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Distribution of Galanin-Like Immunoreactive Elements in the Brain of the Adult Lamprey *Lampetra fluviatilis*

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ABSTRACT

Galanin is a brain-gut peptide present in the central nervous system of vertebrates and invertebrates. The distribution of galanin-like immunoreactive perikarya and fibers in the brain of the river lamprey *Lampetra fluviatilis* (Agnatha) has been studied immunocytochemically by using antisera against rat and porcine galanin. Galanin-like immunoreactive perikarya were seen in the telencephalon and mediobasal diencephalon. In the telencephalon, they were present in the nucleus olfactorius anterior, nucleus basalis, and especially, in the nucleus commissurae anterioris. The diencephalon contained most of the immunoreactive neurons. They were located in the nucleus commissurae praeinfundibularis, nucleus ventralis hypothalami, nucleus commissurae postinfundibularis, nucleus ventralis thalami, and nucleus dorsalis thalami pars medius. Most of the galanin-like immunoreactive infundibular neurons showed apical processes contacting the cerebrospinal fluid. Immunoreactive fibers and terminals were widely distributed throughout the neuraxis. In the telencephalon, the richest galaninergic innervation was found in the nucleus olfactorius anterior, lobus subhippocampalis, corpus striatum, and around the nucleus septi and the nucleus praeopticus. In the diencephalon, the highest density of galanin-like immunoreactive fibers was seen in the nucleus commissurae postopticae, nucleus commissurae praeinfundibularis, nucleus ventralis hypothalami, nucleus dorsalis hypothalami, and neurohypophysis. In the mesencephalon and rhombencephalon, the distribution of immunoreactive fibers was heterogeneous, being most pronounced in a region between the nucleus nervi oculomotorii and the nucleus interpeduncularis mesencephali, in the nucleus isthmi, and in the raphe region. A subependymal plexus of immunoreactive fibers was found throughout the ventricular system. The distribution of immunoreactive neurons and fibers was similar to that of teleosts but different to those of other vertebrate groups. The possible hypophysiotropic and neuroregulatory roles of galanin are discussed.

Indexing terms: **immunocytochemical study, hypothalamus, neurohypophysis, cerebrospinal fluid-contacting neurons, Agnatha**

Galanin (GAL) is a 29-residue peptide that was originally isolated by Tatemoto et al. (1983) from the porcine intestine. Later it was shown that GAL is present in the central nervous system (CNS) of vertebrates. Immunocytochemical studies have revealed galanin-like immunoreactive (GAL-ir) elements in the CNS of mammals, including man (Skofitsch and Jacabowitz, 1985; Melander et al., 1986; Levin et al., 1987; Palkovits et al., 1987; Blasco et al., 1989; Gaymann and Martin, 1989; Gentleman et al., 1989; Walker et al., 1989; Kordower and Mufson, 1990; Meister et al., 1990; Elmquist et al., 1992; Kordower et al., 1992), and in nonmammalian species, such as the dogfish (Vallarino et al., 1991), teleosts (Batten et al., 1990a,b; Cornbrooks and

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Parsons, 1991a,b; Holmqvist and Ekström, 1991; Olivereau and Olivereau, 1991; Yamamoto et al., 1992; Magliulo-Cepriano et al., 1993; Anglade et al., 1994), amphibians (Wolfbauer and Skofitsch, 1989; Lázár et al., 1991; Olivereau and Olivereau, 1992), reptiles (Jiménez et al., 1994) and birds (Józsa and Mess, 1993). In addition, GAL-ir elements have been found in the nervous system of invertebrates (Lundqvist et al., 1991). In mammals, anatomical (Holmqvist and Carlberg, 1992) and physiological (Ottlecz et al., 1986; Koshiyama et al., 1987; Murakami et al., 1987; Sahu et al., 1987; López et al., 1991) studies led to the proposal that GAL is a hypophysiotropic hormone. In the hypothalamus of mammals, GAL colocalizes with growth hormone releasing factor (GRF; Meister et al., 1990), vasopressin (Gai et al., 1990) and oxytocin (Landry et al., 1991). In fish, no such colocalization has been observed (Holmqvist and Ekström, 1991). Tracing studies demonstrated that in the rat, nearly 50% of the neurons of the hypothalamo-neurohypophyseal system contained immunoreactive GAL (Arai et al., 1990). Galanin has been shown to be involved in nociceptive reception (Skofitsch and Jacobowitz, 1985; Wiesenfeld-Hallin et al., 1990), learning and memory (Melander and Staines, 1986; Chan-Palay, 1988; Senut et al., 1989), and in cognitive, sensory, motor and autonomic processes (see Rökaeus, 1987; Kordover and Mufson, 1990). The list of probable functions involving GAL can even be bigger. Since the differentiation of the galaninergic system occurs early in the developing mammalian brain, a role of GAL in CNS differentiation has also been suggested (Sizer et al., 1990; Elmquist et al., 1992). The role of GAL in the CNS of nonmammalian vertebrates is poorly understood. In fish, GAL has been implicated in olfactory/gustatory functions, central visual processing, somatosensory transmission, osmoregulation, and sex-specific behaviors (Cornbrooks and Parsons, 1991b; Holmqvist and Carlberg, 1992).

Cyclostomes are representatives of the most primitive vertebrate group, the Agnatha. Due to this crucial position, the study of the galaninergic system in this group is of great phylogenetic interest. The present investigation was undertaken to study the distribution of GAL immunoreactivity in the brain of a cyclostome, the river lamprey (*Lampetra fluviatilis*).

MATERIALS AND METHODS

Animals

Investigation was performed on six adult (20–35 cm) river lampreys (*Lampetra fluviatilis* L.). Animals were captured in the spring in Söderhamn (Sweden) during their upstream migratory phase, and kept in aerated freshwater aquaria at 4–10°C, with a 12 hour:12 hour light:dark cycle (illumination between 08:00 and 20:00). Four animals were used to obtain transverse sections and two for sagittal sections. All brains used in this study were fixed in July, during the terminal spawning phase.

Immunocytochemical procedure

The animals were anaesthetized with tricaine methanesulphonate (MS-222; 100 mg/1; Sigma, Madrid, Spain) and killed by decapitation. The brains were quickly removed

Abbreviations

AH	adenohypophysis	NP	nucleus praetectalis
C	cerebellar plate	os	optic stratum (tectum opticum)
CGL	corpus geniculatum laterale	P	pineal organ
CSF	cerebrospinal fluid	PP	parapineal organ
cc	commissura cerebelli	pch	plexus choroideus
ch	chiasma opticum	ps	periventricular stratum (tectum opticum)
cp	commissura posterior	Ra	raphe region
cpt	commissura postectalis	Rm	mesencephalic reticular area
cs	central stratum (tectum opticum)	Rr	rhombencephalic reticular area
D1	ganglion habenula dexter	ri	recessus infundibuli
D2	ganglion habenula sinister	rpo	recessus postopticus
D4	nucleus dorsalis thalami	rpos	recessus posterior
D4m	nucleus dorsalis thalami pars medius	rpro	recessus praeopticus
D6	nucleus ventralis thalami	SCO	subcommisural organ
D7	nucleus tuberculi posterioris	ss	superficial stratum (tectum opticum)
D8	nucleus commissurae postopticae	T1	nucleus bulbi olfactorii
D9	nucleus commissurae praeinfundibularis	T2	nucleus septi
D10	nucleus ventralis hypothalami	T3	nucleus olfactorius anterior
D11	nucleus dorsalis hypothalami	T4	nucleus commissurae anterior
D12	nucleus commissurae postinfundibularis	T5	nucleus praeopticus
flm	fasciculus longitudinalis medialis	T6	primordium piriforme
fr	fasciculus retroflexus	T7	primordium hippocampi
GO	glomeruli olfactorii	T8	lobus subhippocampalis
hc	habenular commissure	T9	corpus striatum
I	nucleus isthmi	T10	primordium pallii
III	nucleus nervi oculomotorii	to	tractus opticus
IV	nucleus trochlearis	tv	commissura tegmenti ventralis
LLL	lateral line lobe	V	nucleus motorius nervi trigemini
M1	nucleus commissurae posterioris	VII	nucleus motorius nervi facialis
M2	nucleus fasciculi longitudinalis medialis	Vm	radix motorius nervi trigemini
M3	tectum opticum	Vs	radix sensorius nervi trigemini
M4	torus semicircularis	vd	ventriculus diencephali
M5	nucleus M5 of Schober	vla	ventriculus lateralis anterior telencephali
M6	nucleus interpeduncularis	vm	ventriculus mesencephali
NB	nucleus basalis	vr	ventriculus rhombencephali
NH	neurohypophysis	vt	ventriculus medius telencephali
NL	nucleus lentiformis mesencephali	X	nucleus vagus

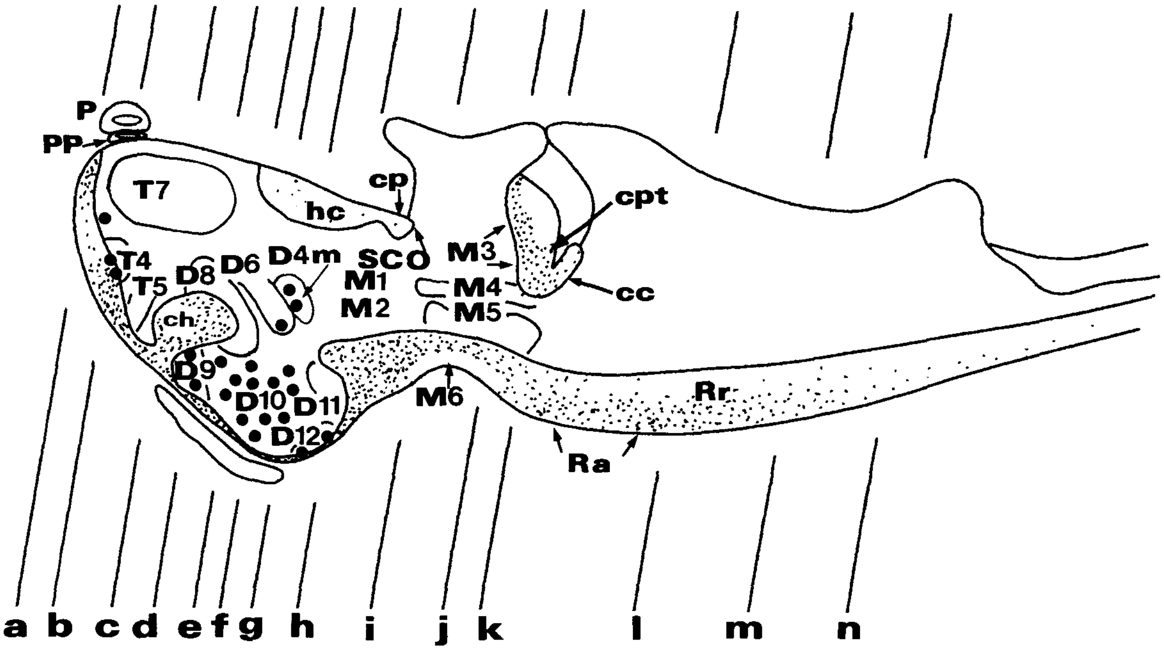

Fig. 1. Schematic drawing illustrating a sagittal view of the brain of the adult lamprey *Lampetra fluviatilis* showing the localization of GAL-ir perikarya (dark circles) and fibers (stippled regions). Levels a to n correspond to transverse sections shown in Figure 2.

and fixed by immersion in Bouin's fluid for 48 hours. Then they were dehydrated and embedded in paraffin. Sagittal and transverse (8-μm-thick) sections were hydrated and immunostained according to the PAP method of Sternberger (1986), using an anti-GAL serum raised in rabbit against porcine GAL (AB 1985, Chemicon, New York, NY; dilution 1:1,500). In addition, for two specimens, parallel sections were incubated with two other antisera against porcine GAL (s-1589; dilution 1:400) and rat GAL (s-1591; dilution 1:400) kindly supplied by Tomas Hökfelt (Stockholm, Sweden). Sections were sequentially incubated in anti-GAL sera for 18 hours, secondary antiserum (obtained in the laboratory of Málaga; dilution 1:40) for 30 minutes, and the PAP complex (Dako, Copenhagen, Denmark; dilution 1:100) for 30 minutes. As electron donor, 0.04% 3.3′ diaminobenzidine tetrahydrochloride (Sigma) was used. It was dissolved in Tris buffer containing 0.04% ammonium nickel sulphate hexahydrate (Fluka, Buchs, Switzerland). All incubations were performed at 22°C under gentle agitation.

All antisera and the PAP complex were diluted in Tris buffer, pH 7.8, containing 0.7% nongelling seaweed gelatin, lambda carrageenan (Sigma), 0.5% Triton X-100 (Sigma) and 0.02% sodium azide. Coplin jars were used for the incubation in the second antibody, whereas incubation for the first antisera and the PAP complex was carried out in a moist chamber.

Control test

To test the immunocytochemical procedure, incubation in the first antiserum was omitted. To verify the specifity of the immunoreaction, the anti-GAL serum (Chemicon) was preabsorbed with porcine GAL (Sigma G 5773). Two aliquots of the diluted antiserum (1:1,500) were mixed with 10 and 20 μg/ml GAL, respectively, for 18 hours at 22°C. The immunoabsorbed antiserum was used as primary antisera on sections adjacent to those submitted to immunostaining with the nonabsorbed anti-GAL. Neither the use of immunoabsorbed anti-GAL nor immunocytochemistry omitting the primary antiserum revealed any stained structures in the sections.

In addition, due to a possible cross-reactivity of the anti-GAL serum with other related peptides, preabsorption with substance P (Sigma S 6883) and physalaemin (Sigma P 2149) was performed for the anti-porcine GAL serum (Chemicon). Immunostaining (intensity and distribution) was not affected by preabsortion either with substance P or physalaemin.

Nomenclature

The nomenclature was according to Schober (1964), in the adult river lamprey *Lampetra fluviatilis*.

RESULTS

Immunoreactive neurons were found in the basal telencephalon and the mediobasal diencephalon. Immunoreactive fibers were widely distributed throughout the telencephalon, diencephalon (hypothalamic and extrahypothalamic areas), mesencephalon and rhombencephalon. The anatomical localization of immunoreactive perikarya and fibers is schematically illustrated in sagittal (Fig. 1) and representative transverse sections (Fig. 2) through the brain of *Lampetra fluviatilis*.

Telencephalon

Perikarya. In the telencephalon, few isolated GAL-ir cell bodies were found in the nucleus basalis (NB) and the nucleus olfactorius anterior (T3) among loosely arranged

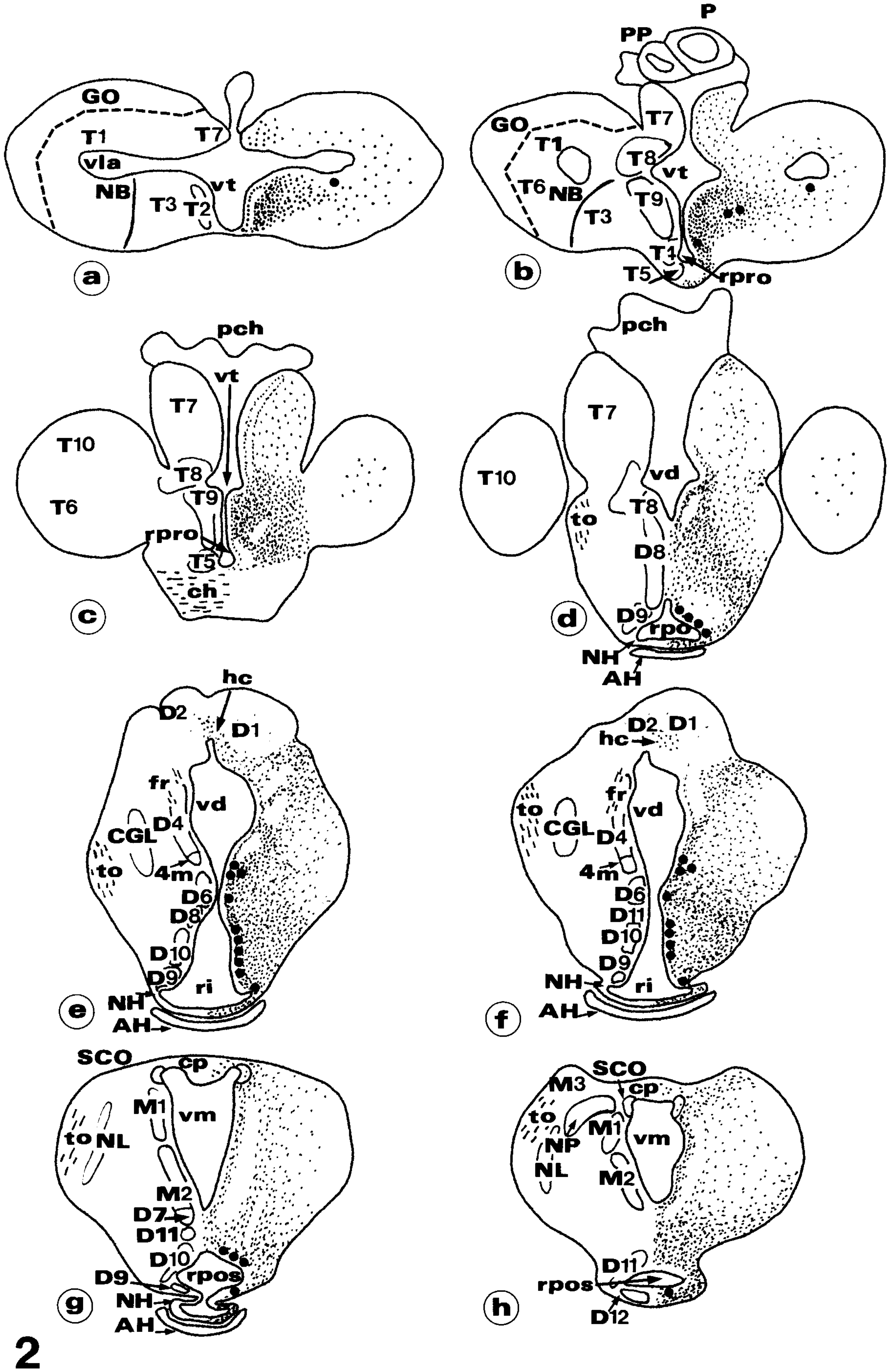

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Fig. 2. Schematic drawing of successive rostrocaudal transverse sections (**a–n**) through the levels indicated in Figure 1. Immunoreactive perikarya (dark circles) and fibers (stippled regions) are represented.

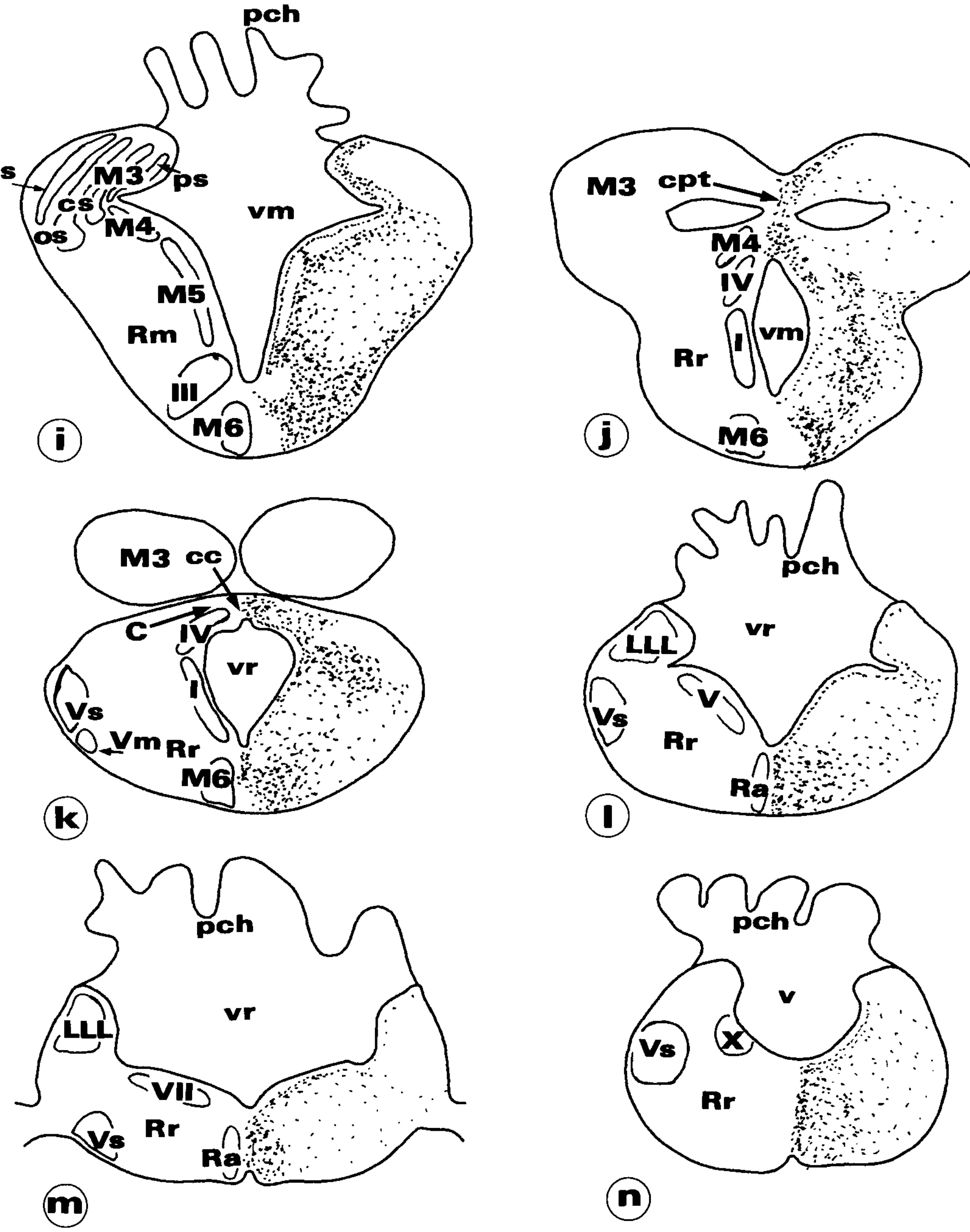

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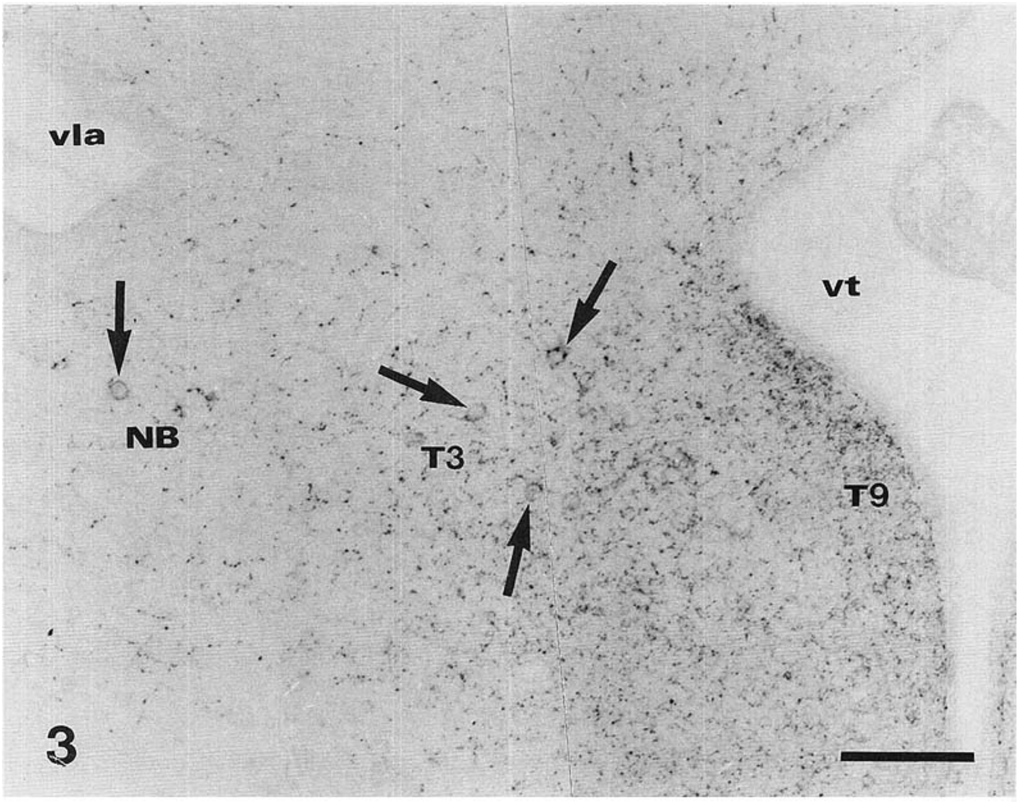
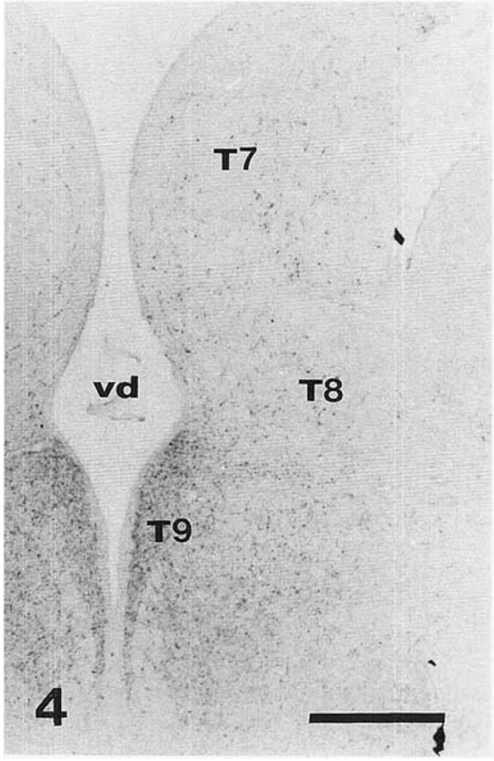
Figure 2 (Continued.)

immunoreactive fibers (Figs. 2a,b, 3). These cells were round and showed a weak immunoreaction. In the nucleus commissurae anterioris (T4; Fig. 2b) many GAL-ir neurons were found in the ependymal and subependymal layers; some of them contacted the cerebrospinal fluid (CSF) of the brain ventricle (recessus praeopticus) by means of an apical process.

Fibers. In the rostral forebrain, the nucleus olfactorius anterior was strongly innervated by GAL-ir fibers (T3; Figs. 2a,b, 3). These immunoreactive fibers outlined the nucleus septi (T2; Fig. 2a), that was almost free of fibers, and reached the subependymal neuropil of the ventriculus medius telencephali. No immunopositive fibers innervated the glomeruli olfactorii (GO; Fig. 2a,b), and only a few GAL-ir fibers were detected in the nucleus bulbi olfactorii (T1; Fig. 2a). Galanin-immunoreactive fibers were abundant in the corpus striatum (T9) but scarce in the lobus subhippocampalis (T8; Figs. 2b–d, 3, 4). Dorsally, the primordium hippocampi (T7; Fig. 3a–d) showed scarce immunoreactive fibers forming a subependymal network. Scattered GAL-ir fibers were found in the primordium piriforme (T6) and primordium pallii (T10), around the





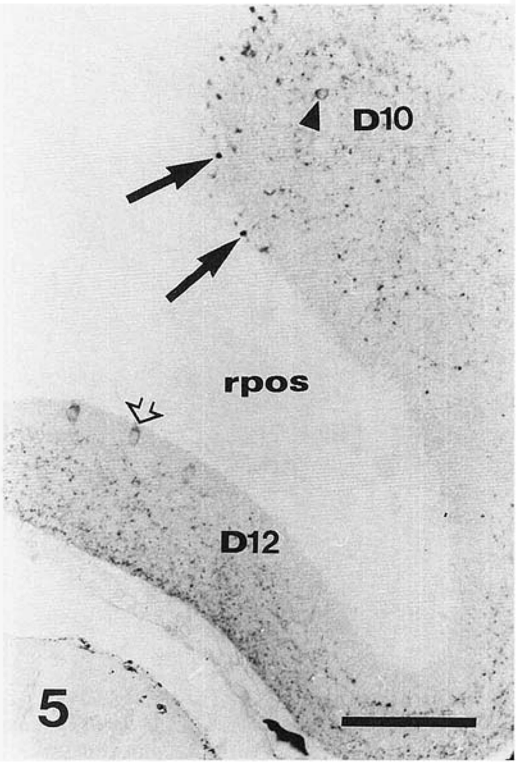


Fig. 3. Transverse section through the anterior telencephalon showing some weakly labeled neurons (arrows) in the nucleus basalis (NB) and the nucleus olfactorius anterior (T3). A dense innervation by immunoreactive fibers is observed in the corpus striatum (T9). Scale bar: 200 μm.

Fig. 4. Survey of a transverse section at the level of Figure 2c. The corpus striatum (T9) shows a dense innervation by GAL-ir fibers. Few fiber profiles are seen in the primordium hippocampi (T7) and the lobus subhippocampalis (T8). Scale bar: 400 μm.

Fig. 5. Sagittal section through the recessus posterior (rpos). The nucleus ventralis hypothalami (D10) shows immunoreactive perikarya (arrowhead). Note bleb endings protruding the ventricle (arrows). The nucleus commissurae postinfundibularis (D12) shows a dense network of fibers and some immunoreactive perikarya located among the ependyma (open arrow). Scale bar: 200 μm.

ventriculus lateralis anterior telencephali (Fig. 2b–d). The area occupied by cell bodies in the nucleus praeopticus (T5) and the commissura anterior were devoid of GAL-ir fibers (Fig. 2b,c).

Diencephalon

Perikarya. The hypothalamus contained most of the GAL-ir neurons in the lamprey brain. Around the recessus postopticus, the nucleus commissurae praeinfundibularis (D9; Figs. 2d–f, 9) showed immunoreactive fusiform cell bodies arranged in 3–4 layers parallel to the ventricle. These cells had their long axis perpendicular to the ependyma and displayed an apical process that reached and protruded into the ventricle, and a long basal process that coursed deep into the lateral neuropil. The apical process passed through a dense subependymal network of GAL-ir fibers (Fig. 9). Some pear-shaped immunoreactive perikarya were also found in the ependymal layer.

The most conspicuous population of GAL-ir cell bodies was found in the nucleus ventralis hypothalami (D10; Figs. 2e–g, 5–7). Immunoreactive cells were located among or beneath the ependyma, arranged into 3–5 layers parallel to the recessus infundibuli. Perikarya were bipolar, with a cerebrospinal fluid contacting process displaying a terminal bleb, and a basal process toward the lateral area. In the nucleus dorsalis thalami pars medius (D4m; Figs. 2e,f, 6) numerous fusiform GAL-ir neurons were arranged into layers parallel to the ventricle. These cells showed immunoreactive beaded basal processes that ran toward the lateral neuropil. In the nucleus ventralis thalami (D6; Fig. 2e,f) few GAL-ir neurons were scattered among a dense immunoreactive plexus.

In the floor of the recessus posterior, the nucleus commissurae postinfundibularis (D12; Figs. 2h, 5, 6) showed few immunoreactive pear-shaped and cerebrospinal fluid-contacting GAL-ir neurons.

Fibers. The hypothalamus of the lamprey displayed a very dense innervation of GAL-ir fibers in the nucleus commissurae postopticae (D8), the nucleus commissurae praeinfundibularis (D9), the nucleus ventralis hypothalami (D10), and the nucleus dorsalis hypothalami (D11; Figs. 2d–h; 5–7; 9). A dense network of immunoreactive fibers was also present in the subependymal region and in the neuropil beneath these nuclei.

In the dorsal thalamus, the corpus geniculatum laterale (CGL) received a moderate innervation of GAL-ir fibers (Fig. 2e,f).

In the epithalamus, the habenula showed some GAL-ir fibers in its ventral part, and a few were seen to run along the habenular commissure (Fig. 2e,f). Galanin-like immunoreactive fibers were absent from the pineal and parapineal organs (Fig. 2b).

In the pretectal region, GAL-ir fibers were seen in the nucleus praetectalis (NP), crossing the commissura posterior and bordering the subcommissural organ (Figs. 2g,h, 8). Occasional GAL-ir fibers coursed among cells of the subcommissural organ (Fig. 8).

The neural lobe of the hypophysis (NH) contained abundant GAL-ir fibers, both in its anterior and posterior aspects. At variance, the adenohypophysis (AH) lacked immunoreactive fibers (Figs. 2d–g, 9).

Within the diencephalon, the main fiber tracts, such as chiasma opticum, tractus opticus, commissura postoptica and fasciculus retroflexus, were free of immunoreactive fibers.

Mesencephalon and rhombencephalon

Perikarya. Galanin-immunoreactive neurons were not detected in the mesencephalon and the rhombencephalon of the lamprey.

Fibers. Despite the absence of immunoreactive neurons, the mesencephalon and rhombencephalon showed a fairly rich innervation by GAL-ir fibers, mainly in the basal plate-derived regions. In the rostral mesencephalon, some GAL-ir fibers innervate the nucleus lentiformis mesencephali (NL; Fig. 2g,h). In the dorsal mesencephalon, the periventricular and central strata of the tectum opticum received a moderate innervation of GAL-ir fibers (Figs. 2i,j, 11). At variance, the superficial and the optic strata were devoid of label. Many immunopositive fibers were found in the commissura posttectalis and around the torus semicircularis (M4; Fig. 2i,j).

In the mesencephalon, the nuclei around the ventriculus mesencephali such as the nucleus commissurae posterioris (M1), the nucleus fasciculi longitudinalis medialis (M2), and the nucleus M5 of Schober (M5; Figs. 2g–i; 10) lacked GAL-ir fibers. However, a subependymal network of immunopositive fibers was noted in these areas. The area occupied by mesencephalic reticular cells (Müller cells) and their dendrites (Rm) showed scarce GAL-ir fibers (Fig. 2i). Immunoreactive fibers were also seen in the neuropil among the nucleus oculomotorii (III), the nucleus interpeduncularis (M6), and the fasciculus retroflexus (Figs. 2i–k, 10).

In the isthmus rhombencephali, the density of GAL-ir fibers was particularly high in the periventricular areas near to the mesencephalon and in the subependymal area at the level of the trochlear nucleus (IV; Fig. 2j,k). Some innervation was detected in the nucleus isthmi (I; Fig. 2j,k). In addition, some immunoreactive fibers were seen in the cerebellar plate and crossing the commissura cerebelli (Figs. 2k, 11).

In the rhombencephalon proper, most GAL-ir fibers distributed in the basal plate, especially in the raphe region (Ra; Fig. 2l,m). The rhombencephalic reticular area (Rr; Figs. 2j–n, 10) contained numerous GAL-ir fibers, whereas other areas such as the lateral line lobe (LLL; Fig. 2l,m) and the motor nuclei (V, VII, X and nucleus glossopharingicus) were almost devoid of GAL-ir innervation. A sparse network of GAL-ir fibers was found in the subependymal region of the ventriculus rhombencephali. Some GAL-ir fibers were also intermingled, and sometimes in close apposition with the giant descending reticular axons.

DISCUSSION

The amino acidic sequence of GAL varies among mammals (cf. Rökaeus and Brownstein, 1986; Kaplan et al., 1988) and among nonmammalian vertebrates (McKeon et al., 1990; Anglade et al., 1994). However, anti-porcine GAL antisera have been reported to cross-react with GAL of mammals, including man (Skofitsch and Jacobowitz, 1985; Melander et al., 1986; Levin et al., 1987; Palkovits et al., 1987; Blasco et al., 1989; Gaymann and Martin, 1989), of nonmammalian vertebrates (teleosts: Batten et al., 1990a,b; Cornbrooks and Parsons, 1991a,b; Holmqvist and Ekström, 1991; Olivereau and Olivereau, 1991; amphibians: Lázár et al., 1991; Olivereau and Olivereau, 1992; reptiles: Jiménez et al., 1994) and of invertebrates (Lundquist et al., 1991). The present study has been carried out using two antiporcine GAL sera and an anti-rat GAL serum in a

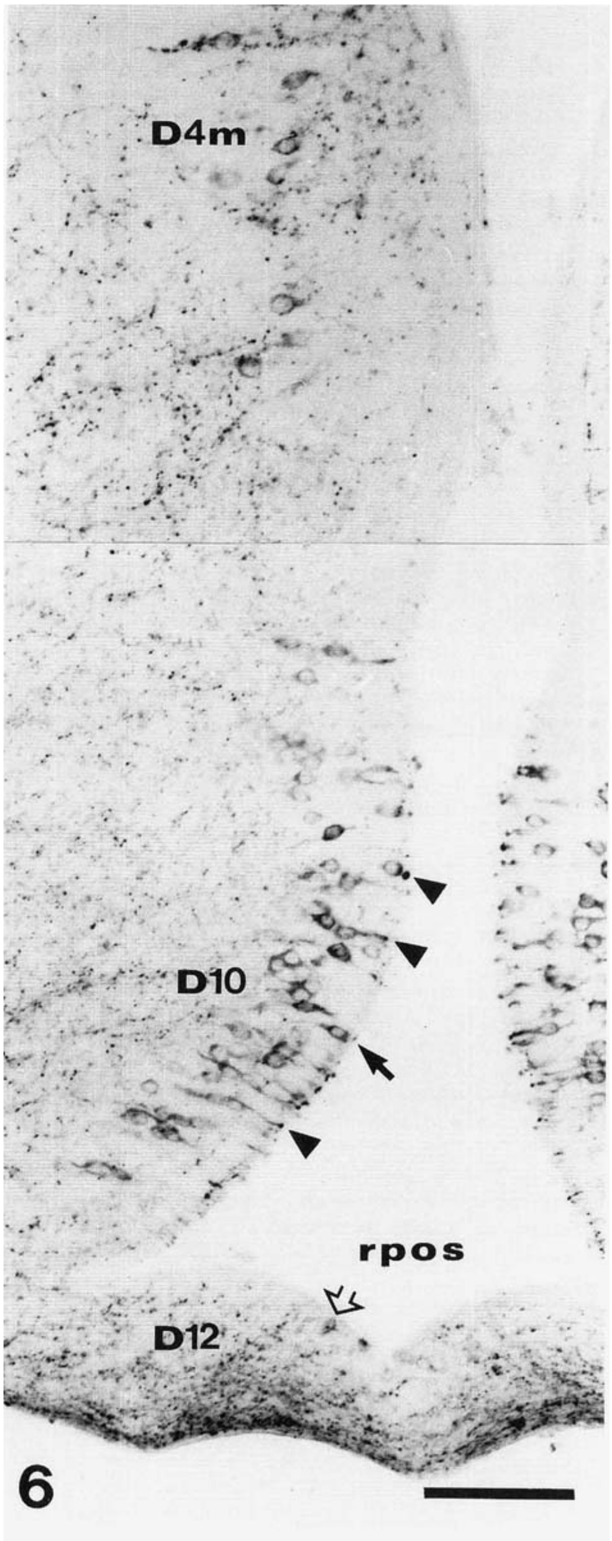


Fig. 6. Transverse section through the hypothalamus and the ventral thalamus. Many labeled perikarya located in the ependyma (arrow) of the nucleus ventralis hypothalami (D10) or subependymally, show apical processes protruding into the ventricle (arrowheads). In the nucleus commissurae postinfundibularis (D12) a dense innervation takes place and occasional immunoreactive neurons are seen (open arrow) near the recessus posterior (rpos). Many GAL-ir perikarya are also observed in the nucleus dorsalis thalami pars medius (D4m). Scale bar: 200 μm.

Fig. 7. Detail of the GAL-ir neurons in the nucleus ventralis thalami shown in Figure 6. Note apical dendrites contacting the cerebrospinal fluid by a bleb (arrowheads). Scale bar: 100 μm.

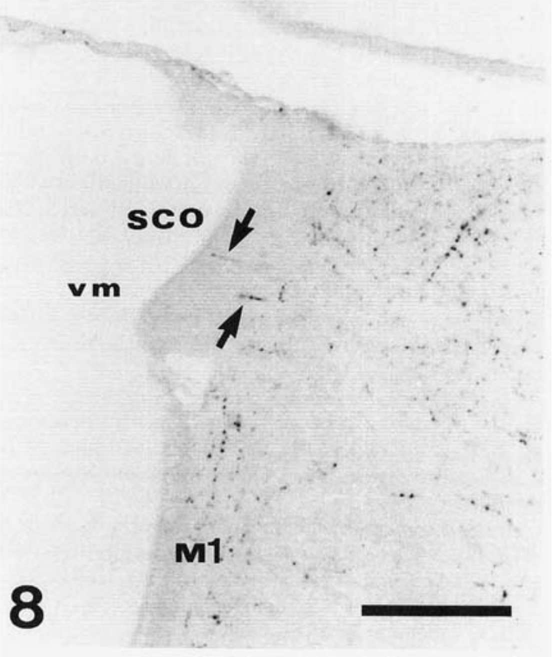
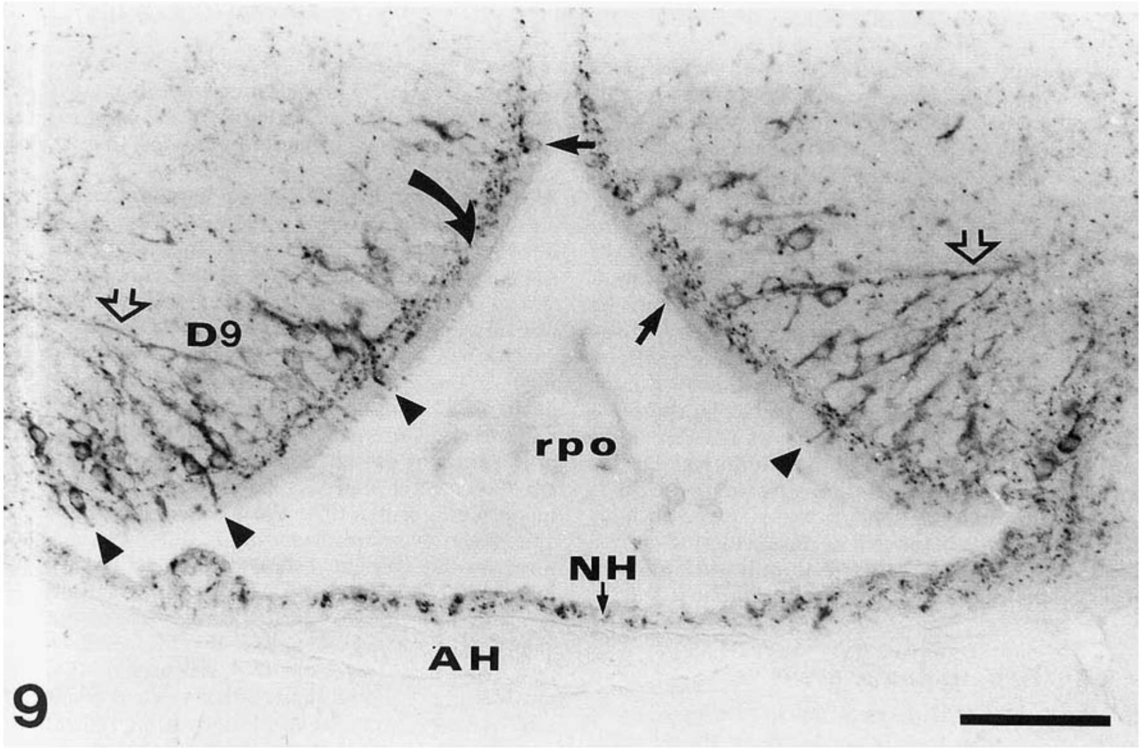
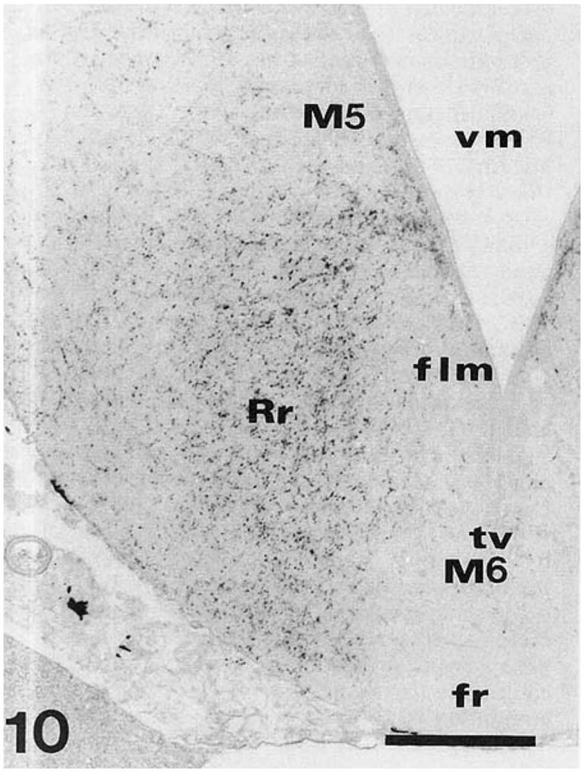


Fig. 8. Transverse section through the pretectal region showing immunoreactive fibers among subcommissural organ ependymal cells (SCO; arrows). Scale bar: 200 μm.





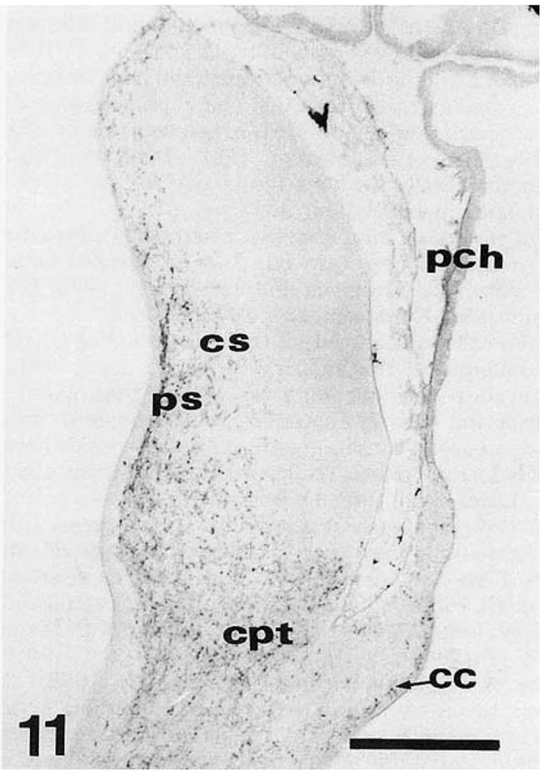


Fig. 9. Transverse section through the recessus postopticus (rpo) showing the nucleus commissurae postinfundibularis (D9) and the hypophysis. Many galanin-immunoreactive neurons send apical processes toward the ventricle (arrowheads) and long basal processes (open arrows). Also a few cells located in the ependymal layer appear positive (arrows). A dense network of immunoreactive fibers is located in the subependymal neuropil (large curved arrow). The neurohypophysis (NH) showed many immunopositive fibers while the adenohypophysis (AH) is devoid of GAL innervation. Scale bar: 160 μm.

Fig. 10. Transverse section through the boundary between the ventral caudal mesencephalon and the rostral rhombencephalon. Many immunoreactive fibers are observed in the rostral part of the rhombencephalic reticular region (Rr). Fibers are absent in the nucleus M5 of Schober (M5) and the nucleus interpeduncularis (M6). Scale bar: 400 μm.

Fig. 11. Midsagittal section through the commissura posttectalis (cpt) and the commissura cerebelli (cc). The tectum opticum contains immunoreactive fibers innervating the periventricular (ps) and the central (cs) strata. Note the presence of some GAL-ir fibers scattered in the commissura cerebelli. Scale bar: 400 μm.

species of an ancient vertebrate line, the adult river lamprey Lampetra fluviatilis. Perikarya and fibers were immunostained throughout the brain identically with all antisera. The reaction was abolished by preincubation with porcine GAL in one antiporcine GAL serum (Chemicon). Thus, a GAL-like peptide or a compound cross-reacting with an antiporcine GAL serum exists in the lamprey brain.

Since there is some molecular similarity among GAL, substance P and physaelamin (Rökaeus et al., 1984), cross-reactions of these substances with GAL antisera could be expected to occur. By use of immunocytochemistry, these closely related molecules have been located in the hypothalamus of teleosts in structures different from those revealed by anti-GAL (Holmqvist and Ekström, 1991). In the present investigation, preincubation of the anti-GAL serum (Chemicon) with substance P and physaelamin was carried out as control. Since immunoreaction was unaffected it is unlikely that galanin-like substance revealed by the antiporcine GAL serum is not substance P or physaelamin.

In general, the distribution of GAL immunoreactivity in the brain of *Lampetra fluviatilis* was quite similar to that in teleosts.

Hypothalamic areas

The distribution of GAL-ir neurons in the lamprey is quite similar to teleosts, having perikarya in the preoptic and tuberal hypothalamus (Batten et al., 1990a,b; Cornbrooks and Parsons, 1991a,b; Holmqvist and Ekström, 1991; Olivereau and Olivereau, 1991; Anglade et al., 1994). In amphibians and birds, in addition to the hypothalamus, the telencephalon, mesencephalon and rhomboencephalon were also reported to display GAL-ir neurons (Lázár et al., 1991; Olivereau and Olivereau, 1992; Józsa and Mess, 1993). In the turtle, we have found GAL-ir perikarya in telencephalon, diencephalon, and rhombencephalon, but not in the mesencephalon (Jiménez et al., 1994). Immunoreactive neurons have been found in extrahypothalamic areas of mammals (Skofitsch and Jacobowitz, 1985; Elmquist et al., 1992; Kordower et al., 1992).

The present results show that the hypothalamus and the ventral thalamus of the lamprey contained many GAL-ir neurons in the periventricular areas. Most of these cells had CFS-contacting apical processes. Cerebrospinal fluid-contacting neurons containing immunoreactive GAL were reported before in teleosts (Holmqvist and Ekström, 1991), anurans (Lázár et al., 1991; Olivereau and Olivereau, 1992) and the turtle (Jiménez et al., 1994). In lampreys, CSF-contacting neurons have been found to be immunoreactive to antisera against several substances such as serotonin (Brodin et al., 1988a, 1990a; Pierre et al., 1992), somatostatin (Wright, 1986; Yáñez et al., 1992), substance P (Nozaki and Gorbman, 1986), cholecystokinin (Brodin et al., 1988b), gonadotropin-releasing hormone (King et al., 1988), the molluscan cardio-excitatory tetrapeptide (Ohtomi et al., 1989) neurotensin (Brodin et al., 1990b), histamine (Brodin et al., 1990a), dopamine and tyrosine hydroxylase (Pierre et al., 1994), and opsins (García-Fernández and Foster, 1994). The brain of lamprey is considered to have conserved many characteristics of the primitive vertebrate brain (Nieuwenhuys, 1977). Neurons of the CSF-contacting type have been considered to reflect a primitive character of the vertebrate brain (Vigh-Teichmann and Vigh, 1989).

In the brain of *Lampetra fluviatilis,* an interesting fact was the presence of an extended subependymal network of immunoreactive fibers in virtually all the ventricular system, including the regions containing nuclei lacking galaninergic innervation. This galaninergic subependymal network could control the activity of other cerebrospinal fluid-contacting neurons or could be related with the activity of the ependymal cells.

Many GAL-ir fibers have been found in the neurohypophysis of *Lampetra fluviatilis.* The anterior neurohypophysis of lampreys is equivalent to the mammalian median eminence, whereas the posterior neurohypophysis corresponds to the neural lobe (see Tsuneki, 1988). The lamprey anterior neurophypophysis displays immunoreactivity to: arginine vasotocin (Goossens et al., 1977), luteinizing hormone-releasing hormone (Nozaki and Kobayashi, 1979), enkephalin (Dores et al., 1984; Nozaki and Gorbman, 1984) and substance P (Nozaki and Gorbman, 1986). The posterior neurohypophysis of the lamprey presents immunoreaction for: arginine vasotocin (Goossens et al., 1977), luteinizing hormone-releasing hormone (Crim et al., 1979; Nozaki and Gorbman, 1986), met-enkephalin (Nozaki and Gorbman, 1984), prolactin and growth hormone-like (Wright, 1986), and somatostatin (Yáñezet al., 1992). The presence of GAL-like immunoreactivity in the lamprey anterior and posterior neurohypophysis suggests that GAL could act either as a blood-borne hormone and/or as a hypophysiotropic factor. Alternatively, GAL could have a role as a neuromodulator of the release of neurohypophyseal hormones and hypothalamic releasing factors.

Evidence for the control of adenohypophysis by GAL has been reported in vertebrates. Galanin can be released to the portal capillaries in the outer layer of the median eminence of amphibians (Lázár et al., 1991), the turtle (Jiménez et al., 1994), birds (Józsa and Mess, 1993) and mammals (Skofitsch and Jacobowitz, 1985; Melander et al., 1986; Palkovits et al., 1987; Beal et al., 1988; Gaymann and Martin, 1989; Walker et al., 1989; Kordower et al., 1992). In this way it could modulate the activity of adenohypophyseal cells. Indeed, in mammals, GAL has been reported to exert a hypophysiotropic role affecting the release of adenohypophyseal hormones (Ottlecz et al., 1986; Koshiyama et al., 1987; Murakami et al., 1987; Sahu et al., 1987; López et al., 1991). In teleosts lacking a hypophyseal portal vascular system, direct projections of GAL-ir fibers to the adenohypophysis were reported (Batten et al., 1990a,b; Holmqvist and Ekström, 1991; Olivereau and Olivereau, 1991; Anglade et al., 1994); this is in agreement with the presence of GAL receptors in the adenohypophysis of teleosts (Moons et al., 1991). Like teleosts, lampreys lack a hypothalamo-hypophyseal portal system; but unlike teleosts, the adenohypophysis of the lamprey is not directly innervated from the hypothalamus. Hence, different mechanisms have been proposed to exist for hypothalamic control of the adenohypophysis in lampreys; these include releasing of the neurosecretory materials in the external layer of the neurohypophysis and subsequent transport through the underlying connective tissues or, less likely, releasing to the systemic circulation in the neurohypophysis or, indirectly, via the cerebrospinal fluid, after releasing in the third ventricle (see King et al., 1988). In *Lampetra fluviatilis,* abundant fibers were found in all layers of the neurohypophysis including the external layer. The exact mechanism of delivery from hypothalamic neurons to adenohypophyseal target cells, if any, is unknown in *Lampetra fluviatilis.*

Extrahypothalamic areas

Mammals and birds have GAL-ir cell bodies in the septum (rat: Melander et al., 1986; monkey: Melander and

Staines, 1986; Kordower et al., 1992; human: Kordower et al., 1992; chicken: Józsa and Mess, 1993). In this same location GAL-ir fibers were described in anuran (Lázár et al., 1991; Olivereau and Olivereau, 1992) and the turtle (Jiménez et al., 1994). In teleosts, a rich network of fibers is located rostrally in the telencephalic floor (Batten et al., 1990a,b; Holmqvist and Ekström, 1991; Olivereau and Olivereau, 1991; Anglade et al., 1994). In *Lampetra fluviatilis* few GAL-ir fibers were observed in the septum itself, but many of them were distributed in the periventricular and lateral neuropil bordering this area. In mammals, the septum has been involved in the control of water balance (Miselis, 1981; Tanaka et al., 1988).

It is known that the striatum of several vertebrates is involved in processing sensory information and in motor control. In mammals, GAL coexists with acetylcholine in cholinergic neurons of the basal forebrain involved in learning and memory (Ogren et al., 1992), and it was proposed that GAL could inhibit the activity of cholinergic neurons (Melander et al., 1986; Chan-Palay, 1988). Galanin also exerts an inhibitory effect in the serotoninergic system either by a presynaptic inhibition (Sundström and Melander, 1988) or by being an inhibitor of serotonin receptors (cf. Fuxe et al., 1988). A strong acetylcholinesterase activity has also been demonstrated in the corpus striatum of lampreys (Wächtler, 1974), while the surrounding neuropil has been found to contain immunoreactive fibers to antisera against serotonin (Brodin et al., 1988a, 1990a; Pierre et al., 1992), substance P (Nozaki and Gorbman, 1986), neurotensin (Brodin et al., 1990b), histamine (Brodin et al., 1990a), dopamine and tyrosine hydroxylase (Pierre et al., 1994). The dense GAL innervation of the corpus striatum of *Lampetra fluviatilis* shown in this study suggests that GAL may modulate cholinergic and/or serotoninergic and other activities in this telencephalic region.

In the present study, no immunoreactive neurons were found in the brainstem of *Lampetra fluviatilis*. This is also the case of some teleosts (see: Holmqvist and Ekström, 1991; Olivereau and Olivereau, 1991; Anglade et al., 1994). In contrast, mammals showed GAL-ir neurons throughout the brainstem (Skofitsch and Jacobowitz, 1985; Melander et al., 1986; Kordower et al., 1992). Although lacking GAL-ir neurons, the brainstem of the lamprey is richly innervated by GAL-ir fibers, indicating some role of GAL in this area.

In the tectum opticum of *Lampetra fluviatilis*, GAL-ir fibers were found near the ventricle. A similar pattern was reported for teleosts (Batten et al., 1990a,b; Holmqvist and Ekström, 1991; Olivereau and Olivereau, 1991), anurans (Lázár et al., 1991; Olivereau and Olivereau, 1992), and the turtle (Jiménez et al., 1994). At variance, few GAL-ir fibers were described in the tectum opticum of the chicken (Józsa and Mess, 1993) and the superior colliculus of the rat (Skofitsch and Jacobowitz, 1985; Melander et al., 1986). In this respect, differences seem to exist between birds and mammals on one hand, and fishes, amphibians and the turtle on the other.

Unlike teleosts, the cerebellar plate of *Lampetra fluviatilis* showed few GAL-ir fibers. This characteristic therefore seems to be exclusive of Agnatha.

Some scattered GAL-ir fibers were found between the descending reticular axons in the ventromedial rhombencephalon. It is known that reticulospinal neurons play a crucial role in the initiation of locomotion, by conveying information from the brainstem to the spinal cord (Rovainen, 1967; Wickelgren, 1977; Brodin et al., 1988b). Sometimes GAL-ir fibers were seen to be closely associated with reticular axons, establishing putative en passant contacts. Galanin could therefore modulate this descending reticulospinal pathway.

CONCLUSION

The present study indicates that a GAL-like substance is present in the lamprey brain. The galaninergic system in the brain of lampreys is well developed as compared with that described in teleosts, but presents some differences with respect to other vertebrate groups.

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