

The Monoamine-Containing Neurons in the Brain of the Garfish, *Lepisosteus osseus*

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PARENT, A. AND R. G. NORTHCUTT. *The monoamine-containing neurons in the brain of the garfish, Lepisosteus osseus.* BRAIN RES. BULL. 9(1-6) 189-204, 1982.—The morphological organization of monoamine (MA)-containing neurons in the brain of the longnose gar (*Lepisosteus osseus*) was studied by means of fluorescence histochemical methods. In this species, one of the few living representatives of the holostean group of actinopterygian fishes, by far the largest number of MA cells is found within the preoptico-hypothalamic complex. Multitudinous small-sized MA cells, with a short club-like process protruding into the third ventricle, are present along the ependymal wall of the lateral and posterior hypothalamic recesses. This population of CSF-contacting MA cells, comprising both catecholamine (CA) and serotonin (5-HT) type cells, gives rise to numerous efferent fibers, some of which proceed rostrally toward the telencephalon while others course dorsally to reach the midbrain tegmentum and optic tectum. Many fibers, however, arborize directly within the hypothalamus, particularly around blood vessels where they form patches of highly fluorescent material. Small CA cells are also scattered along the preoptic recess wall. They do not directly contact the CSF but also appear to contribute to the CA innervation of telencephalon. At brain stem levels, a few CA cells are scattered at the base of the rostral midbrain, in the isthmal tegmentum, and in the central and dorsal (vagal lobe) portions of the medulla. Some CSF-contacting CA cells are also present around the central canal at upper spinal cord levels. One of the most striking features of the MA systems in *Lepisosteus* is the remarkable development of the 5-HT neuronal network. A prominent 5-HT cell column extends rostrocaudally in the raphe region from the caudal midbrain to upper spinal cord levels. In the caudal midbrain and isthmus, the 5-HT cells also invade the lateral tegmentum and profusely innervate various brain stem structures as well as large portions of telencephalon, particularly the dorsal nucleus of area ventralis (Vd). The CA innervation of telencephalon is relatively weak, except in the olfactory bulb where numerous CA varicosities were found. These findings in *Lepisosteus* suggest that the pattern of MA system organization in the holostean brain is far more similar to that seen in primitive vertebrates, such as cyclostomes—where the 5-HT systems are highly elaborated relative to the CA systems—than it is to the pattern in more advanced fishes such as teleosts.

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| Brain monoamine neurons | Nonmammalian vertebrates | Histofluorescence methods |
| Comparative neuroanatomy | <i>Lepisosteus osseus</i> | Holostean fishes |

DESPITE the fact that central monoaminergic systems are known to be phylogenetically ancient and are thought to subserve fundamental brain functions in all vertebrates [26], very little is yet known of their anatomical organization in nonmammals. This lack of information is particularly obvious in fishes. Until now, the cellular localization of these putative neurotransmitters has been studied by means of fluorescence histochemical methods in the brains of only a few teleosts among all bony fishes (see Refs. in [27-32]). The teleosts, however, are known to be the most specialized representatives of a broad radiation (actinopterygian radiation) of bony fishes. Hence, a clear picture of the general patterns of organization of the monoaminergic systems in the brain of fishes has not yet emerged.

In contrast to teleosts, chondrosteans (sturgeons and paddlefish) and holosteans (gars and bowfin) are represented

by only a few extant species. They are of interest in that their brain organization is more simple than that in teleosts and, therefore, they may serve as a model of the primitive actinopterygian stock [23]. Thus, it was thought of interest to undertake a histofluorescence investigation of the brain of the longnose gar (*Lepisosteus osseus*), a holostean, in the hope of reaching a greater understanding of the organization of monoamine (MA)-containing neuronal systems in the brains of bony fishes. It was expected, among other things, that such a study in holosteans would reveal important clues as to how the actinopterygian radiation differs from land vertebrates in regard to the arrangement of MA neuronal systems.

METHOD

A total of 36 specimens of longnose gars, *Lepisosteus*

LIST OF ABBREVIATIONS

| | | | |
|------|--|-------|---|
| A | Auricle of cerebellum | nX | Motor nucleus of vagal nerve |
| C | Corpus of cerebellum | OB | Olfactory bulb |
| CC | Cerebellar crest | OC | Optic chiasm |
| CN | Cerebellar nucleus | ON | Olfactory nerve |
| CP | Central posterior thalamic nucleus | PC | Posterior commissure |
| D | Area dorsalis telecephalic | PG | Preglomerular complex |
| Dd | Dorsal zone of D | PP | Periventricular preoptic nucleus |
| DI | Lateral zone of D | PR | Posterior recess of the third ventricle |
| Dm | Medial zone of D | PT | Central pretectal nucleus |
| Dp | Posterior zone of D | PV | Periventricular pretectal nucleus |
| DT | Dorsal thalamus | SC | Spinal cord |
| ECL | External cell layers of olfactory bulb | SG | Secondary gustatory nucleus |
| EG | Eminentia granularis | SMC | Spinal motor cells |
| EN | Entopeduncular nucleus | SN | Suprachiasmatic nucleus |
| FN | Funicular nucleus | SOD | Supraoptic decussation |
| FR | Fasciculus retroflexus | SOF | Secondary olfactory fiber layer of olfactory bulb |
| GL | Glomerular layer of olfactory bulb | SR | Superior raphe nucleus |
| H | Habenula | TC | Tela chorioidea |
| HA | Hypophysis, pars anterior | TEC | Optic tectum |
| HP | Hypophysis, pars posterior | TEL | Telencephalon |
| ICL | Internal cell layer of olfactory bulb | TL | Torus longitudinalis |
| IL | Inferior lobe of hypothalamus | TPP | Periventricular nucleus of posterior tuberculum |
| IP | Interpeduncular nucleus | TS | Torus semicircularis |
| IRN | Inferior reticular nucleus | V | Area ventralis telencephali |
| LR | Lateral recess of third ventricle | VA | Valvula |
| LT | Lateral tegmental nucleus | Vd | Dorsal nucleus of V |
| MC | Mitral cells | VL | Vagal lobe |
| MLF | Medial longitudinal fasciculus | VI | Lateral nucleus of V |
| MN | Magnocellular octavolateralis nucleus | Vn | Other nucleus of V |
| MO | medulla oblongata | VOT | Nucleus of ventral optic tract |
| MOL | Medial octavolateralis nucleus | Vp | Postcommissural nucleus of V |
| MR | Medial reticular nucleus | VT | Ventral thalamus |
| NI | Nucleus isthmi | Vv | Ventral nucleus of V |
| NOT | Nucleus of olfactory tract | II | Optic nerve |
| NPT | Nucleus posterior thalami | III | Oculomotor nerve |
| NT | Nucleus taenia | V-VII | Trigemino-facial complex |
| NTL | Nucleus of torus lateralis | VIII | Octavus nerve |
| NVL | Nucleus ventralis lateralis | IX | Glossopharyngeal nerve |
| nVII | Motor nucleus of facial nerve | X | Vagus nerve |

osseus (= *Lepidosteus osseus*), were used in the present study. The monoamines were visualized histochemically either by means of the standard Falck-Hillarp paraformaldehyde method [13,14] (21 cases), or with the help of the sucrose-potassium phosphate-glyoxylic acid (SPG) procedure [9,10] (15 cases). All the animals (body length ranging from 23 to 35 cm) were sacrificed by decapitation and their brains dissected out as rapidly as possible. The brains to be processed according to the Falck-Hillarp histofluorescence method were first frozen in liquid propane, then freeze-dried for a period of five days, and subsequently treated with paraformaldehyde (70% relative humidity) for one hour at 80°C. The freeze-dried brains were embedded in paraffin *in vacuo* and sectioned at 10 μ m in the transverse plane. The other brains were placed in a cryostat and sectioned at 20–30 μ m either in the transverse, horizontal or sagittal plane. The brain sections were treated according to the SPG histofluorescence procedure described by de la Torre [9]. The fluorescence microscopy was performed with a Zeiss or a

Leitz microscope, the latter equipped with a Leitz Ploem-pack-2 system for reflected light microscopy. In addition, complete series of brain sections processed with Bodian, Nissl, Golgi-Cox, and Klüver-Barrera methods and cut in the 3 standard anatomical planes were available for the study of nuclear groups and fiber tracts in *Lepisosteus*. In the present study no attempt was made to distinguish between norepinephrine and dopamine, as both primary catecholamines yield a similar yellow-green fluorescence after paraformaldehyde treatment.

RESULTS

The overall distribution of catecholamine (CA)- and serotonin (5-hydroxytryptamine, 5-HT)-containing neurons in the brain of *Lepisosteus osseus* is illustrated in a series of semi-schematic transverse sections displayed in a caudo-rostral order (Figs. 2–6). In these figures the nomenclature used for the telencephalon follows that of Nieuwenhuys [21]

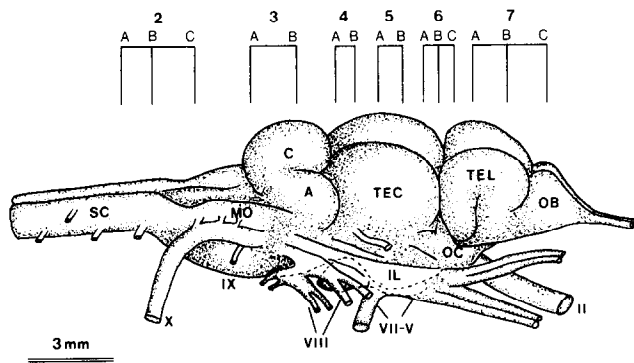


FIG. 1. Lateral view of the brain of the garfish, *Lepisosteus osseus*. Numbered lines indicate the levels of the transverse sections in Figs. 2 to 7.

as modified by Northcutt and Braford [22], whereas the nomenclature for the rest of the brain is in general usage (see [3]).

Medulla Oblongata (Figs. 2, 3, 8, 9D)

Numerous monoamine (MA)-containing cells are present at the level of the junction between the medulla and the spinal cord. First, there are small-sized (mean diameter: 10–15 μm) CA cells located beneath and partly within the ependymal layer of the central canal (Figs. 2A, 8A, B). They possess a short club-like process protruding directly into the lumen of the central canal. No other process belonging to these CA cells could be visualized. Second, 5-HT neuronal somata occur along the lateral border of, and in between, the medial longitudinal fasciculi (Figs. 2A, 8A). The 5-HT neurons located in between the medial longitudinal fasciculi are mostly bipolar, and their thick and varicose processes are orientated along the vertical axis of the midline. These processes are often seen in close proximity to large midline blood vessels. The 5-HT cells lying along the lateral border of the medial longitudinal fasciculi are slightly larger than the medially located ones. They appear to give rise to a multitude of very fine 5-HT axonal varicosities closely surrounding the neuronal somata of the voluminous spinal motor cells (Fig. 2A, B). Numerous CA axonal varicosities can also be visualized in the lower medulla. They are characteristically larger and more intensely fluorescent than the 5-HT varicosities. The CA varicosities abound in the core (neuropil region) of the so-called funicular nucleus where they are closely packed (Figs. 2A, B; 9D), and along the ventrolateral border of the medulla (Fig. 2).

More rostrally in the medulla oblongata, 5-HT nerve cell bodies are found exclusively within the raphe region (Figs. 2C, 3, 8C), whereas CA cells occur in the vagal lobe (Figs. 2C, 3) and within the central portion of the medulla, particularly lateral to the facial motor nucleus (Figs. 3A, 8D). In the vagal lobe the CA cells are mainly found ventromedially in the caudal half (Fig. 2C) and dorsomedially in the rostral half of this nucleus (Fig. 3). These neurons are of small size (maximum diameter: 10–15 μm) and appear to give rise to numerous CA axonal varicosities distributed mainly in the lateral portion of the vagal lobe. The CA neurons lying in the central portion of the medulla are larger (maximum diameter:

20–30 μm) than those in vagal lobe. They give rise to numerous axonal varicose profiles which accumulate along the ventrolateral border of the medulla. Some of these CA axonal profiles can be seen to cross the midline beneath the bulk of the 5-HT neurons of the raphe region (Fig. 2C).

Isthmus (Figs. 4, 9B)

The 5-HT nerve cell bodies are strikingly abundant at the isthmal level. They predominate in the raphe region but some of them are also found more laterally in the tegmentum, particularly around the medial longitudinal fasciculi (Fig. 4). These neurons give rise to numerous 5-HT axonal varicosities which accumulate in very large number along the lateral border of the ventricle, in a rather well-circumscribed area corresponding more or less to the so-called cerebellar nucleus (Fig. 4A). This ill-defined periventricular region appears to receive one of the heaviest 5-HT innervations yet disclosed in the *Lepisosteus* brain (Fig. 9B).

In regard to CA neurons, a few small-sized CA cells are found in the dorsolateral portion of the isthmal tegmentum, lateral to the secondary gustatory nucleus (Fig. 4A). These are rather weakly fluorescent cells surrounded by numerous brightly fluorescent CA axonal profiles. The latter appear to be part of a complex fiber system consisting of a paramedian and of a more laterally located array of straight CA profiles which course vertically through the isthmal tegmentum. In transverse sections the CA fascicles appear to emerge from the periventricular gray regions bordering both the medial and lateral recesses of the cerebral ventricle (Fig. 4B). However, the exact origin and final destination of the CA axons forming this impressive radiation of fluorescent tegmental fibers remain to be investigated. It is nevertheless worth noting that at the isthmal level the periventricular gray itself contains a multitude of thick CA axonal varicosities often interspersed between the 5-HT cells dorsolaterally surrounding the medial longitudinal fasciculi (Fig. 4B).

Midbrain (Figs. 5, 9A, 9E)

Nerve cell bodies of the 5-HT type can be found only in the caudal half of the midbrain tegmentum where they closely surround the medial longitudinal fasciculi. Most of them are smaller (maximum diameter: 20 μm) than the more caudally located 5-HT cells. They can be seen to give rise to fine 5-HT axonal profiles particularly abundant at the periphery of the interpeduncular nucleus (Fig. 9A).

A few isolated and weakly fluorescent CA cells are present at the base of the rostral midbrain tegmentum, medial to the oculomotor nerve root fibers (Fig. 5A). Despite the scarcity of CA neuronal somata in the midbrain proper, numerous thick CA axonal varicosities are found in various mesencephalic structures such as the nucleus of the torus lateralis (Figs. 5, 9E), the torus semicircularis (Fig. 5), the lateral tegmental nucleus (Fig. 5A), and the optic tectum (Figs. 4, 5, 6A). In the optic tectum the CA varicosities are especially numerous in the stratum periventriculare where most of them closely surround the cell bodies of tectal neurons (Figs. 4, 5).

Diencephalon (Figs. 5, 6, 7A, 10)

The vast majority of the MA-containing cells in the brain of *Lepisosteus* are found within the preoptico-hypothalamic complex. At hypothalamic levels there is a multitude of small bipolar cells (maximum diameter: 10 μm) of both CA and

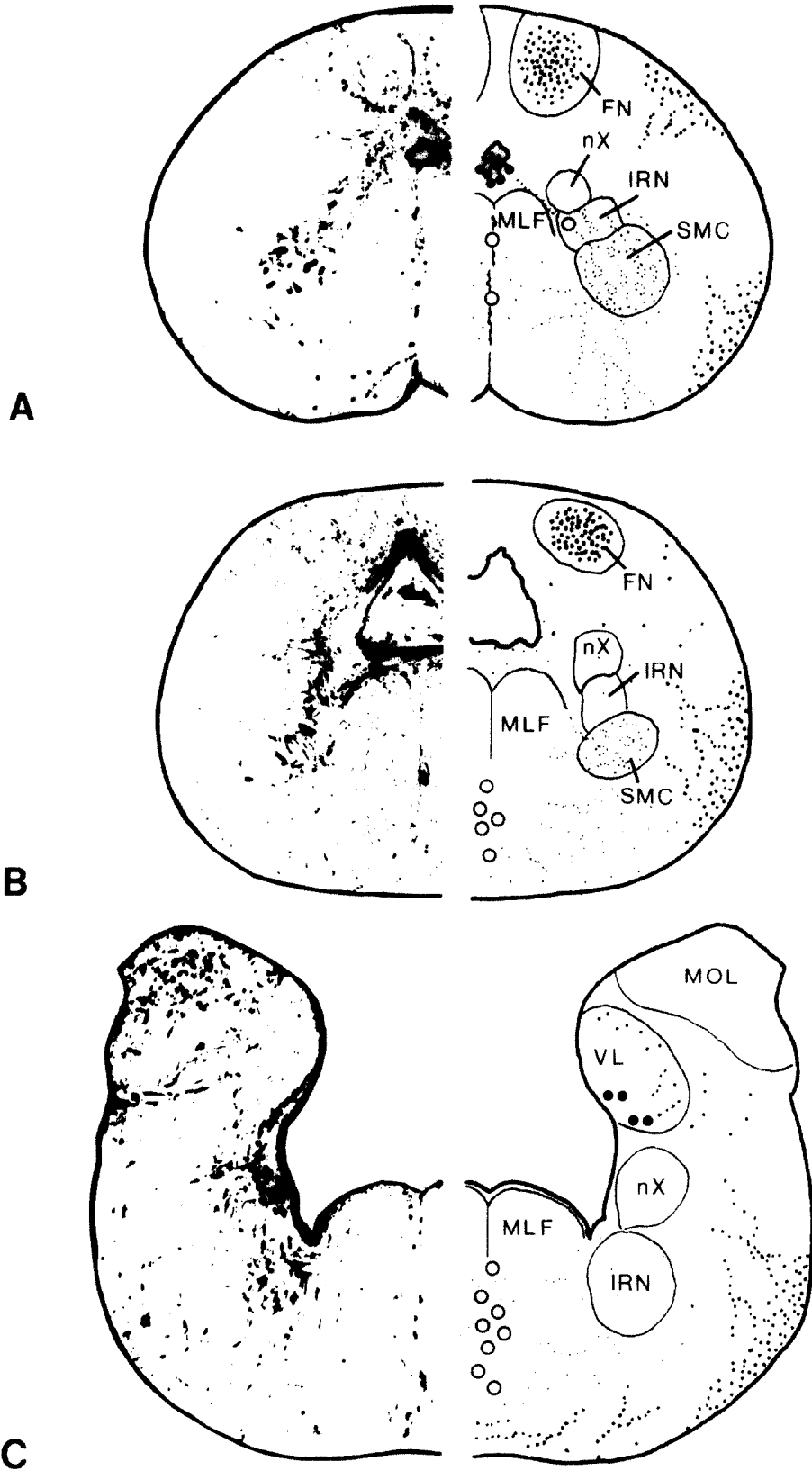


FIG. 2.

FIGS. 2-7. Series of transverse sections through the brain of *Lepisosteus* illustrating, on the right side, the distribution of CA-containing cell bodies (full circles) and CA axonal varicosities (large dots), and of 5-HT-containing cell bodies (open circles) and 5-HT axonal varicosities (small dots). In these figures, the left side is a high-contrast photomicrograph of a corresponding transverse section stained with cresyl violet.

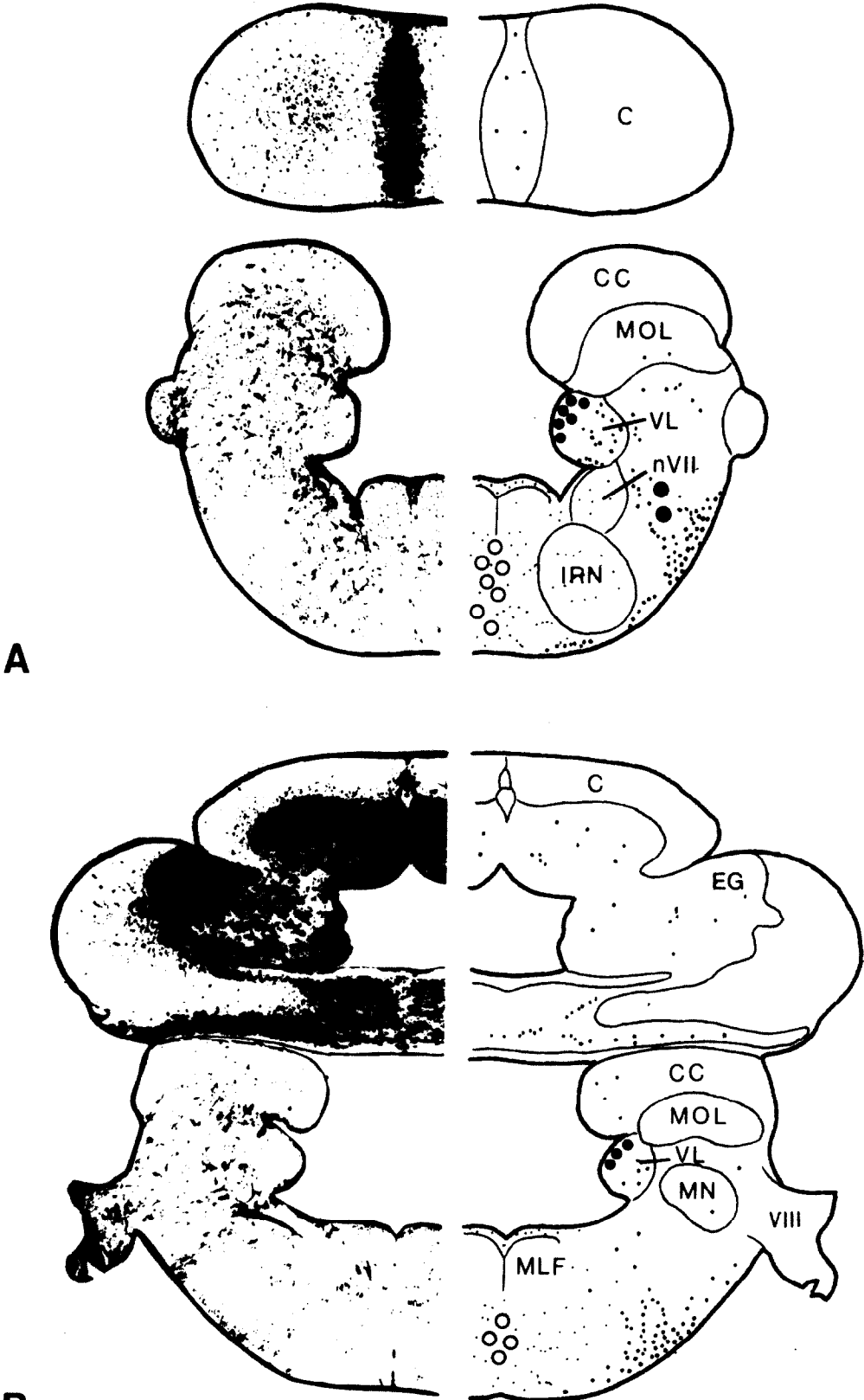
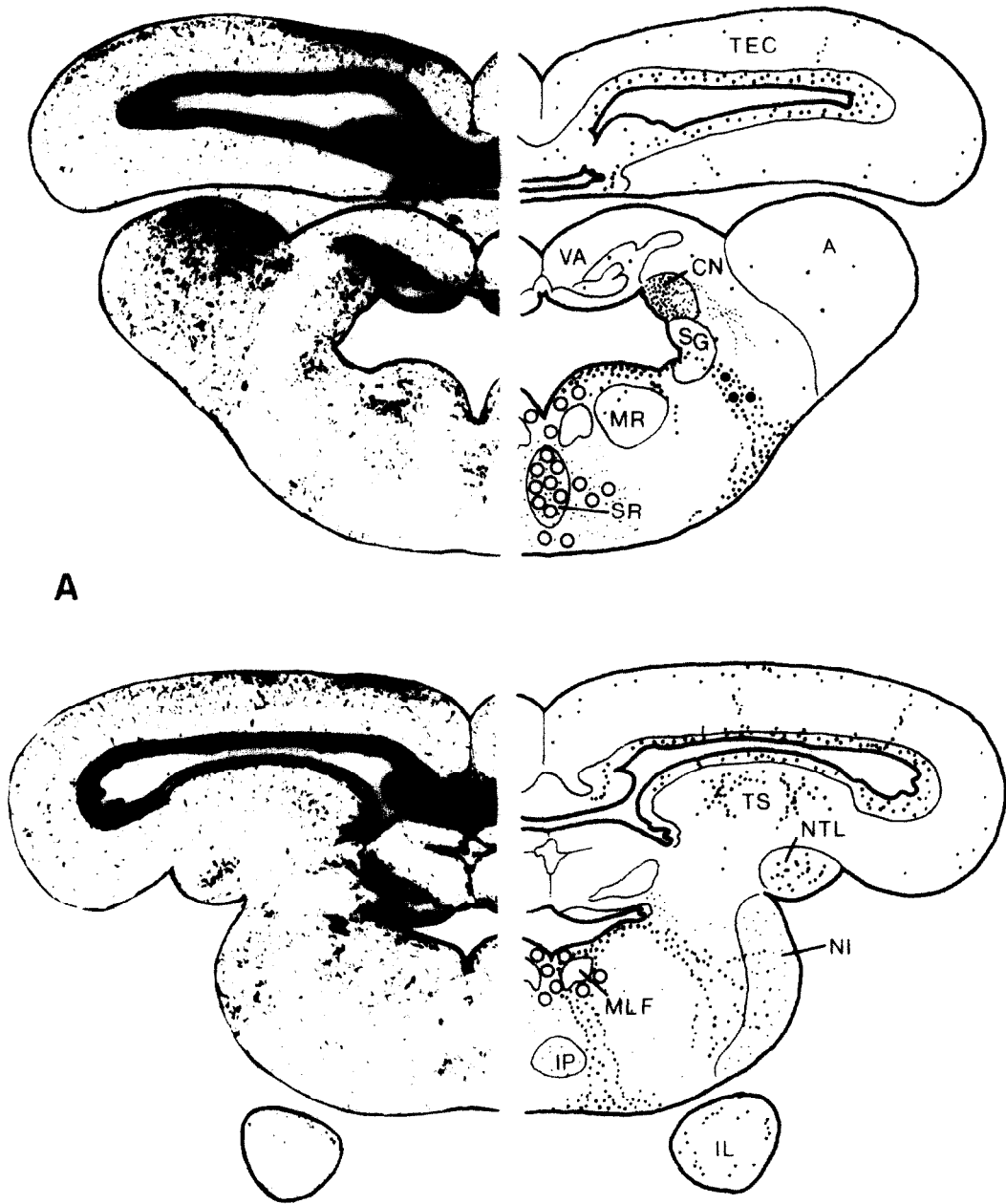
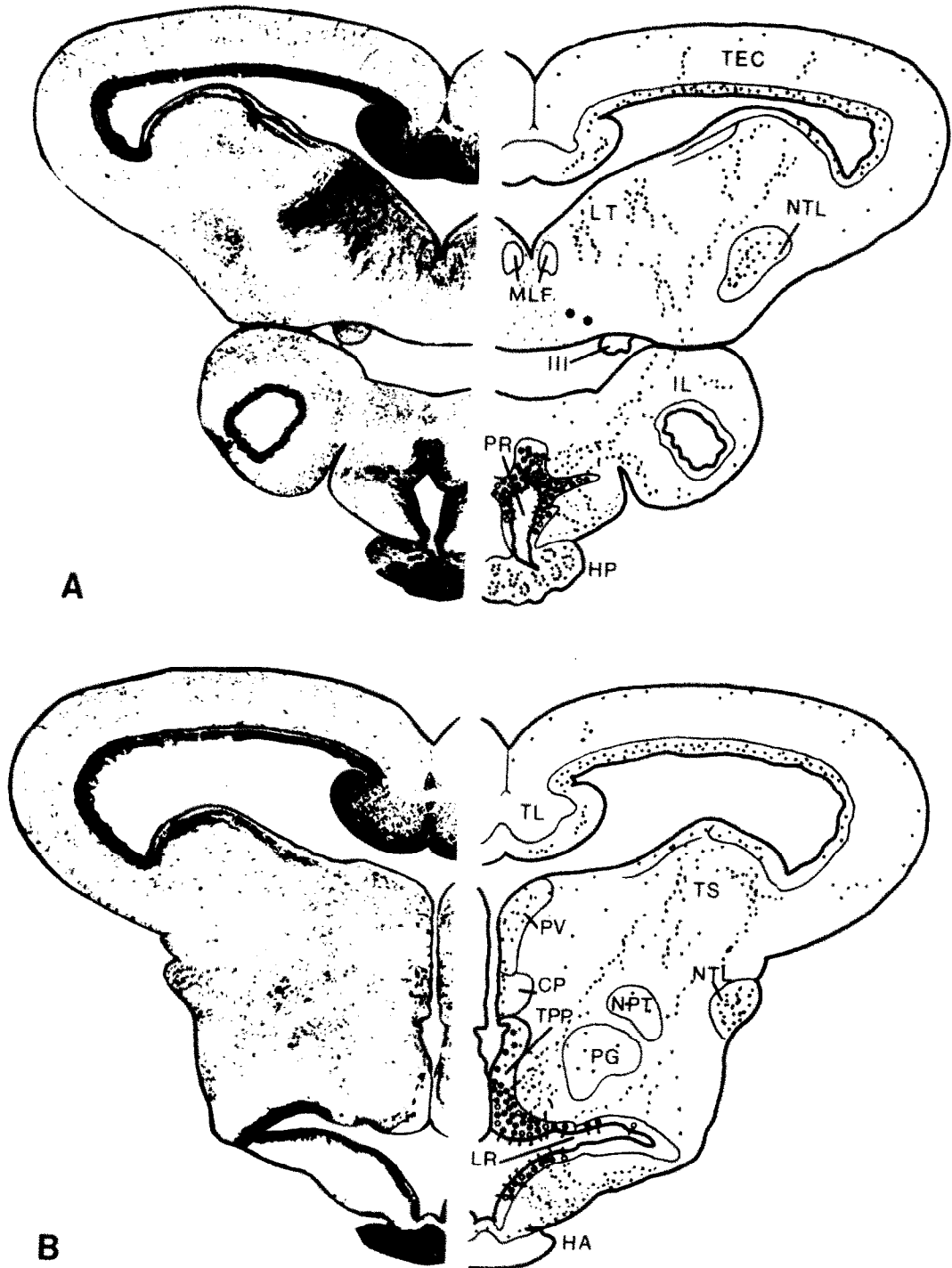


FIG. 3. **B**



B
FIG. 4.



B
FIG. 5.

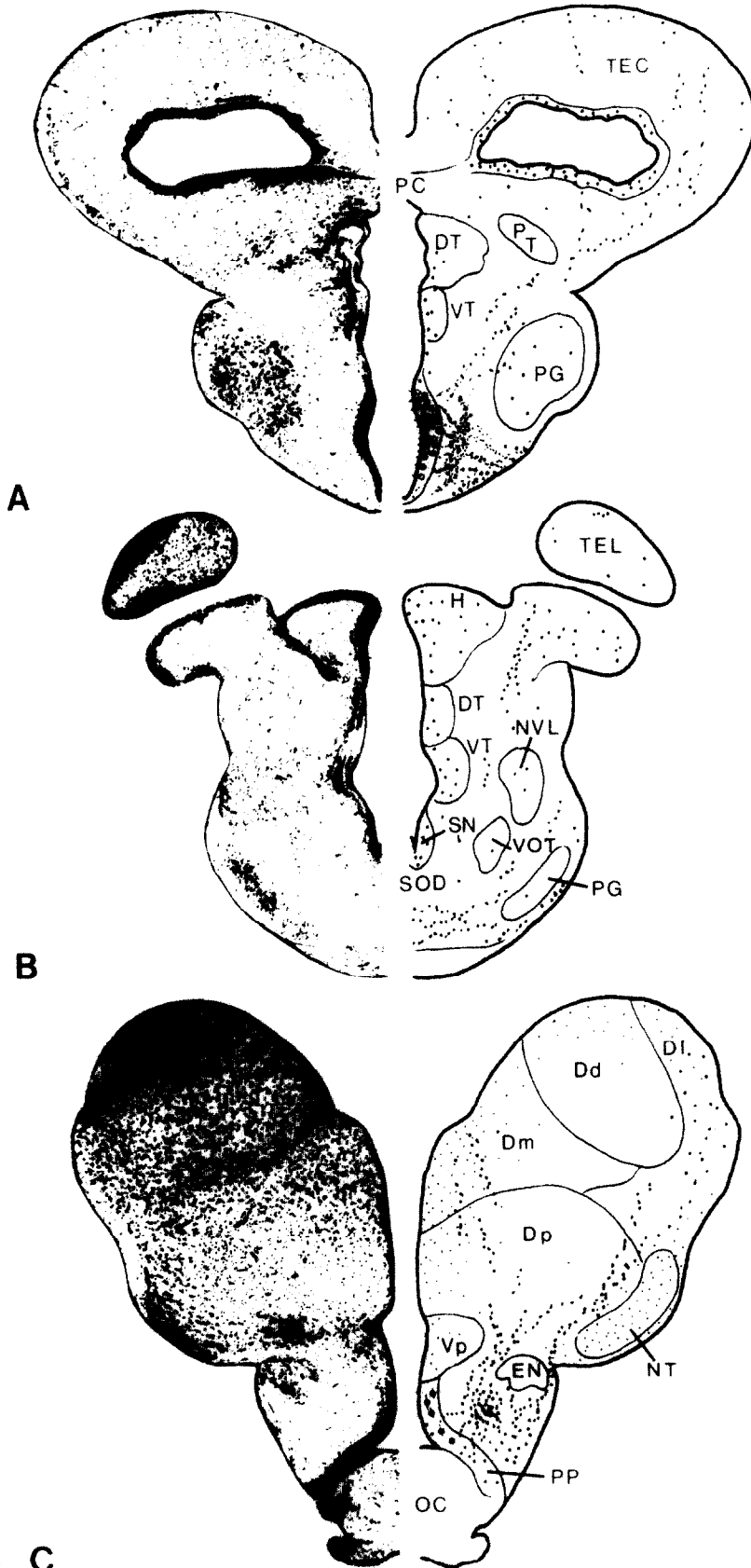


FIG. 6. C

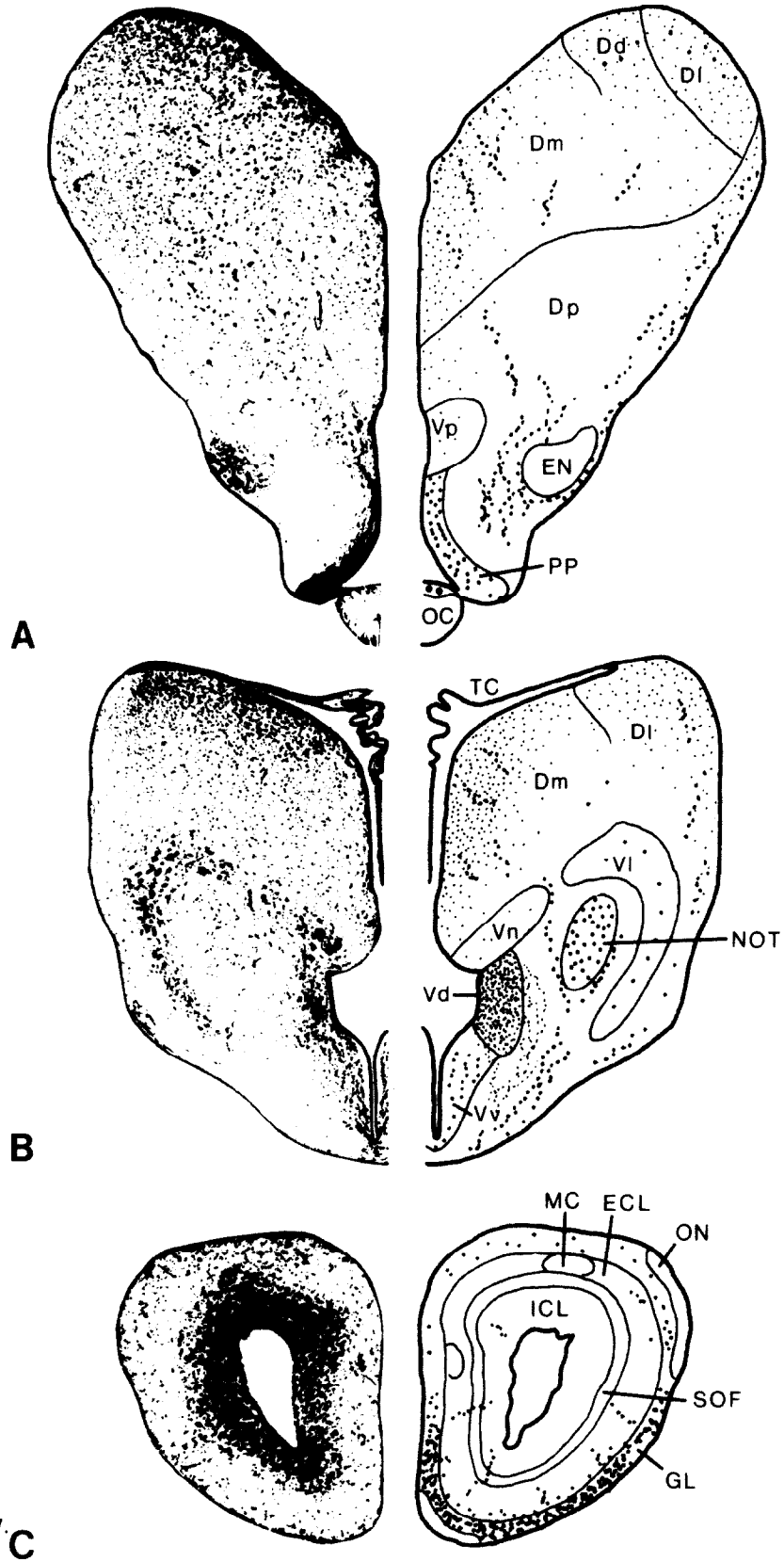


FIG. 7. C

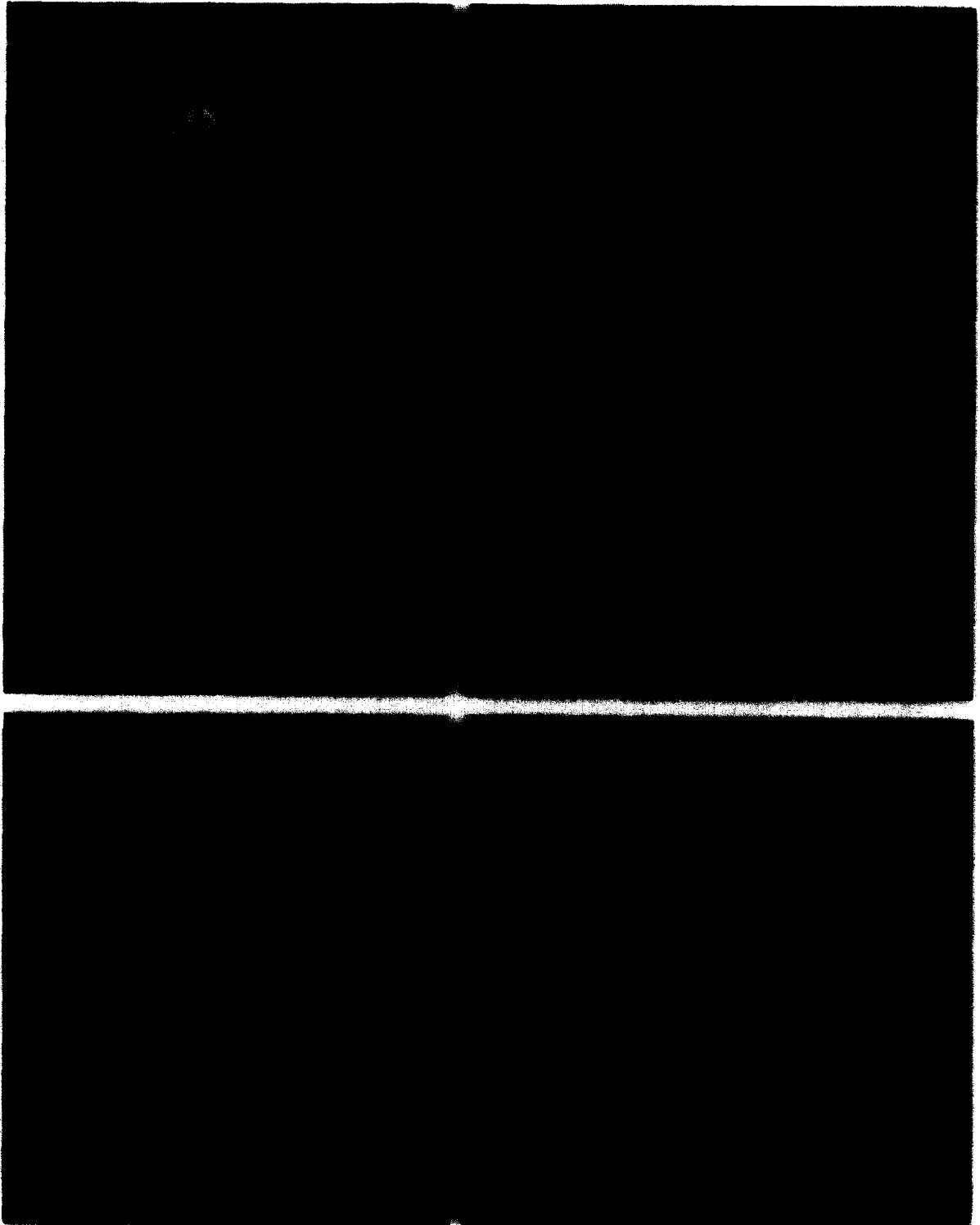


FIG. 8. A-B: Photomicrographs of transverse (A) and horizontal (B) sections through the medullo-spinal junction (see also Fig. 2A) showing numerous CSF-contacting CA cells (arrows in A and all cells in B) located near the lumen (L) of the central canal. Some 5-HT cells (crossed-arrows in A) are seen in-between the medial longitudinal fasciculi (MLF). C-D: Photomicrographs of transverse sections through the upper medulla (see also Fig. 3A) depicting 5-HT cells within the raphe region in-between the MLF (C), and CA cells located in the central portion of the medulla, near the ventricle (VT) (D). Falck-Hillarp method (A, C, D) and SPG procedure (B).

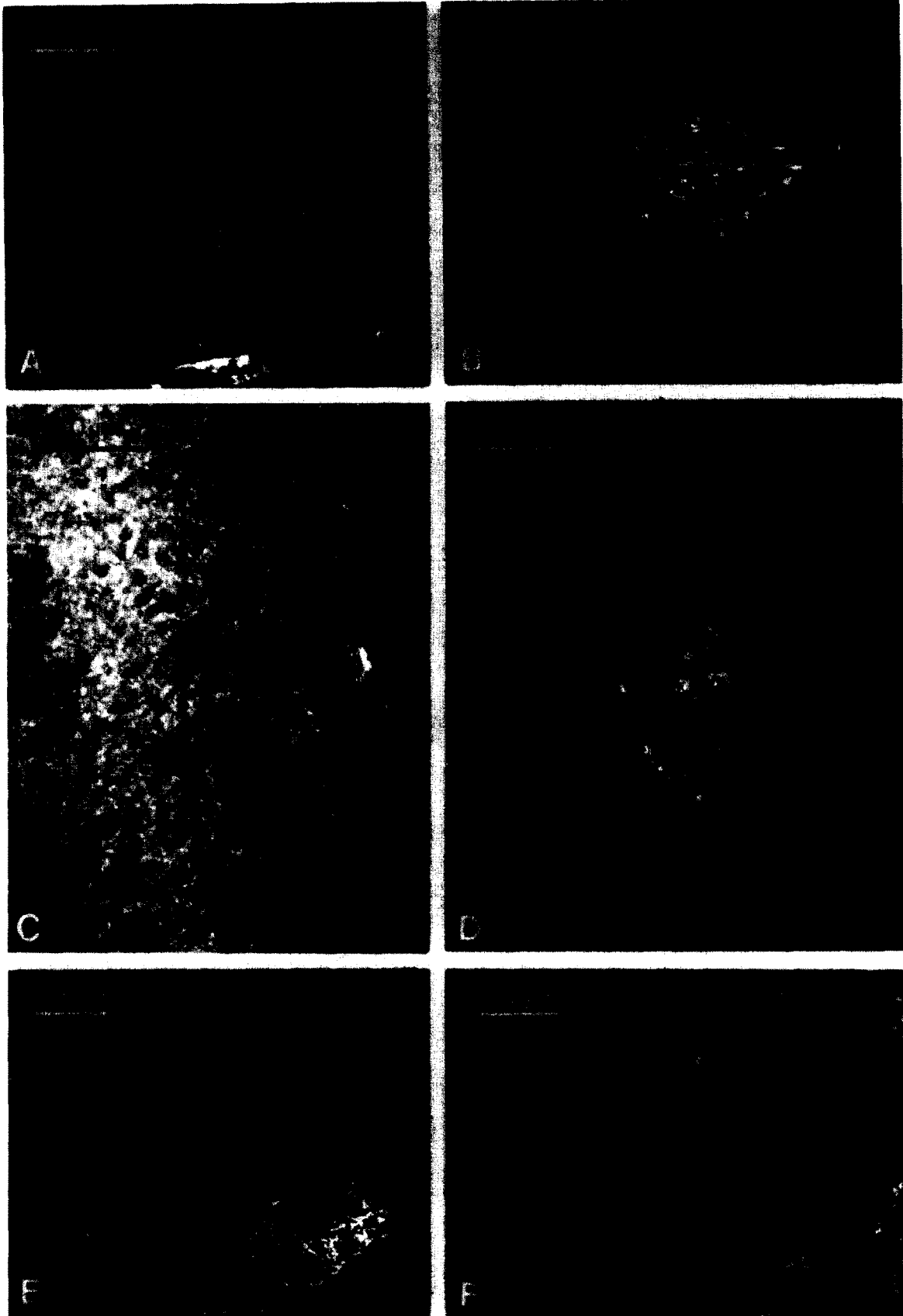


FIG. 9. A–C: Photomicrographs showing numerous 5-HT axonal varicosities present around the interpeduncular nucleus (IP in A) (see also Fig. 4B), within the cerebellar nucleus (B) (see also Fig. 4A), and in the dorsal nucleus of area ventralis telencephali (C) (see also Fig. 7B). D–F: Photomicrographs depicting abundant CA axonal varicosities in the funicular nucleus (D) (see also Fig. 2B), in the nucleus of torus lateralis (E) (see also Fig. 5A), and in the olfactory bulb (F) (see also Fig. 7C). The arrows in F point to CA varicose fibers en route to the glomerular layer (GL). Transverse (A, C, D, E) and horizontal (B, F) sections. Falck-Hillarp method (A–E) and SPG procedure (F).

5-HT types distributed along and partly within various portions of the ependymal wall of the third ventricle. These cells are characteristically provided with a short club-like process protruding directly into the third ventricle. A much thinner and longer ependymofugal process can also be visualized. Rostrocaudally, such CSF-contacting MA cells are first encountered in the middle third of the hypothalamus. There they lie along the small sulcus which divides the hypothalamus into its dorsal and ventral portions (Fig. 6A). As we proceed more caudally into the hypothalamus the number of CSF-contacting MA cells increases, particularly along the dorsal border of the lateral recess (nucleus recessus lateralis of certain authors, see [31]) (Fig. 5B), and around the posterior recess (Fig. 5A) (nucleus recessus posterioris) where up to ten rows of closely-packed MA cells can be found. The 5-HT cells appear to be less numerous than the CA cells within these various regions of the hypothalamic periventricular gray. It has been roughly estimated that the 5-HT cells represent only about 30–40% of the total population of CSF-contacting MA cells. However, the 5-HT cells appear to be uniformly scattered among the CA cells and no preferential distribution has been noticed. The numerous CSF-contacting MA cells in the hypothalamus of *Lepisosteus* contribute to the innervation of various hypothalamic as well as extrahypothalamic structures. First, the thin ependymofugal processes of some of these CA and 5-HT cells can be seen to form dense patches of fluorescent material which often surround blood vessels at the base of the hypothalamus (Figs. 6A, 10A). These numerous patches, which are more or less continuous with one another, appear to consist of a multitude of brightly fluorescent and closely-packed axonal varicosities (Fig. 10A). Secondly, other ependymofugal processes, mainly of the CA type, can be traced as far as the inferior lobe of hypothalamus, laterally, and the torus semicircularis and optic tectum of the midbrain, dorsocaudally (Figs. 5, 6A). The latter projections are particularly well outlined in parasagittal sections prepared according to the SPG method. The same preparations also reveal that some CA fibers arising from CSF-contacting MA cells in the hypothalamus ascend within the lateral zone of the preoptico-hypothalamic complex to reach the telencephalon. A certain proportion of the CA fibers present in the lateral preoptico-hypothalamic region can be seen to cross the midline within the supraoptic decussation (Fig. 6B). Also worth noting is the presence of small clusters of fluorescent material dispersed throughout the posterior half of the hypophysis (Fig. 5A). These clusters, which appear to be formed by numerous closely-packed CA varicosities, are particularly abundant along the extensive perivascular system present within the neurointermediate hypophysial complex (see also [28,31]). In contrast, the anterior half of the hypophysis, comprising the adenohypophysis, is completely devoid of fluorescent material (Fig. 5B).

Small-sized CA cells are also present along the ependymal wall of the preoptic recess, among the non-fluorescent neurons of the magnocellular and parvocellular parts of the periventricular preoptic nucleus (Figs. 6C, 7A). The most rostrally located CA cells of this group lie just above the rostral pole of the optic chiasm (Fig. 7A). The CA neurons found along the preoptic recess display a fluorescence whose intensity varies from one cell to the other (Fig. 10B). Their processes do not seem to contact directly the CSF of the third ventricle but instead are mostly aligned parallel to the ventricular wall (Fig. 10C). These CA cells are also seen to contribute fibers which appear to ascend toward the telen-

cephalon through the lateral preoptic region (Fig. 6C). They may also provide part of the very dense pericellular CA innervation disclosed at the level of the periventricular preoptic nucleus itself (Fig. 7A).

In contrast to the hypothalamus, the thalamus does not contain MA cell bodies and appears relatively poorly innervated by MA axons. A moderately dense CA innervation occurs within the periventricular gray of the thalamus (Figs. 6A, B), whereas only a few, uniformly scattered, CA and 5-HT axonal varicosities are found in other thalamic regions.

Telencephalon (Figs. 6B, 6C, 7, 9C, 9F)

Despite the fact that MA-containing cell bodies are not found within the telencephalon, this portion of the brain nevertheless is densely innervated by MA fibers, particularly 5-HT axons, seemingly originating from the brain stem and hypothalamus. On the one hand, numerous ascending CA fibers can be visualized in the lateral preoptic region (Figs. 6C, 7A). Some of them sweep dorsally around the entopeduncular nucleus to reach the medial (Dm) and lateral (DI) zones of the area dorsalis telencephali where they appear to terminate (Figs. 6C, 7A). However, the majority of the ascending CA axons continue their rostral course, at the base of the telencephalon to reach the nucleus of the olfactory tract (Fig. 7B) and also the glomerular layer of the olfactory bulb (Figs. 7C, 9F) where they arborize profusely. On the other hand, a multitude of very fine 5-HT axonal varicosities can be visualized within the telencephalon. These varicosities are extremely abundant in the dorsal nucleus of area ventralis telencephali (see Fig. 7B), and moderately so in the dorsal (Dm) and lateral (DI) zones of area dorsalis telencephali (Figs. 7A, B). A significant number of 5-HT varicosities occurs also in nucleus taenia (Fig. 6C). The 5-HT innervation of the dorsal nucleus of area ventralis telencephali is one of the densest MA innervations to be observed in the entire brain of *Lepisosteus*. There, the 5-HT varicosities are very closely-packed and this whole area displays an intense and diffuse yellowish fluorescence (Fig. 10C). Within the dorsal and lateral zones of area dorsalis telencephali the 5-HT varicosities are more abundant near the brain surface and decrease progressively in number as we proceed more deeply into the telencephalic vesicle. Their distribution is more uniform in nucleus taenia.

DISCUSSION

The 5-HT-Containing Neuronal Systems

The 5-HT-containing neuronal systems in the brain of *Lepisosteus* are remarkably well-developed relative to those in teleosts such as the sunfish (*Lepomis gibbosus*). In *Lepisosteus* the raphe 5-HT neurons form a prominent cell column extending rostrocaudally from midbrain to upper spinal cord levels; whereas in *Lepomis* 5-HT cells are encountered in the raphe region only at caudal midbrain and isthmal levels. Furthermore, the 5-HT neurons at isthmal levels in *Lepisosteus* are not confined to the raphe region but extend through the lateral portion of the tegmentum. Such lateralization of the 5-HT systems has been noticed in some mammals [18], but is particularly obvious in birds where the 5-HT neurons massively invade the lateral tegmentum and become closely intermingled with the CA neurons of the locus coeruleus and subcoeruleus [11].

Axonal branches of these numerous 5-HT neurons in the brain stem of *Lepisosteus* arborize profusely at all levels of

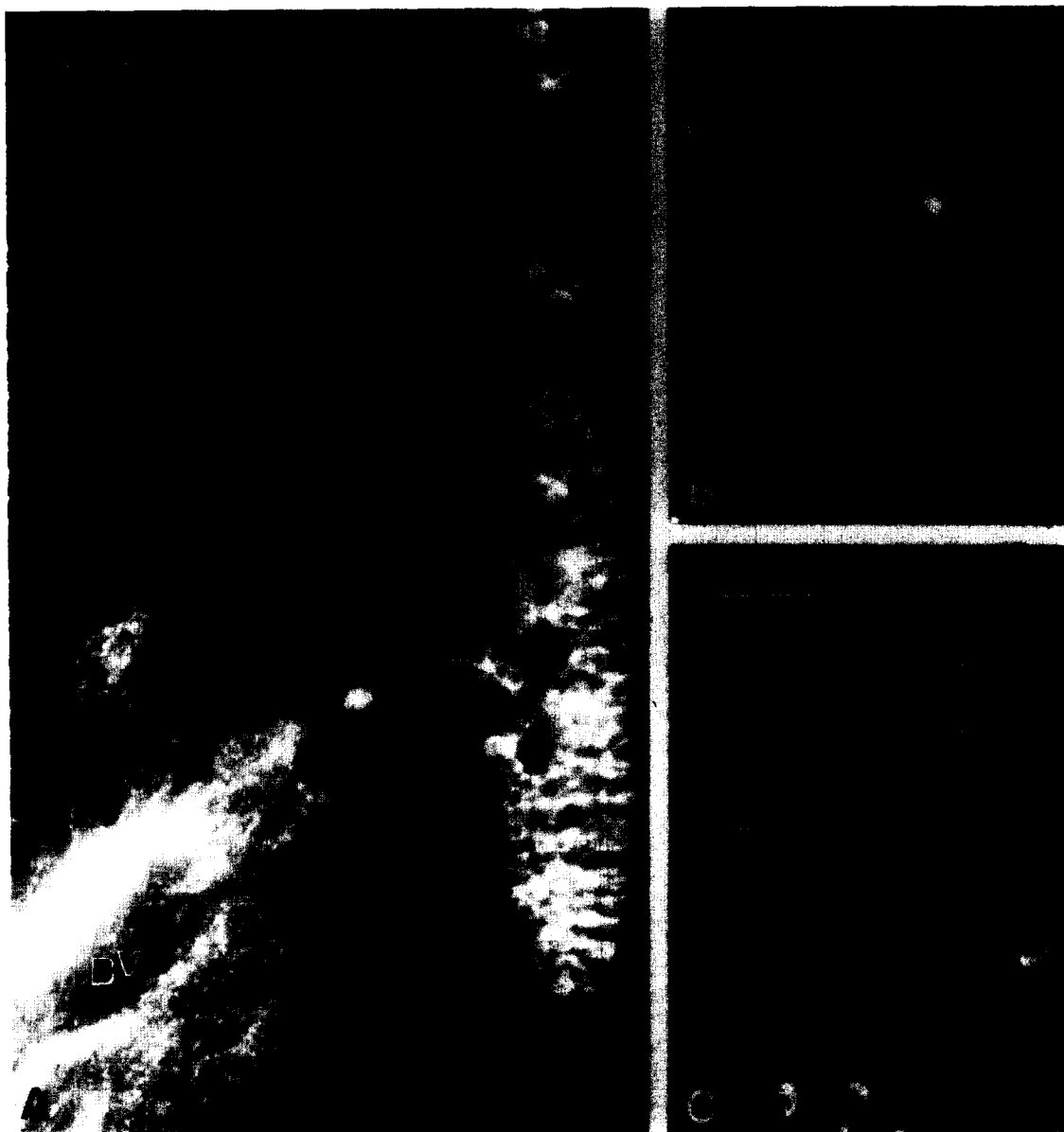


FIG. 10. A: Photomicrograph showing numerous CSF-contacting cells of both CA and 5-HT types bordering the third ventricle at hypothalamic level (see Fig. 6A). Abundant ependymofugal processes arising from some of these cells can be seen to form a dense patch of fluorescent material surrounding a longitudinally-sectioned blood vessel (BV). B-C: Photomicrographs depicting CA cell bodies present within the periventricular preoptic nucleus. Some of these cells display thick varicose processes (C) aligned parallel to the ventricular wall. Horizontal (A) and transverse (B, C) sections. Falck-Hillarp method.

the neuraxis. The structures most densely innervated by 5-HT axons include the spinal motor cells, the so-called cerebellar nucleus, and the dorsal nucleus of area ventralis telencephali.

In mammals, 5-HT is known to exert a potent influence on the activity of spinal motor cells [2,6]. This effect is apparently mediated by 5-HT-producing neurons located in nuclei raphe magnus and pallidus of the lower medulla, whose descending axonal processes arborize profusely around the neuronal somata of the spinal motor cells. Our study strongly suggests that a similar condition exists in holostean fishes. In contrast to mammals, however, the

5-HT varicosities around the spinal motor neurons in *Lepisosteus* arise from the axons of 5-HT-containing neurons whose cell bodies lie directly within the spinal cord, near the lumen of the central canal.

The "cerebellar nucleus" and the dorsal nucleus of area ventralis telencephali receive by far the heaviest 5-HT innervation in the brain of *Lepisosteus*. It is difficult to homologize the "cerebellar nucleus" to similarly located structures in other bony fishes. To our knowledge the dense, well-circumscribed 5-HT innervation in the periventricular gray of the isthmal region in *Lepisosteus* has not been demonstrated in any of the other vertebrate, and the very

dense 5-HT innervation of the dorsal nucleus of area ventralis telencephali may also be unique. Such dense 5-HT innervation is not known in the teleost telencephalon which is rather sparsely innervated by 5-HT axons [27,32]. In a recent reinterpretation of the organization of the telencephalon of bony fishes, Northcutt and Braford [22] proposed that the dorsal nucleus of area ventralis telencephali in actinopterygians is homologous to part of the septal region in land vertebrates. Similarly the septal region, particularly the medial septal nucleus, has been shown to contain a moderate number of fine 5-HT axonal varicosities in most land vertebrates investigated thus far. The density of the 5-HT innervation disclosed in the area ventralis telencephali in *Lepisosteus*, however, is far greater than that observed in the septal region in amphibians, reptiles or mammals [26].

The 5-HT systems are also well-developed in the hypothalamus of *Lepisosteus* where numerous CSF-contacting cells of the 5-HT type are intermingled among cells of the CA type along the wall of the third ventricle and its various recesses. Serotonergic CSF-contacting cells could not be detected with certainty among the multitude of CA cells which occurs in the hypothalamus of *Lepomis*. Although the presence of monoaminergic CSF-contacting cells appears to be one of the most constant features of the MA systems in the hypothalamus of non-mammalian vertebrates, the number of these cells, as well as the relative proportion of CA versus 5-HT elements, varies significantly among vertebrate groups [24]. In the eel (*Anguilla anguilla*), microspectrofluorometric methods have demonstrated that about 80% of the CSF-contacting hypothalamic cells are dopaminergic, and that the remaining cells contain 5-HT or 5-HTP [15]. A similar proportion of CA versus 5-HT CSF-contacting cells has been revealed in the hypothalamus of various other teleosts [12, 31, 32]. In *Lepisosteus*, the proportion of CSF-contacting cells of the 5-HT type is slightly higher (30–40%) than that found in teleosts. In mammals, there appear to be no hypothalamic 5-HT cells in direct contact with the CSF, although the third and lateral ventricles contain a dense supra- and subependymal plexus of 5-HT axons originating chiefly in the dorsal raphe nucleus [1,29]. Therefore, it appears that all vertebrates, including mammals, possess 5-HT neurons that form an important informative link between the CSF and the neural tissue.

The CA-Containing Neuronal Systems

In the brain of *Lepisosteus*, the CA-containing neuronal systems are rather poorly developed in comparison to the 5-HT systems. Their organization is similar to that in teleosts, but with some differences [19, 27, 32].

Among the most striking similarities are (1) the presence of a small number of CA perikarya in the central and dorsal (vagal lobe) regions of the medulla oblongata and (2) the existence of a multitude of small CSF-contacting cells of the CA type within the hypothalamus.

The CA perikarya in the central portion of the medulla in *Lepisosteus* are probably comparable to the CA cells of the A₁ and A₃ groups first described in the rat by Dahlström and Fuxe [8]. In mammals, these CA cells lie around and partly within the lateral reticular nucleus and form the medullary part of the so-called lateral tegmental CA cell system [20]. The CA perikarya in the dorsal (vagal lobe) region of the medulla in *Lepisosteus* are apparently homologous to the CA cells of the A₂ group in the rat [8]. In mammals, the CA cells of group A₂ are located mainly within and around the nucleus

of the solitary tract, and in the area postrema where their number varies markedly among different species [16]. This disseminated cell group is also referred to as the dorsal medullary CA cell system [20]. The presence of CA cells in both the central and dorsal portions of the medulla has been revealed in all vertebrate groups investigated thus far, including amphibians, reptiles, birds, and mammals [11,25]; thus, this feature of CA systems appears to be remarkably constant throughout phylogeny.

The abundant CSF-contacting cells of the CA type in the hypothalamus in *Lepisosteus* are very similar in topographical distribution and morphological characteristics to the hypothalamic CA cells in *Lepomis* [27] and other teleosts [15,31]. This large monoaminergic cell population in the hypothalamus is comparable to the less prominent MA cell group referred to as the paraventricular organ, which is present in the hypothalamus of amphibians, reptiles, and birds, but apparently absent in mammals (see review in [24]). In *Lepisosteus*, as in *Lepomis*, numerous ependymofugal processes of these CSF-contacting CA cells give rise to a very dense, patch-like innervation of the basal hypothalamus. Frequently, patches of fluorescent material, consisting of numerous fine, closely packed MA varicosities, are seen surrounding small capillaries; a situation resembling that found in the external layer of the median eminence of the hypothalamus in mammals [7]. In addition, the use of the glyoxylic acid method has allowed us to visualize other efferent projections from the CSF-contacting CA cells in *Lepisosteus*. These projections could be traced as far as the telencephalon and the midbrain tegmentum and tectum. Therefore, in addition to their highly probable role at hypothalamic and pituitary levels, the CSF-contacting hypothalamic CA cells may exert a significant effect on numerous brain structures in *Lepisosteus* and, perhaps, other bony fishes.

The most obvious differences in the organization of the CA systems in *Lepisosteus* and *Lepomis* include (1) the presence of CSF-contacting CA cell at upper spinal cord levels in *Lepisosteus* but not in *Lepomis*, and (2) only a few small-sized CA cell bodies at isthmal levels in *Lepisosteus* relative to the well-developed group of large-sized CA perikarya in the same region in *Lepomis*.

Although no CSF-contacting CA cells have been revealed at upper spinal cord levels in *Lepomis* [27] it should be noted that a local system of fluorescent neurons surrounds the central canal along the whole length of the spinal cord in the Lamprey (*Lampetra japonica*) [17]. In the pike (*Esox lucius*), a system of intensely fluorescent norepinephrine-containing cells lies among the basal ependyma of the central canal at lower spinal cord levels [5,30]. These cells, which are particularly abundant in the region of the urophysis, send varicose axonal processes which terminate on the neurosecretory cells of this structure in the pike. Although the lower segments of the spinal cord in *Lepisosteus* were not investigated in our study, it can be assumed that the CSF-contacting CA cells disclosed at upper spinal cord levels in the gar are part of a system extending all along the spinal cord similar to that revealed in the pike. It is also worth noting that a small group of CA cells was observed at the medullo-spinal junction in *Lepomis*; however, these CA cells do not lie within the cervical spinal cord but above it, immediately caudal to the obex of the fourth ventricle. A similarly located group of CA cells, termed the post-obocular CA nucleus, was recently revealed in another teleost, *Myoxocephalus scorpius* [32].

Unlike the isthmal tegmentum in *Lepomis*, with its

numerous large CA cells comparable to the locus coeruleus of land vertebrates [27], the corresponding region in *Lepisosteus* contains only a few small CA perikarya. Accordingly, the telencephalic vesicle in *Lepomis* and other teleosts [32] is densely innervated by CA axons, presumably originating in some part from the isthmal CA neurons, whereas the telencephalon in *Lepisosteus* except for the olfactory bulb region contains relatively fewer CA axonal varicosities. In this regard, *Lepisosteus* is similar to amphibians, who reveal only a very small group of CA cells in the isthmal tegmentum and very few CA axonal varicosities in pallial telencephalic regions [25].

Conclusions

In the brain of *Lepisosteus osseus*, a member of the holostean group of actinopterygian fishes, the MA-containing neuronal systems are organized very differently from those reported in teleost brains. Perhaps the most significant difference is that the central 5-HT-containing neuronal systems are remarkably more developed in holosteans than in

teleosts, whereas the converse is true for the CA systems. The pattern of MA system organization in the holostean brain is far more similar to that seen in primitive vertebrates, such as cyclostomes—where the 5-HT systems are highly elaborated relative to the CA systems [4]—than it is to the pattern in more advanced fishes such as teleosts. Because actinopterygians form a wide and divergent phylogenetic radiation with a high degree of interspecific variations, many species will have to be investigated to establish the significance of these morphological differences in the organization of central MA systems in holosteans and teleosts.

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