity of the immunostaining, controls were performed by replacement of primary antiserum with normal rabbit serum, omission of primary antiserum and incubation of sections with primary antiserum preabsorbed with 20 μg/ml of porcine GAL (Sigma, St Louis, MO). The sections were mounted in an aqueous mounting medium for microscopy (Aquatex, Merck, Darmstadt, Germany). The precise localization of GAL-immunoreactive (GAL-ir) fibres and cells was determined with the help of a recently developed brain atlas of *S. senegalensis* (Rodríguez-Gómez *et al*. in press).

Results

GAL-ir cell bodies

GAL-ir cell bodies in the brain of Senegalese sole were detected only in the rostral preoptic area and the caudoventral hypothalamus. In the rostral pole of the preoptic recess, immunoreactive perikarya were observed within the pars parvocellularis of the nucleus preopticus parvocellularis, in a periventricular position (Figures 1C, 2A,B). However, GAL-ir cells in this nucleus did not appear to be neurons in contact with cerebrospinal fluid (Figure 2A). These cells started slightly rostral to the opening of the preoptic recess, ventral to the anterior commissure, and slightly caudally, they lie lateral to the root of the rostral ventricle (Figure 2A). More caudally, as the preoptic recess enlarges and the magnocellular preoptic nucleus starts, the nucleus preopticus parvocellularis leaves its periventricular position and GAL-ir cells migrated laterally, being concentrated mainly in the dorsolateral zone of the nucleus preopticus parvocellularis. Two types of GAL-ir cells were distinguished: small round and ovoid cells and medium-sized polygonal cells (Figure 2B). These larger cells exhibit two or more intensely stained processes exiting from the cell bodies (Figure 2B).

A second population of GAL-ir cells was observed more caudally, in the ventromedial hypothalamus, within the cytoarchitectonic limits of the nucleus recessus lateralis pars ventralis (Figures 1E–G, 2C,D,F). These GAL-ir cells, small and round in shape, appeared lateral to the medial ventricle, just rostral to the opening of the lateral recess (Figure 2C). Slightly caudally, the lateral recess commence, with the GAL-ir neurons adopting a ventral position in relation to it (Figure 2D). More caudally, the lateral recess joined the medial ventricle and GAL-ir cells occupied its ventromedial border (Figure 2F). Finally, the medial expansions of the lateral recess obliterated and a few GAL-ir cells were observed at the caudal pole of the nucleus recessus lateralis pars ventralis, dorsally to the nucleus recessus posterioris.

GAL-ir fibres

The GAL-ir fibres were widely distributed throughout the entire brain of the Senegalese sole. The olfactory bulbs showed a high density of immunoreactive axons, especially in their caudal portion. These fibres were more abundant in the internal and external cell layers; but they were also evident near the terminal nerve ganglion cells (Figure 1A). The ventral area of the telencephalon displayed an intense GAL-ir innervation. Many of these fibres appeared within the medial olfactory tract (Figure 1B). A high density of GAL-ir fibres was observed in the ventral (Vv), dorsal (Vd) and central (Vc) components of the ventral telencephalon (Figure 1B). More caudally, the supracommissural (Vs) and postcommisural (Vp) nuclei of the ventral telencephalon, as well as the nucleus entopeduncularis, also exhibited evident immunostained fibres (Figure 1C). It was notable the presence of dense plexuses of GAL-ir axons running above and below the anterior commissure, which was devoid of immunoreactivity (Figures 1C, 2A). In the dorsal telencephalon, the GAL-ir innervation was much more sparse, with most of the GAL-ir fibres concentrated in the medial (Dm2) and central (Dc) zones (Figure 1B,C).

The rostral preoptic area of the Senegalese sole, where GAL-ir cells were located, contained a very high density of GAL-ir fibres (Figures 1C,D, 2A). These fibres appeared

laterally to GAL-ir cells and were organized in tracts which ran caudally in the ventrolateral surface of the brain, between the fibres of the lateral forebrain bundles (Figure 2A). The density of GAL-ir fibres in the preoptic area decreased from rostral to caudal positions. The hypothalamus also exhibited dense networks of GAL-ir fibres (Figure 1D–H). This innervation was more evident around the nucleus recessus lateralis pars ventralis, which contained GAL-ir perikarya, but also in the nucleus lateralis tuberis pars medialis and the nucleus recessus posterioris (Figures 2C,D,F, 3A). In the ventral surface of the hypothalamus, GAL-ir axons could be observed entering the pituitary stalk (Figures 1D,E, 2C). These fibres exhibited intensely stained varicosities and seemed to originate from preoptic GAL-ir cells. An intense innervation of the pituitary was observed in the Senegalese sole (Figures 1D,E, 2E). GAL-ir fibres were very evident in the neurohypophysis, and terminals were also observed in the proximal pars distalis of the adenohypophysis (Figure 2E). Furthermore, moderate GAL-ir fibre bundles ascended from hypothalamic cells to innervate the thalamus, the pretectal area and the posterior tuberculum (Figures 1D–H, 3A). In the mesencephalon, the optic tectum received a distinct GAL-ir innervation (Figures 1F–J, 3B). Most axons appeared in the deep white zone but also

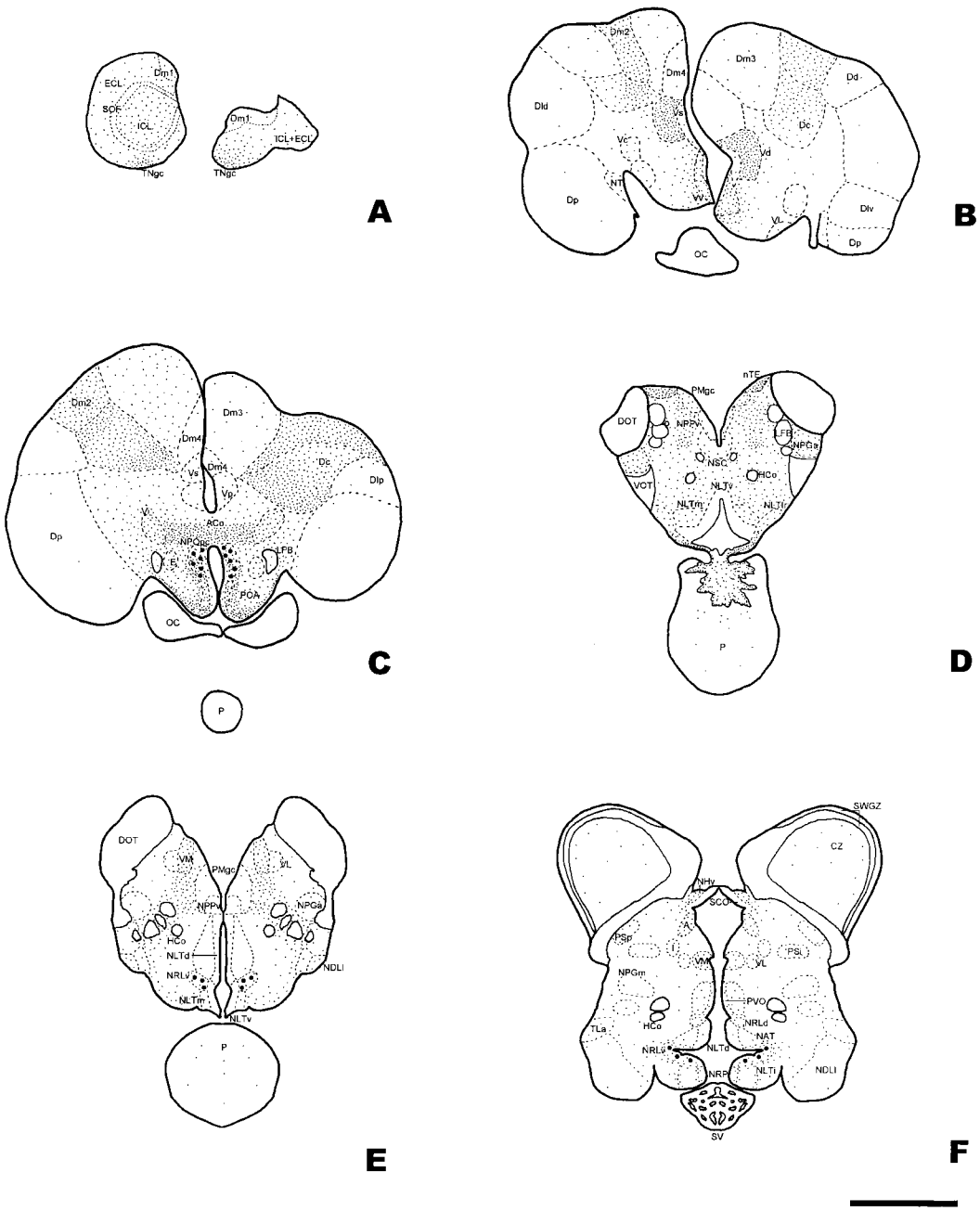

Figure 1.

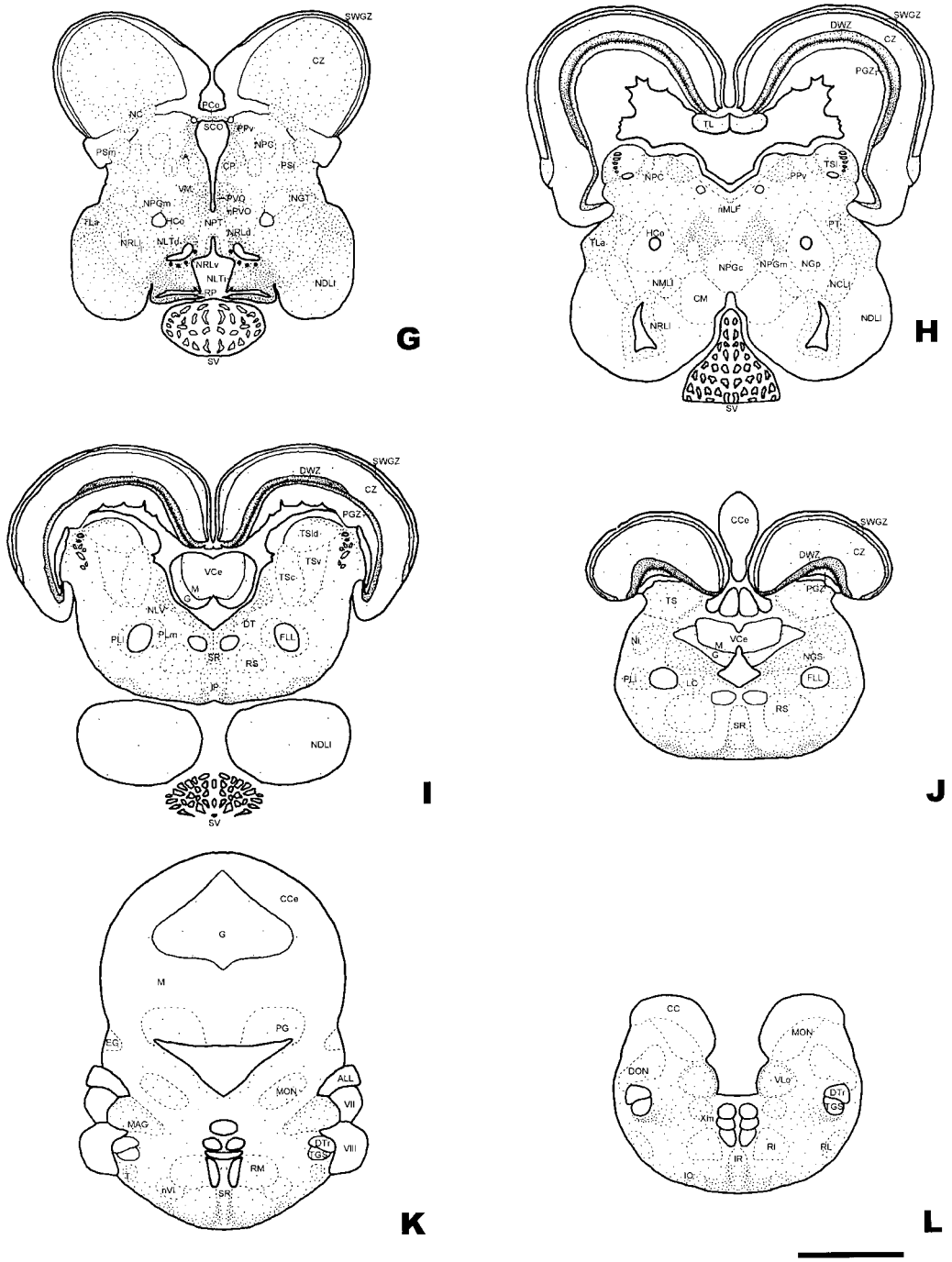

Figure 1. Series of transverse sections through the brain of *S.senegalensis*, from rostral to caudal, showing the distribution of GAL-ir cells (filled circles) and fibres (small dots). A constitutes the rostral-most section and L the caudal-most one. Bar scale = 1 mm. For abbreviations see Table 1.

at the limits with the central zone (Figure 3B). In the lateral zone of the mesencephalic tegmentum, the torus semicircularis also contained a moderate density of GAL-ir fibres, as well as in the vicinity of the mesencephalic ventricle (Figure 1H–J). The rhombencephalon also exhibited conspicuous GAL-ir projections. These fibres extended from the dorsal aspects of the brainstem to the medial and ventral zones. Higher densities were observed in the viscerosensory rhombencephalon, median reticular formation (raphe area) and in the inferior olivary region (Figure 1I–L). Only a scarce GAL-ir innervation was present in the cerebellum of the Senegalese sole.

Immunostaining was not evident in fibres and cells of adjacent control sections.

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
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
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 bundle
M: molecular layer of the cerebellum
MAG: nucleus magnocellularis
MON: nucleus octavolateralis medialis
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
NH: neurohypophysis
NI: nucleus isthmi
NLT: nucleus lateralis tuberis
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 fascicle
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
NPOpc: nucleus preopticus parvocellularis pars parvocellularis
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
NSC: nucleus suprachiasmaticus
NT: nucleus taenia
nTE: nucleus eminentia thalami
nVI: nucleus nervi abducentis
OB: olfactory bulbs
OC: optic chiasma
OlN: olfactory nerve fibres
P: pituitary
PCo: posterior commissure
PG: periventricular granular cell mass
pgd: nucleus periglomerulosus dorsalis
PGZ: periventricular grey zone
PL1: nucleus perilemniscularis pars lateralis
PLL: posterior lateral line nerve
PLm: nucleus perilemniscularis pars medialis
PMgc: nucleus preopticus magnocellularis pars gigantocellularis
POA: preoptic area
PPD: proximal pars distalis
PPv: nucleus pretectalis periventricularis pars ventralis
PSi: nucleus pretectalis superficialis pars intermedia
PSm: nucleus pretectalis superficialis pars magnocellularis
PT: nucleus posterior thalami
PVO: paraventricular organ
RI: nucleus reticularis inferioris
RL: nucleus reticularis lateralis
RP: recessus posterioris
RS: nucleus reticularis superioris
SCO: subcommissural organ
SOF: secondary olfactory fibres
SR: nucleus raphes superior
SV: saccus vasculosus
SWGZ: superficial white and grey zone
T: nucleus tangentialis
TGS: tractus gustatorius secundarius
TL: torus longitudinalis
TLa: nucleus tori lateralis
TNgc: terminal nerve ganglionar cells
TS: torus semicircularis
TSc: torus semicircularis pars centralis
TSl: torus semicircularis pars lateralis
TSld: torus semicircularis pars lateralis dorsalis
TSlv: torus semicircularis pars lateralis ventralis
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
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

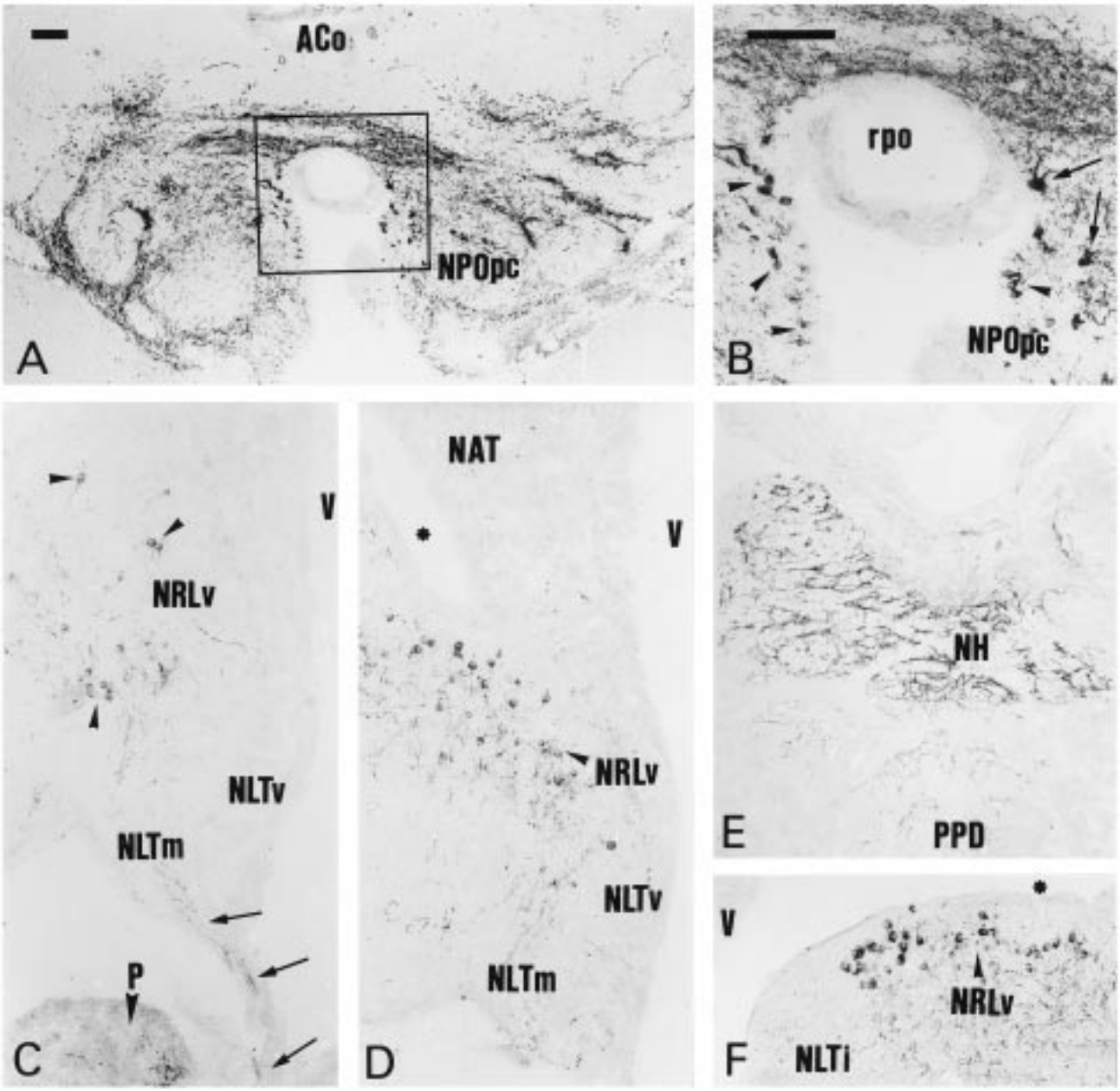

Figure 2. Photomicrographs of transverse sections of the brain of *S. senegalensis*, showing GAL-ir cells. (A) Rostral preoptic area. GAL-ir perikarya in the nucleus preopticus parvocellularis and profuse GAL-ir fibre tracts. Framed area is enlarged in B. (B) Detail of small round (arrowheads) and medium-sized polygonal (arrows) GAL-ir cells in the nucleus preopticus parvocellularis. (C) Ventromedial hypothalamus. GAL-ir cells (arrowheads) in the anterior pole of the nucleus recessus lateralis pars ventralis, just rostral to the rise of the lateral recess. Arrows mark varicose GAL-ir axons entering the pituitary stalk. (D) Ventromedial hypothalamus.GAL-ir cells in nucleus recessus lateralis pars ventralis (arrowhead), ventral to the rostral onset of the lateral recess (asterisk) and lateral to the ventricle (V). (E) GAL-ir fibres running into the neurohypophysis (NH) and reaching the proximal pars distalis (PPD) of the adenohypophysis. (F) GAL-ir cells in the caudal zone of NRLv, ventral to the medial evagination of the lateral recess (asterisk). Bars represent 100 μm. Bar in B is also valid for C, D, E and F. For other abbreviations see Table 1.

Discussion

This study describes the immunohistochemical distribution of a GAL-like peptide in the brain and hypophysis of a flatfish, the Senegalese sole, using an antiserum against porcine GAL. Whereas GAL-ir cells appeared restricted to the preoptic area and the ventral hypothalamus, GAL-ir fibres were widely distributed in the brain of this species. The characterization of GAL in trout and its comparison with tetrapod galanins have revealed that the N-terminal region of this peptide is strongly conserved during evolution (Anglade *et al*. 1994). Thus, antisera against pig GAL would seem to be a reliable tool for locating homolog peptides in fish brain, as has been demonstrated in other studies (Olivereau & Olivereau

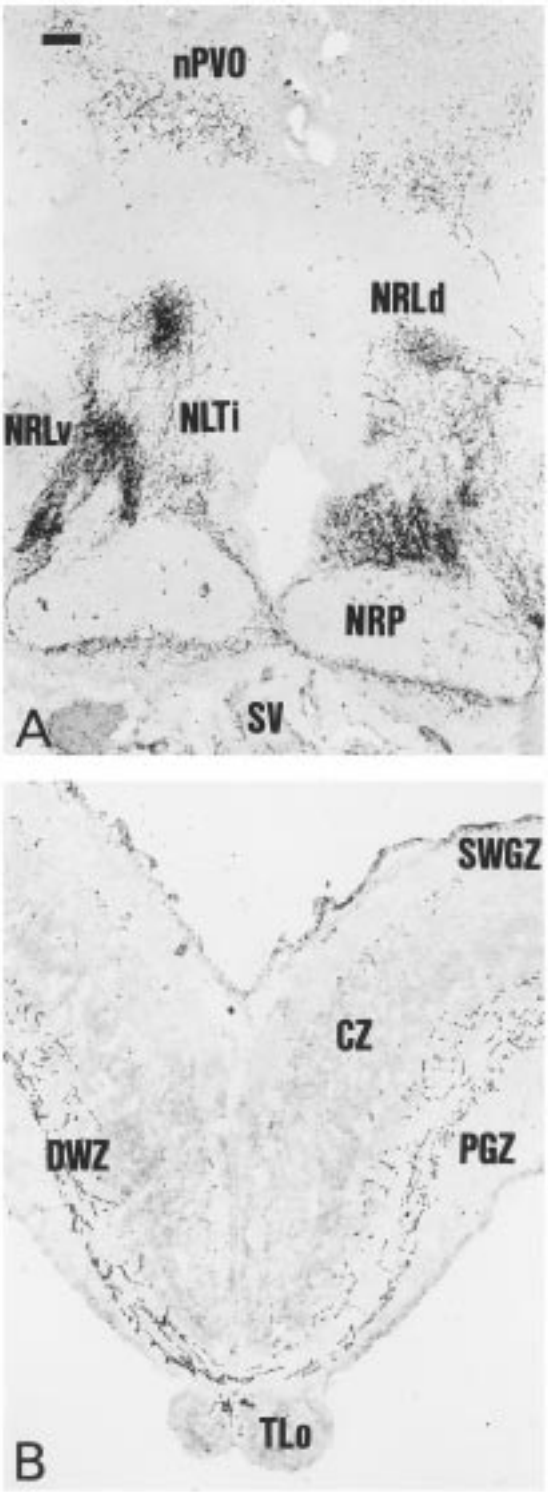

Figure 3. Photomicrographs of transverse sections of the brain of *S. senegalensis*, showing GAL-ir fibres. (A) Ventrocaudal hypothalamus. Abundant GAL-ir fibres are observed in the caudal end of the nucleus recessus lateralis and around the nucleus recessus posterioris. A moderate innervation is also observed in the posterior tuberculum. (B) Optic tectum. Note the presence of GAL-ir fibres in the interne zone of CZ and in DWZ. Bar in A represents 100 μm and is also valid for B. For other abbreviations see Table 1.

1991a, Yamamoto *et al*. 1992, Anglade *et al*. 1994, Power *et al*. 1996).

The distribution of GAL-like immunoreactive cells in the brain of the Senegalese sole does not differ markedly from that reported in cyclostomes (Jiménez *et al*. 1996) and teleosts (Batten *et al*. 1990a,b, Cornbrooks & Parsons 1991a,b, Holmqvist & Ekström 1991, Olivereau & Olivereau 1991a, Anglade *et al*. 1994). Although GAL-ir cell masses appear in a similar location in other teleosts (Batten *et al*. 1990b, Cornbrooks & Parsons 1991a, Holmqvist & Ekström 1991, Olivereau *et al*. 1991a, Yamamoto *et al*. 1992, Anglade *et al*. 1994, Power *et al*. 1996), they do not form compact preoptic or hypothalamic nuclei as in the Senegalese sole. In this species, GAL-ir cell bodies appear restricted to the pars parvocellularis of the nucleus preopticus parvocellularis, and to the pars ventralis of the nucleus recessus lateralis. In the Senegalese sole, the most rostral GAL-ir cell bodies of the nucleus preopticus parvocellularis appear just anterior to the opening of the preoptic recess. Anglade *et al*. (1994) reported the presence of GAL-ir cells in the caudal telencephalon of rainbow trout. From their illustrations, these cells appear to correspond to the most rostral cells of the nucleus preopticus parvocellularis of the sole. Our GAL-ir cells of the nucleus preopticus parvocellularis seem to represent the cells described in the nucleus recessus anterior, the nucleus preopticus parvocellularis, the nucleus preopticus periventricularis, the anterior part of the nucleus preopticus periventricularis or the nucleus preopticus of different teleosts (Batten *et al*. 1990, Olivereau & Olivereau 1991a, Cornbrooks & Parsons 1991a,b, Holmqvist & Ekström 1991, Yamamoto *et al*. 1992, Anglade *et al*. 1994, Power *et al*. 1996). Furthermore, the surges of the magnocellular preoptic nucleus in Senegalese sole displaces the nucleus preopticus parvocellularis laterally, from its periventricular position. Interestingly, most of the caudal GAL-ir cells of the preoptic area were located laterally, confirming their cytoarchitectonic position within the nucleus preopticus parvocellularis. In rainbow trout, GAL-ir cells identified in a similar position were ascribed to the nucleus anterioris periventricularis (Anglade *et al*. 1994). In rainbow trout and sea bream, the nucleus posterioris periventricularis also exhibited GAL-ir cells (Anglade *et al*. 1994, Power *et al*. 1996) whereas in the Senegalese sole, the nucleus posterioris periventricularis did not.

The second and most caudal group of GAL-ir cells is located in the nucleus recessus lateralis of the caudomedial hypothalamus. The nucleus recessus lateralis pars ventralis of Senegalese sole represents a similar cell mass to that of the nucleus recessus lateralis inferior of *Apteronotus* (Yamamoto *et al*. 1992), which also contained GAL-ir cells. The presence of GAL-ir cells in the nucleus recessus lateralis has also been identified in *Anguilla anguilla, Salmo fario* (Olivereau & Olivereau 1991a), rainbow trout (Anglade *et al*. 1994) and sea bream (Power *et al*. 1996).

In other teleosts, additional hypothalamic nuclei such as the nucleus lateralis tuberis and nucleus recessus posterioris also exhibited GAL-ir cells (Anglade *et al*. 1994, Holmqvist

and Ekström 1991, Power *et al*. 1996). The rostralmost and caudalmost GAL-ir cells of the nucleus recessus lateralis pars ventralis lie slightly anterior and posterior to the opening and the closure of the medial extensions of the lateral recess, respectively. Although these cells appear in the proximity of the nucleus lateralis tuberis and the nucleus recessus posterioris we consider that they belong cytoarchitectonically to the nucleus recessus lateralis pars ventralis.

The presence of labelled fibres in the olfactory bulbs has been reported in other fish (Olivereau & Olivereau 1991a, Anglade *et al*. 1994). In the trout, the axons are restricted to the internal cell layer and the medial olfactory tract (Anglade *et al*. 1994), while in a cyclostome, the lamprey (Jiménez *et al*. 1996), the distribution is very similar to that reported in *S. senegalensis*. In the lamprey, as occurs in the Senegalese sole, the nucleus olfatorius anterior, probably equivalent to our terminal nerve ganglion cells, is strongly innervated by GAL-ir fibres. The abundant presence of immunoreactive axons in the ventral telencephalon and the sparse innervation of the dorsal aspect seems to be a conserved characteristic of the GAL systems because is evident in reptiles (Jiménez *et al*. 1994), cyclostomes (Jiménez *et al*. 1996), and teleosts (Olivereau & Olivereau 1991a, Anglade *et al*. 1994). As in rainbow trout (Anglade *et al*. 1994), GAL-ir fibres in the ventral telencephalon of Senegalese sole increase from rostral to caudal positions as they approach the nucleus preopticus parvocellularis, suggesting a possible origin of this innervation in GAL-ir preoptic cells.

In the Senegalese sole, a dense network of GAL-ir fibres is observed in the preoptic area. Some of these axons run ventrolaterally and probably enter the hypophysis. A strong GAL-ir innervation is also present in the neurohypophysis of *S. senegalensis*, which reaches the proximal pars distalis of the adenohypophysis. A similar observation has been reported in some teleosts (Batten *et al*. 1990a,b, Cornbrooks & Parsons 1991a, Holmqvist & Ekström 1991, Olivereau & Olivereau 1991a, Yamamoto *et al*. 1992, Anglade *et al*. 1994, Power *et al*. 1996), but not in the *Anguilla* (Olivereau & Olivereau 1991a), *Apteronotus* (Yamamoto *et al*. 1992) or lamprey (Jiménez *et al*. 1996). This region of the sole adenohypophysis contains thyrotrophic, somatotrophic and gonadotrophic cells (Rendón *et al*. 1997) suggesting that GAL may modulate the activity and/or secretion of these endocrine cells. At least in the sea bass (Moons *et al*. 1991) and Atlantic salmon (Holmqvist & Carlberg 1992), GAL-binding sites are present in the anterior pituitary. Ultrastructural studies in teleosts have also demonstrated frequent contacts between GAL-ir fibres and endocrine cells of the adenohypophysis (Moons *et al*. 1989, Batten *et al*. 1990b). GAL-ir fibres entering the hypophysis of the Senegalese sole seem to originate, principally, in GAL-ir cell bodies of the nucleus preopticus parvocellularis. In this species, the nucleus preopticus parvocellularis also displayed gonadotropin-releasing hormone-immunoreactive neurons (F.J. Rodríguez-Gómez, C. Rendón, C. Sarasquete & J.A. Muñoz-Cueto, submitted). In mammals, a coexpression of GAL and gonadotropin-releasing hormone in cells of the anterior preoptic area has been described (Merchenthaler *et al*. 1990). Furthermore, GAL has stimulatory effects on gonadotropin release and reinforces the effects of gonadotropin-releasing hormone (López *et al*. 1993). In eels, oestrogens and androgens increased GAL levels in preoptic neurons (Olivereau & Olivereau, 1991b). This observation supports the existence of a reproductive seasonality in GAL content in relation to the steroid environment. At least in trout, the cells of the nucleus preopticus parvocellularis exhibit oestrogen receptors and projects to the pituitary (Anglade 1994, Linard *et al*. 1996). In molly, a sexual dimorphism in GAL-ir cells of the preoptic nucleus, as well as in preoptico-spinal GAL-ir projections, have been described (Cornbrooks & Parsons 1991a,b). These authors proposed that this dimorphism could mediate sex-specific behaviours. Taken together, these findings are evidences indicating a role for GAL in reproduction in fish.

In conclusion, the localization of GAL-ir structures in the brain and pituitary of the Senegalese sole supports the existence of conserved GAL systems in the forebrain of teleosts. However, some discrepancies exist in the identification of GAL-ir cell masses. Future research should determine whether these discrepancies only reflect inconsistencies in neuroanatomical nomenclatures, indicate the simplicity of the organization of GAL systems in the Senegalese sole, or are related to differences in physiological states of animals. The widespread distribution of GAL-ir structures in the brain and pituitary of the Senegalese sole suggests that GAL plays an important role in neuroendocrine regulation of brain and adenohypophyseal functions. Further studies should be directed towards elucidating whether gonadotrophic cells receive direct GAL-ir inputs and the putative role of GAL in the reproductive activity of the Senegalese sole.

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