Distribution of Neuropeptide Y-Like Immunoreactivity in the Brain of the Senegalese Sole (*Solea senegalensis*)

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ABSTRACT

We present the results of an immunohistochemical study aimed at localizing the neuropeptide Y (NPY) in the brain of the Senegalese sole, *Solea senegalensis*, using an antiserum raised against porcine NPY and the streptavidin-biotin-peroxidase method. In this species, we have identified immunoreactive cells in the ventral and dorsal telencephalon, caudal preoptic area, ventrocaudal hypothalamus, optic tectum, torus longitudinalis, synencephalon and isthmic region. NPY-immunoreactive fibers were profusely distributed throughout the brain, also reaching the adenohypophysis. The extensive distribution of NPY suggests an important role for this neuropeptide in a variety of physiological processes, including the neuroendocrine control of adenohypophyseal functions. Our results are compared with those obtained in other teleosts and discussed in relation to putative functions of NPY in the control of metabolism and reproduction in the Senegalese sole. Anat Rec 262:227–237, 2001.

Key words: neuropeptide Y; reproduction; metabolism; brain; sole; pleuronectiformes; teleost

Neuropeptide Y (NPY) is a 36-amino acid peptide, belonging to the pancreatic polypeptide molecular family that was originally isolated and characterized from the porcine brain (Tatemoto, 1982; Tatemoto et al., 1982), and later characterized in other vertebrates (Blomqvist et al., 1992). NPY is widely distributed in the CNS of dipnoans (Vallarino et al., 1995; Trabucchi et al., 2000), elasmobranchs (Vallarino et al., 1988; Chiba and Honma, 1992a), cyclostomes (Rawitch et al., 1992; Chiba et al., 1993), teleosts (Noe et al., 1989; Pontet et al., 1989; Danger et al., 1991; Chiba et al., 1996), amphibians (Danger et al., 1985; Cailliez et al., 1987; Perroteau at al., 1988), birds (Aste et al., 1991) and mammals (Smith et al., 1985; Bons et al., 1990). The extensive distribution in all vertebrate taxons, and the strong evolutionary conservation of the NPY gene (Blomqvist et al., 1992), suggest that this peptide plays important roles in the regulation of brain functions.

In mammals, many physiological functions have been assigned to NPY: inhibition of sexual behavior (Kalra et al., 1987), control of cardiovascular physiology (Gibbins and Morris, 1988; Martin et al., 1988), control of circadian rhythms (Albers and Ferris, 1984; Card and Moore, 1989) and regulation of adenohypophyseal functions and endocrine secretion (Wahlestedt et al., 1987; Danger et al., 1987). NPY also plays an important role in the regulation of feeding activity and behavior (Morley, 1987; Sahu et al., 1988). Furthermore, a direct relationship between dietary quality and reproduction has been established. Thus, fasting or deficiency in dietary energy produces negative effects on gonadotropin secretion and ovulation (Kile et al., 1991).

In teleosts, NPY has also been involved in stimulating release of gonadotropin (GTH) and growth hormone (GH)

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(Breton et al., 1989, 1990, 1991; Kah et al., 1989; Peng et al., 1990, 1993a–c; Danger et al., 1991; Peng and Peter, 1997). NPY-immunoreactive fibers have been identified in the hypophysis of the sea bass (Moons et al., 1989), goldfish (Pontet et al., 1989), trout (Danger et al., 1991), platyfish (Magliulo-Cepriano and Schreibman, 1993), and ayu (Chiba et al., 1996). In goldfish, a seasonal pattern of NPY actions on GH and GTH II release has been demonstrated to be dependent on the steroid environment and seems to be mediated, at least in part, by enhanced stimulation of GnRH release (Peng et al., 1993c). Also, NPY gene expression is regulated by gonadal steroids (Peng et al., 1994).

Recent data suggest that NPY is also involved in the regulation of food intake in teleosts (Himick and Peter, 1995; Peng and Peter, 1997). In sea bass, it has been demonstrated that dietary composition affects oocyte development, gonadotropin, GnRH, vitellogenin and steroid profiles and reproductive performance (Cerdá et al., 1994a–c, 1995; Kah et al., 1994; Navas et al., 1995). Furthermore, nutritional influences on the endocrine control of growth and metabolism have been clearly established (Zanuy et al., 1993). Taken together, these results suggest that NPY represents a key factor mediating the neuroendocrine interactions of metabolic and reproductive processes.

In recent years, the number of papers describing the distribution of NPY in the brain and hypophysis of fish has increased (Vallarino et al., 1988; Pontet et al., 1989; Chiba and Honma, 1992a,b; García-Fernández et al., 1992; Pickavance et al., 1992; Reiner and Northcutt, 1992; Vecino and Ekström, 1992; Chiba et al., 1993; Magliulo-Cepriano and Schreibman, 1993; Chiba and Honma, 1994; Vecino et al., 1994; Zandbergen et al., 1994; Vallarino et al., 1995; Chiba et al., 1996; Subhedar et al., 1996; Trabucchi et al., 2000). To date, however, this type of study has not been undertaken in the pleuronectiform species, which represents one of the most evolved orders of euteleosts.

The Senegalese sole, *Solea senegalensis*, is a pleuronectiform species extensively exploited in marine aquaculture (Drake et al., 1984; Dinis, 1992). Currently, considerable effort is being directed at inducing the species to spawn in captivity and at cultivating their larvae, but results are unpredictable and a wide range of variability in the quality and viability of the spawn is generally obtained. These problems could reflect, at least in part, disruptions in neuroendocrine mechanisms involved in the control of reproduction and metabolism, caused by conditions associated with intensive cultivation. Thus, basic information on the anatomical localization of NPY in the Senegalese sole could provide useful data before the study of seasonal variations of NPY and putative interactions of NPY with other endocrine factors (e.g., GnRH, gonadal steroids) in the regulation of metabolic and reproductive processes. In this study, we present an immunohistochemical study of the distribution of NPY-immunoreactive cells throughout the whole brain of the Senegalese sole.

MATERIALS AND METHODS

Adult female and male specimens of Senegalese sole, *Solea senegalensis* (n = 12), with a mean length of 17 cm, 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 86-23, revised 1985) and the Spanish laws. 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). Brains with the pituitary attached were then carefully removed and further postfixed in the same fixative for 6 hr in darkness at 4°C. After fixation, tissues were cryoprotected in 15% sucrose in 0.1 M phosphate buffer for 6 hr, 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.

Immunocytochemical 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) with 0.1% Triton X-100 (CBT) for 30 min. Before immunostaining, 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 a rabbit antiserum against porcine NPY (donated by Dr. Tramu), at 1:5,000 dilution. Sections were washed in CBT and incubated for 1 hr at room temperature with biotinylated anti rabbit-IgG diluted 1:1,000 in CBT. After washing in CBT, sections were incubated for 1 hr at room temperature with streptavidin-peroxidase complex diluted 1:1,000 in CBT. Finally, sections were washed with CBT followed by Tris-HCl (0.05 M, pH 7.4) and peroxidase activity was detected in Tris-HCl 0.05 M pH 7.6, containing 0.025% 3,3 diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.05% hydrogen peroxide. To confirm the specificity of the immunostaining, controls were performed by absorption of primary antisera with porcine NPY (Sigma), replacement of primary antisera with normal rabbit serum and omission of primary antisera. The sections were dehydrated and mounted in Eukitt. Finally, for an accurate location of the NPY-immunoreactive fibers and cells, we have used a specific atlas of the *Solea senegalensis* brain recently developed in our laboratory (Rodríguez-Gómez et al., 2000a).

RESULTS

Immunohistochemistry demonstrated the presence of NPY-immunoreactive (ir) material throughout the entire brain of the Senegalese sole (Fig. 1). The rostralmost NPY-ir cell bodies were detected in the telencephalon and specifically in the pars centralis (Vc, Figs. 1B, 2A,B) and the pars lateralis (Vl, Figs 1B, 2A) of the ventral telencephalon, as well as in the pars centralis of the dorsal telencephalon (Dc, Figs. 1B,C, 2B). In the preoptic area, NPY-ir cells were observed in the nucleus posterioris periventricularis (NPPv, Figs. 1D, 2D). In the hypothalamus, NPY-ir perikarya appear in the nucleus recessus posterioris (NRP), around the posterior recess (Figs. 1E, 3A). The presence of blood vessels in the proximity of these NPY cells can be observed in Figure 3A. Medium-to-large sized NPY-ir cell bodies were found in the lateral zone of the nucleus of the medial longitudinal fasciculus (Figs. 1F, 3D). In the mesencephalon, small granular NPY-ir cell bodies were observed in the periventricular gray zone (PGZ) of the optic tectum (Figs. 1F–H, 3B) and in the torus longitudinalis (Figs. 1F, 3C). The most caudal NPY-ir

TABLE 1. Abbreviations for figures

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
NLTi, nucleus lateralis tuberis pars inferioris
NLTlr, nucleus lateralis tuberis pars lateralis rostral
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
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
NT, nucleus taenia
nTE, nucleus eminentia thalami
nVI, nucleus nervi abducentis
OB, olfactory bulbs
OC, optic chiasm
OIN, olfactory nerve fibers
P, pituitary
PCo, posterior commissure
PG, periventricular granular cell mass
pgd, nucleus periglomerulosus dorsalis
PGZ, periventricular grey zone
PLl, nucleus perilemniscularis pars lateralis
PLL, posterior lateral line nerve
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
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 fibers
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
TS, torus semicircularis
TSc, torus semicircularis pars centralis
TSl, torus semicircularis pars lateralis
TSld, torus semicircularis pars lateralis dorsal
TSlv, torus semicircularis pars lateralis ventral
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

perikarya appear in the isthmic region, at the transition between the mesencephalon and the rhombencephalon. These large NPY cells are confined within the locus coeruleus (Figs. 1H, 3E).

NPY-ir fibers are also widely distributed in the brain of the Senegalese sole. In the olfactory bulbs, we found only a few NPY-ir fibers in the ICL and ECL (Fig. 1A). The dorsal telencephalon exhibits numerous ir axons, particu-

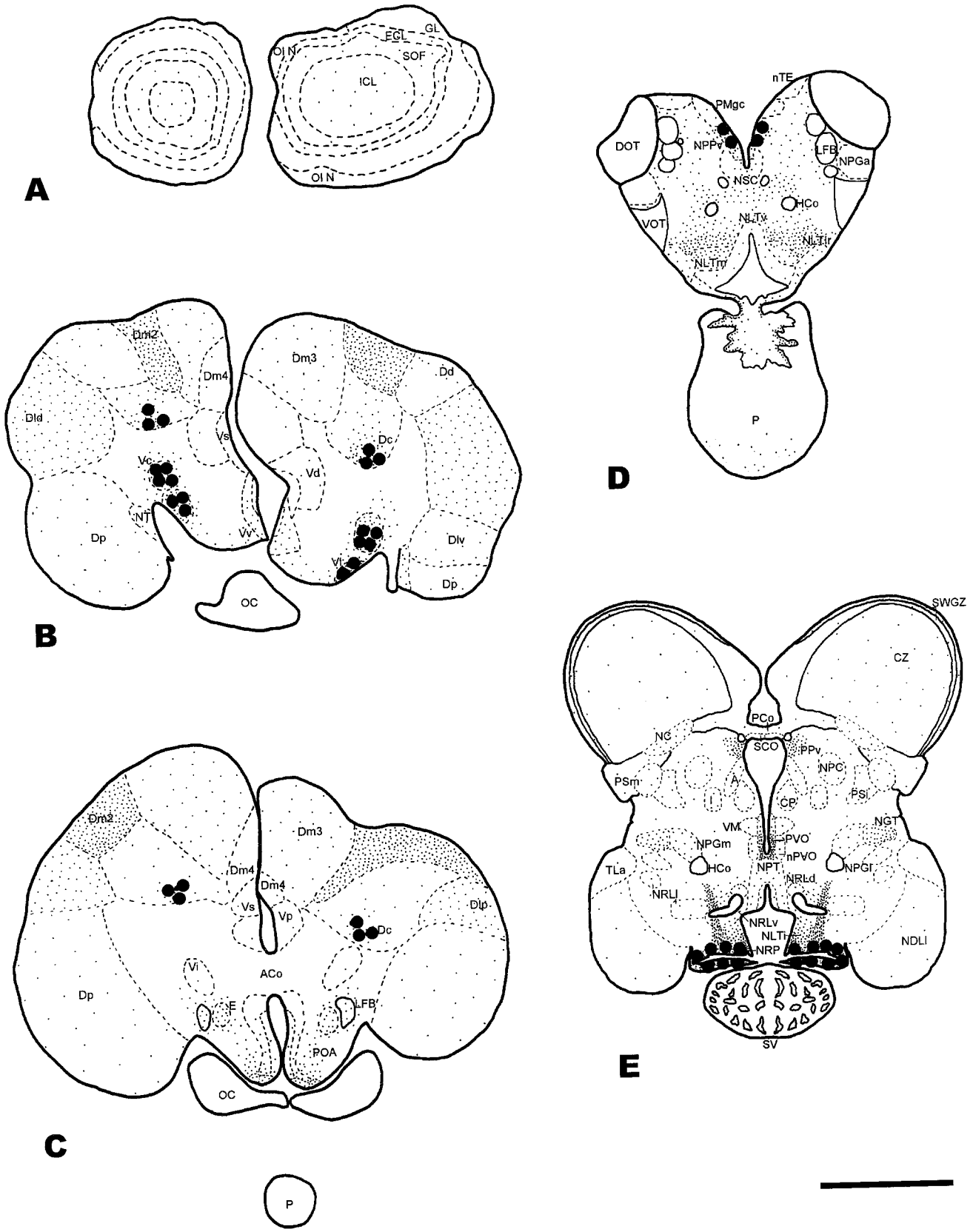
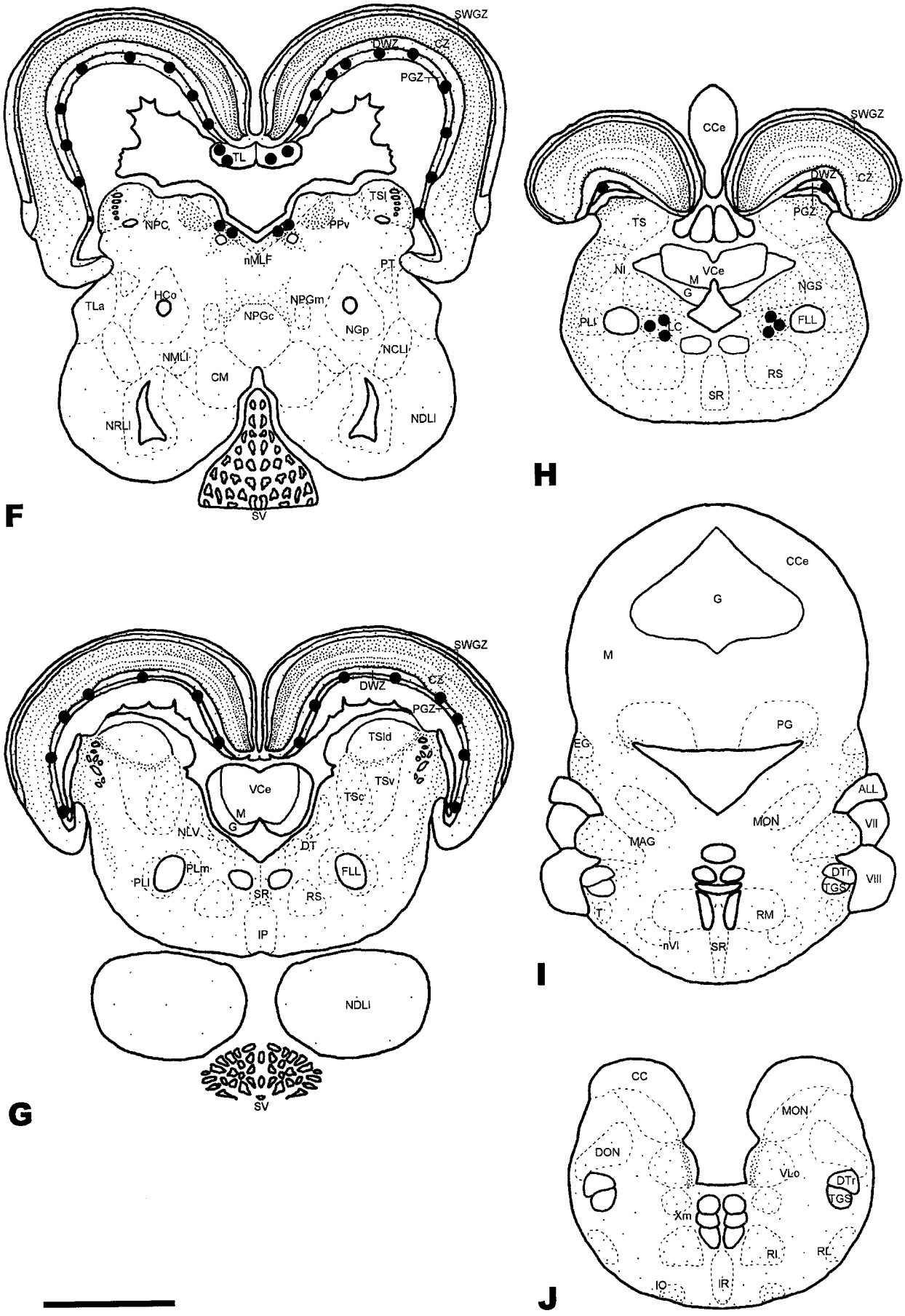


Fig. 1. Series of transverse sections through the brain of *Solea senegalensis*, from rostral to caudal, showing the distribution of neuropeptide Y-immunoreactive cells (large filled circles) and fibers (small dots). A constitutes the rostralmost section and J the caudalmost one. Scale bar = 1 mm. For abbreviations see Table 1.


SWGZ
DWZ
CZ
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TL
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NPC
PPv
nMLF
PT
HCo
TLa
NPGm
NPGc
NGp
NCLi
NMLi
CM
NRLi
NDLi
SV
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DWZ
CZ
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Figure 1

larly in the dorsomedial subdivision (Dm2, Figs. 1B, 1C), but also in the Dld (Fig. 1B). In the ventral telencephalon, numerous NPY-ir fibers appear concentrated around Vc, Vl and the nucleus entopeduncularis (E), and in general, around LFB (Figs. 1B,C, 2A,B). NPY-ir fibers were also observed in the preoptic area, especially around NPPv (Figs. 1D, 2D), whereas in the hypothalamus most NPY-ir axons appear in different subdivisions of the nucleus lateralis tuberis and around the posterior recess (Figs. 1D,E, 2C, 3A). Only a few ir terminals, however, were observed in the inferior lobes of the hypothalamus (Figs. 1E–G). Labeled fibers in the ventral hypothalamus were traced into the neurohypophysis, arriving at the proximal pars distalis (Figs. 1D, 2C) and pars intermedia. We have also found important plexuses of NPY-ir fibers in the periventricular pretectum, as well as in the preglomerular complex (Figs. 1E, 1F). The optic tectum contains a large number of NPY-ir fibers, especially in the central zone (Figs. 1E–H, 3B). In the synencephalon, we have identified NPY-ir fibers around nMLF, that seem to originate in NPY-ir cell bodies of the same nucleus (Fig. 3D). In the mesencephalic tegmentum, the torus semicircularis exhibits an important NPY-ir innervation (Figs. 1F–H) as also occurs around the locus coeruleus (Fig. 1H). Finally, the rhombencephalon exhibits abundant NPY-ir axons, that become more evident in viscerosensory areas (Figs. 1G–J), whereas the cerebellum contains only NPY-ir fibers in the eminentia granularis and periventricular granular zone of the lobus vestibulolateralis (Fig. 1I).

DISCUSSION

Our results demonstrate that NPY-like substance is widely distributed in the brain of the Senegalese sole, *Solea senegalensis*, with the highest density found in the forebrain, especially in the ventral telencephalon and hypothalamus.

In contrast to results obtained in goldfish (Pontet et al., 1989), *Polypterus senegalus* (Reiner and Northcutt, 1992) and ayu (Chiba et al., 1996), NPY-like cells were not found in the olfactory bulbs, and only a few positive fibers were identified in ICL and ECL of *Solea senegalensis*. NPY-ir cells were also observed in the nucleus olfactorius retinalis or the ganglion of nervus terminalis of cloudy dogfish (Chiba and Honma, 1992a), bichir (Chiba, 1997a), platyfish (Magliulo-Cepriano and Schreibman, 1993), killifish (Subhedar et al., 1996), ayu (Chiba et al., 1996) and masu salmon (Chiba, 1997b). The ganglion cells of the terminal nerve, however, did not exhibit NPY immunoreactivity in the sole. In teleosts, these neurons and fibers have been reported to contain both GnRH and FMRFamide (Schreibman et al., 1984; Kah et al., 1986; Grober et al., 1987; Subhedar and Rama-Krishna 1988; Rama-Krishna and Subhedar, 1992; Rodríguez-Gómez et al., 1999) and seem to represent an important neuroendocrine/neuromodulator area in fishes.

The NPY-containing neurons in the ventral telencephalon (Vl, Vc) constitute the major component of the NPY-system in the Senegalese sole. In the ventral telencephalon, NPY-ir cells were also identified in Vl of *Salmo salar* and *Gambussia affinis* (García-Fernández et al., 1992), *Xiphophorus maculatus* (Magliulo-Cepriano and Schreibman, 1993), *Clarias gariepinus* (Zandbergen et al., 1994), *Carassius auratus* (Pontet et al., 1989; Peng et al., 1994) and *Fundulus heteroclitus* (Subhedar et al., 1996). In turn, the Vc also contains NPY-ir cells in *Salmo* and *Gambussia* brains (García-Fernández et al., 1992) and NPY-ir cells were also observed in the ventral telencephalon of *Acipenser transmontanus* (Chiba and Honma, 1994). NPY-like-ir neurons, however, were not reported in Vl or Vc of other teleosts (Danger et al., 1991; Magliulo-Cepriano and Schreibman, 1993; Zandbergen et al., 1994; Chiba et al., 1996) or they were reduced in number (Magliulo-Cepriano and Schreibman, 1993). It is probable that NPY-expressing cells associated with the ventrolateral surface of the telencephalon, belonging to the nucleus entopeduncularis of different species (Pontet et al., 1989; Danger et al., 1991; Magliulo-Cepriano and Schreibman, 1993; Peng et al., 1994; Zandbergen et al., 1994; Chiba et al., 1996; Subhedar et al., 1996), actually represent Vc or Vl cells, according to the cytoarchitectonic criteria of Northcutt and Davis (1983). It should be noted that the Vl of teleosts has been homologized to the olfactory tubercles of higher vertebrates (Northcutt, 1995), in which abundant NPY-ir cell bodies have also been described (Chronwall et al., 1985). In goldfish, a high degree of co-location of NPY and somatostatin was noted in Vl and entopeduncular neurons (Pickavance et al., 1992).

In the dorsal telencephalon, Dc is the only subdivision that shows NPY-ir cells in the Senegalese sole. These positive neurons were also described in *Salmo salar* (García-Fernández et al., 1992) and goldfish (Pickavance et al., 1992), in which a co-location of NPY with somatostatin has also been observed. Further, this cell group seems to correspond to the area dorsalis telencephali, pars centralis dorsalis of *Fundulus heteroclitus*, that also exhibits NPY-ir cells (Subhedar et al., 1996).

In the preoptic area, the nucleus posterioris periventricularis of Senegalese sole also exhibited NPY-ir neurons. Similar NPY-ir cells were also identified in the preoptic area of goldfish (Pontet et al., 1989; Peng et al., 1994), Senegal bichir (Reiner and Northcutt, 1992; Chiba, 1997a) and platyfish (Magliulo-Cepriano and Schreibman, 1993). The presence of this neuropeptidergic cell population, however, does not appear to be a conserved characteristic of ray-finned fishes because it seems to be absent in many other species (García-Fernández et al., 1992; Vecino and Ekström, 1992; Chiba and Honma, 1994; Chiba et al., 1996; Subhedar et al., 1996). In the Senegalese sole, as in goldfish (Kah et al., 1984), the nucleus posterioris periventricularis also contains catecholaminergic cells (Rodríguez-Gómez et al., 2000b), whereas a co-localization of NPY and GABA has been described in the preoptic area of the African lungfish (Trabucchi et al., 2000).

The neurohypophysis of *Solea senegalensis* showed intense NPY immunoreactivity. Danger et al. (1991) suggested that NPY might regulate the endocrine secretion of adenohypophyseal cells, acting at both hypothalamic and pituitary levels. In support of the hypophysiotropic functions of NPY in fish, it has been observed that high concentrations of NPY binding sites occur in the adenohypophysis of the lungfish *Protopterus annectens* (Vallarino et al., 1998). Different morphofunctional and physiological studies in rainbow trout (Breton et al., 1989, 1990; Danger et al., 1991) and goldfish (Kah et al., 1989; Pontet et al., 1989; Peng et al., 1990, 1993a–c) indicate NPY stimulatory actions on GTH and GH release. In *Solea senegalensis*, NPY-ir fibers entering the neurohypophysis reach the proximal pars distalis of the adenohypophysis, where GTH and GH cells are found (Rendón et al., 1997), sug-

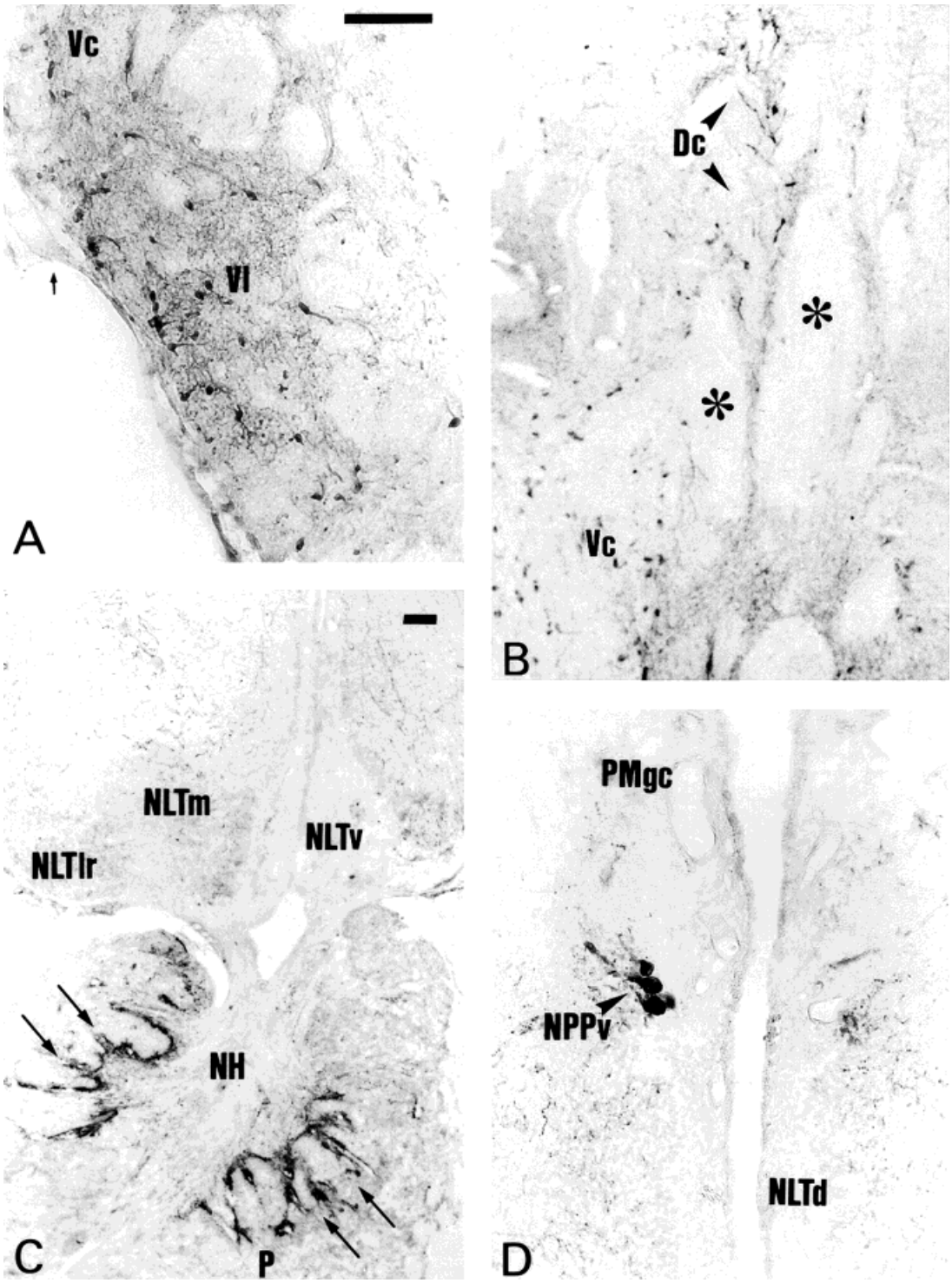

Fig. 2. Photomicrographs of transverse brain sections of *Solea senegalensis*, showing NPY-ir cells and fibers. **A:** Central (Vc) and lateral (Vl) parts of the ventral telencephalon. **B:** NPY-ir cells in the central zone of the dorsal telencephalon (Dc, arrowheads) and in the central zone of the ventral telencephalon (Vc). Asterisks mark fibers tracts. **C:** NPY-ir fibers in the nucleus lateralis tuberis of the ventral hypothalamus and entering the hypophysis (arrows). **D:** NPY-ir cells (arrowheads) in NPPv of the preoptic area. For abbreviations see Table 1. Scale bar = 100 μm.

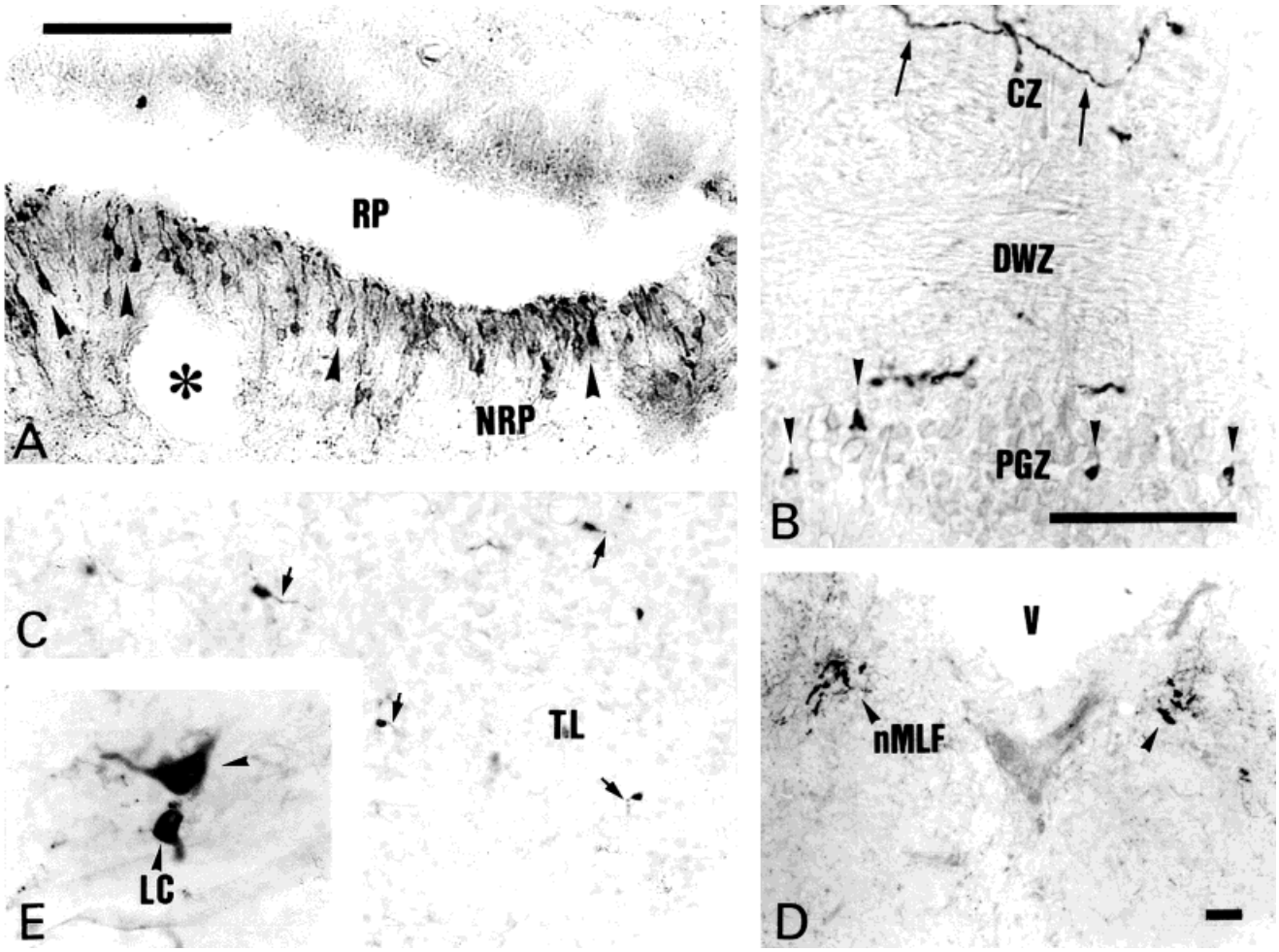

Fig. 3. Photomicrographs of transverse brain sections of *Solea senegalensis*, showing NPY-ir cells and fibers. **A:** Positive cerebrospinal fluid-contacting cells (arrowheads) in the nucleus recessus posterioris of the hypothalamus. Asterisk marks a blood vessel in the proximity of NPY-ir cells. **B:** NPY-ir perikarya (arrowheads) in the periventricular gray zone of the optic tectum. The presence of NPY-ir fibers (arrows) in the central zone is also shown. **C:** Small NPY-ir cells in the torus longitudinalis (arrows). **D:** NPY-ir cells and fibers in the lateral zone of the nucleus of the medial longitudinal fasciculus. **E:** Large NPY-ir cells in the locus coeruleus. Scale bar = 100 μm. For abbreviations see Table 1.

gesting a possible role for NPY in the regulation of GTH and GH secretion. A direct effect of NPY on gonadotropin release is not evident, however, in platyfish (Magliulo-Cepriano and Schreibman, 1993) and catfish (Zandbergen et al., 1994) because their GTHs cells do not receive NPY-ir innervation. The occurrence of NPY-immunoreactive fibers in the pars intermedia of sole suggests that NPY may be involved in the control of melanotropin secretion. Actually, NPY has been shown to inhibit the activity of melanotrope cells in the frog *Rana ridibunda* (Danger et al., 1986, 1987; Chartrel et al., 1991; Valentijn et al., 1994) and in the toad *Xenopus laevis* (Verburg van Kemenade et al., 1987). Thus, a role of NPY in melanotrope cell activity and skin color adaptation in the sole, that are very important for its survival in benthonic habitats, cannot be discarded.

In the ventral hypothalamus of *Solea senegalensis*, cerebrospinal fluid (CSF)-contacting NPY-ir cells were observed in the nucleus recessus posterioris, that also contains abundant NPY-ir fibers. According to our results, immunoreactive CSF-contacting cells and fibers were also detected in the NRP of *Acipenser transmontanus* (Chiba and Honma, 1994). The presence of CSF-contacting NPY-ir neurons has been reported in the diencephalon of the arctic lamprey (Chiba and Honma, 1991), scyliorhinid dogfish (Vallarino et al., 1988; Chiba and Honma, 1992a), bichir (Chiba, 1997a), killifish (Subhedar et al., 1996) and in some tetrapods (Perroteau et al., 1988; Medina et al., 1992), but not in the hagfish (Chiba et al., 1993), goldfish (Pontet et al., 1989) or trout (Danger et al., 1991). These cells seem to be involved in monitoring the homeostatic state of the organism, taking chemical information from CSF or secreting NPY or a related molecule into CSF. Furthermore, the presence of blood vessels in the proximity of NPY-ir cells is a common feature of the NRP in Senegalese sole. Together, this evidence suggests that NPY-ir cells of NRP could be implicated in the control of metabolic processes and food intake in the Senegalese sole.

In this species, the synencephalon shows NPY-like-ir cells in the lateral zone of the nMLF. In fishes, there is some controversy on the distribution of NPY-ir cells in this brain area. Thus, whereas similar ir neurons have been described in green molly (Batten et al., 1990), *Salmo salar* (Vecino and Ekström, 1992; García-Fernández et al., 1992), *Gambusia affinis* (García-Fernández et al., 1992) and ayu (Chiba et al., 1996), they seem to be absent in goldfish (Pontet et al., 1989), killifish (Subhedar et al., 1996), white sturgeon (Chiba and Honma, 1994), and cloudy dogfish (Chiba and Honma, 1992a).

In the Senegalese sole, the granular cells of the torus longitudinalis also exhibit NPY immunoreactivity. To our knowledge, similar NPY-ir neurons have not been reported previously. These cells are smaller in size but have a similar appearance to the NPY cells of the optic tectum. This result might reinforce a common embryonic origin of granular cells in PGZ and torus longitudinalis in fishes. On the other hand, the torus semicircularis complex displayed an important plexus of NPY-ir fibers, but did not show NPY-ir cells, as has also been described in *Cyprinus carpio* (Cuadrado and Coveñas, 1993).

In the Senegalese sole, the cerebellum is devoid of NPY-ir cells, but NPY-ir fibers can be observed in the lobus vestibulolateralis. Although the presence of NPY-ir cells has been described in the cerebellum of dogfish (Chiba and Honma, 1992a), similar observations have not been reported in teleosts (Pontet et al., 1989; García-Fernández et al., 1992; Chiba et al., 1996).

The locus coeruleus is the only nucleus in the brainstem of the Senegalese sole possessing NPY-ir cells. This cell group is also a source of NPY in goldfish (Pontet et al., 1989; Vecino et al., 1994), *Salmo salar* (García-Fernández et al., 1992; Vecino and Ekström, 1992; Peng et al., 1994) and *Gambusia affinis* (García-Fernández et al., 1992). The locus coeruleus has also been reported as a noradrenergic nucleus, not only in teleosts but also in most vertebrates (Smeets and Reiner, 1994). In the Senegalese sole, TH-ir neurons have also been described in this nucleus (Rodríguez-Gómez et al., 2000b). In mammals, the NPY system coexists with noradrenaline, both in the central and peripheral nervous system (Everitt et al., 1984). In the rhombencephalon, NPY-ir fibers are also abundant, especially in viscerosensory areas such as the glossopharyngeal and vagal lobes. These areas represent primary gustatory centers (Wullimann, 1998), supporting a possible role for NPY in the control of feeding in the Senegalese sole.

In conclusion, this study shows the precise distribution of NPY-immunoreactive systems in the brain of the Senegalese sole. The wide distribution of NPY-ir structures in the brain and hypophysis of this species suggests that NPY may be involved in the regulation of different physiological functions, including reproductive, metabolic and color adaptation processes. In fact, NPY-ir fibers arrive to the proximal pars distalis and pars intermedia of Senegalese sole, that contains GTH, GH and MSH cells. The anatomical location of NPY in the Senegalese sole brain will provide useful information for the study of seasonal variations of NPY and putative interactions of NPY with other endocrine factors (e.g., GnRH, catecholamines, gonadal steroids) in the regulation of metabolic and reproductive processes in this species.

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