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Distribution of Monoamine-containing Neurons in the Brain of a Teleost, *Carassius auratus* (Cyprinidae)

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With 9 Figures and 1 Table

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Summary: The occurrence and distribution of monoamine-(MA) containing neurons and fibres in the brain of *Carassius* was investigated by formaldehyde-induced fluorescence (FIF) histochemistry (Falck-Hillarp technique). Many brightly green-fluorescent nerve cell perikarya were found in the nucleus dorsolateralis and ventromedialis, in the nucleus posterioris periventricularis, in the nucleus recessus lateralis and posterioris. They also occurred in the mesencephalic nucleus lateralis valvulae, in the metencephalic nucleus gustatorius secundus and near the ventricular borders of the facial and vagal lobes in the myelencephalon.
Many fluorescent fibres and nerve terminals were localized in the frontal and medio-lateral parts of the telencephalon, showing fluorescent connections to the caudal parts. In the diencephalon, MA-fibres branched in a horizontal and ventral tract, leading to the medulla oblongata and the hypothalamic nuclei, respectively. There were laterally situated fibres connecting the hypothalamic nuclei with the medulla and the nucleus gustatorius secundus. Many fluorescent fibres were found in the middle layers of the tectum opticum, in the torus semicircularis, in the lobus inferior and in the medulla oblongata. Considerably fewer fibres occurred in the corpus cerebelli and in the dorsal parts of the hindbrain lobes.
These results are compared with the MA-system in the brains of other fish.

Key words: Teleostean brain — Catecholamines — Fluorescence microscopy — *Carassius auratus* (Teleostei)

Introduction

Many catecholamine- and serotonin-containing neurons have been found in various tissues since Falck and Hillarp (Falck et al. 1962) introduced their fluoroscopic method for the localization of biogenic amines. There are early biochemical findings on the presence of monoamines (MA) in the brains of fishes (Bogdanski et al. 1963; Juorio 1973). However, since then, most work has focussed on the MA-system in the mammalian CNS, particularly in the rat brain. Much less is known about the distribution of monoaminergic neurons in non-mammalian brains (for literature see Santer, 1977; Parent et al. 1984).

Bertler et al. (1963) were the first to describe MA-specific fluorescence in the fish brain, followed by several studies on the MA-distribution in the whole brain of fishes (Lefranc et al. 1969; Parent et al. 1978; Watson 1980; Kotrschal and Adam 1983; and Parent and Northcutt 1982). Other studies investigated the hypothalamic area only (Honma and Honma 1970; Wilson and Dodd 1973; Ekengren 1975; Swanson et al. 1975; Fremberg et al. 1977; Terlou et al. 1978; Batten et al. 1979; Ekström and Van Veen 1982). Two of these, Baumgarten and Braak (1967) and Braak (1967), studied the goldfish.

The aim of the present study is to show the distribution of the MA-system in the whole brain of *Carassius auratus*.

Material and Methods

The distribution of monoaminergic neurons in the brain of the goldfish was studied by means of the Falck-Hillarp histofluorescence method (Falck and Owman 1965). A total of 94 goldfish (5–10 cm body length) were used for the present study. All fish were obtained from a commercial dealer.

1) FIF.

Fourty one animals were used for formaldehyde-induced fluorescence microscopy (FIF). Twelve animals were processed for FIF according to the modification of Loren et al. (1976) by intracardial perfusion. Eight animals were treated with the CA-precursor L-Dopa (Sigma, 400 mg/kg i.m., 30 and/or 6 h before sacrifice) or the MAO-inhibitor nialamide (Roche, 100 mg/kg i.m., 31 and/or 6 h before sacrifice). These drugs were used to increase the MA-concentrations in the tissues.

One animal was treated with reserpine (Serpasil, Ciba, 10 mg/kg i.m., 18 h before sacrifice). This was one of the tests for specificity of the observed fluorescence.

The animals were killed with MS 222 (Sandoz) and the brains were rapidly dissected out, shock frozen, freeze-dried,

gassed for 1 h at 80 °C with p-formaldehyde (70% rel. humidity) prepared according to HAMBERGER et al. (1965), and then vacuum-embedded in liquid paraplast. Sequential transverse or sagittal sections (10—20 μm) were mounted on clean slides, briefly dipped in celloidine (0.5—1%) and then covered with Depex-xylene (9 : 1). Where no celloidin-cover was used, adjacent slices were mounted and stained by standard histological techniques for precise localization of the fluorescent structures. Four brains similarly treated but not exposed to the formaldehyde gas, served as another control for MA-specific fluorescence.

2) Fluorescence microscopy.

The slices were examined with an Ortholux II fluorescence microscope (Leitz) using Leitz filter set D (Excitation; BP 355—425; beam splitter: RKP 455; barrier filter: LP 460). The mechanical stage of the microscope was modified with an additional stage for fine movements which was connected to an X-Y-plotter (HP 7035 B, Hewlett Packard) via two potentiometer-controlled d.c.-circuits, one for the X- and one for the Y-axis. All structures of the brain were recorded on paper using a cross-hair microscope eyepiece.

3) Histology.

Complete series of transverse, sagittal and horizontal sections of 30 goldfish brains, and whole decalcified heads, were stained according to Klüver-Barrera, Masson-Goldner, Azan, or silverimpregnated according to Bielschowsky, Bodian or Cajal (ROMEIS 1968).

4) HPLC.

The brains of 17 *Carassius auratus* were homogenized and their MA-concentrations measured electrochemically after separation by high performance liquid chromatography (HPLC; KISSINGER et al. 1981).

5) Microspectrofluorometry.

The emission-spectra of 80 green- and yellow-fluorescent perikarya and of varicose fibres in 2 *Carassius* brains were measured microspectrofluorometrically with an MPV-2 microscope (Leitz). This set-up was equipped with a Xenon high pressure lamp (Osram, XBO 75), filter set D (Leitz), a photomultiplier (EM 9558) and an S 20 cathod. The aperture could be closed to 2—3 μm. The measurements were made from 430 to 700 nm in 2 nm steps. Calibration of the photomultiplier and the correction of the spectra were according to Leitz-Mitteilungen (16/01.76).

The presence of noradrenaline was recognized by beadlike (varicose) fluorescent fibres (FALCK 1962). The discrimination of other monoamines (and their precursors) was difficult because all show green to yellow fluorescence after Falck-Hillarp treatment. The subjective fluorescence of CA at high concentrations only differs from 5-HT-/5-HTP-fluorescence by its slow fading (BJÖRKLUND et al. 1975). Because of a possible coexistence of CA and 5-HT/5-HTP in the same neuron the specifically fluorescent neurons were referred to as monoaminergic.

Results

The whole brain and the cranial nerves of *Carassius auratus* are shown in Fig. 1. The distribution of monoamine-containing neurons in the CNS is schematically indicated in a series of transversal sections (Fig. 2).

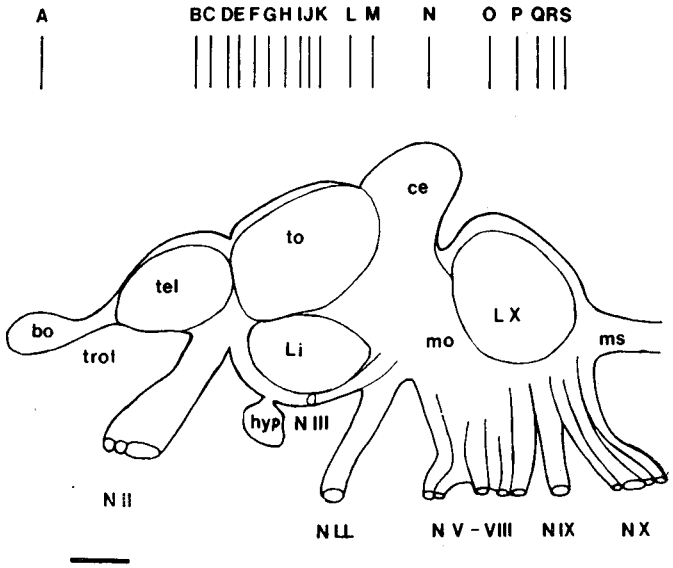

Fig. 1. Lateral view of the whole brain of *Carassius auratus*. Planes of transverse sections are indicated alphabetically in Fig. 2. Unless indicated otherwise, dorsal is up in all figures; bar = 500 μm. Abbreviations: bo bulbus olfactorius; ce — cerebellum; hyp hypophysis; Li lobus inferior; L X lobus vagi; mo medulla oblongata; ms medulla spinalis; NII nervus opticus; N III-X cranial nerves; tel telencephalon; trol tractus olfactorius.

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Fig. 2. Schematized transverse sections of the goldfish brain in rostro-caudal order. On the left, the distribution of CA-containing neurons (filled circles), of CA-fibres (dots or lines) and of yellow-fluorescent perikarya (open circles) is shown. The relevant anatomical structures are shown on the right. Bar = 0.5 mm. Abbreviations bo bulbus olfactorius; ca commissura anterior; cc corpus cerebelli; ce cerebellum; cm corpus mamillare; co chiasma opticum; cp commissura posterior; dc area dorsalis telencephali pars centralis; dd Fa. dors. tel. pars dorsalis; dl Fa. dors. tel. pars lateralis; dm Fa. dors. tel. pars medialis; eg eminentia granularis; fll fasciculus longitudinalis lateralis; flm fasciculus longitudinalis medialis; inf infundibulum; L VII lobus facialis; L X lobus vagi; mo medulla oblongata; nah nucleus anterior hypothalami; nap nuc. anterior periventricularis; nat nuc. anterior tuberis; ndl nuc. dorsolateralis thalami; ndli nuc. difusus lobi inferioris; ndtl nuc. diff. tori lateralis; ne nuc. entopeduncularis; ng nuc. glomerulosus; ngs nuc. gustatorius secundus; nh nuc. habenularis; nltp nuc. lateralis tuberis pars post.; nlv nuc. lat. valvulae cerebelli; nm nuc. medialis; np nuc. praetectalis; npo nuc. praeopticus; npp nuc. praeopticus periventricularis; nppv nuc. posterior periventricularis; npt nuc. posterior tuberis; nrl nuc. recessus lateralis; nrp nuc. rec. posterior; nsv nuc. saccus vasculosus; ntp nuc. post. thalami; nvm nuc. ventromedialis thalami; N II nervus opticus; teg tegmentum; tl torus longitudinalis; to tectum opticum; tol tractus olfactorius lateralis; tro tractus opticus; ts torus semicircularis; va valvula cerebelli; vd area ventralis telencephali pars dorsalis; vl a. ventr. tel. pars lateralis; vv A. ventr. tel. pars ventralis; v III ventriculus tertius; v IV ventriculus quartus.

Fig. 2H—M and 2N—S see page 532/533.

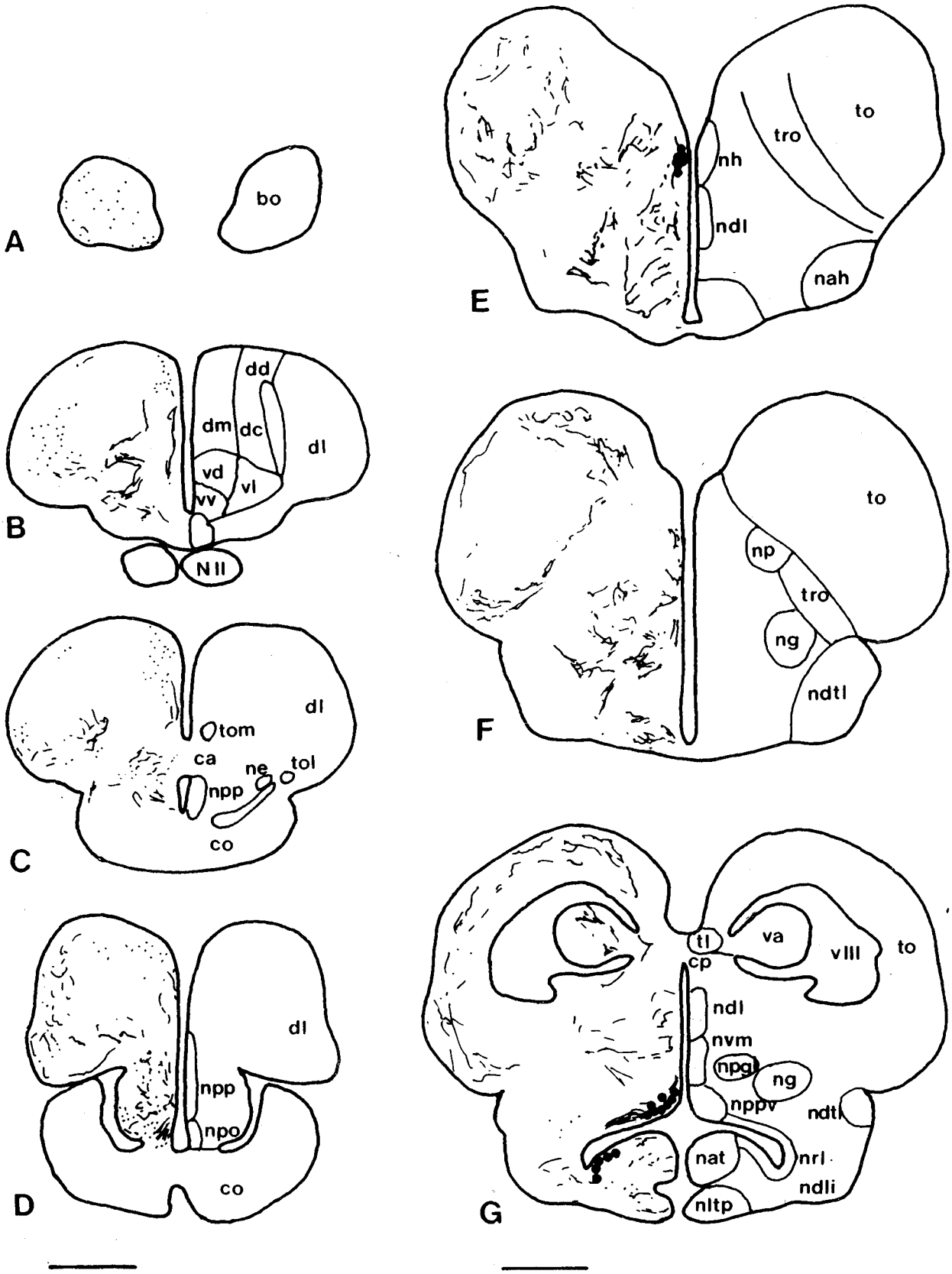
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Fig. 2A–G

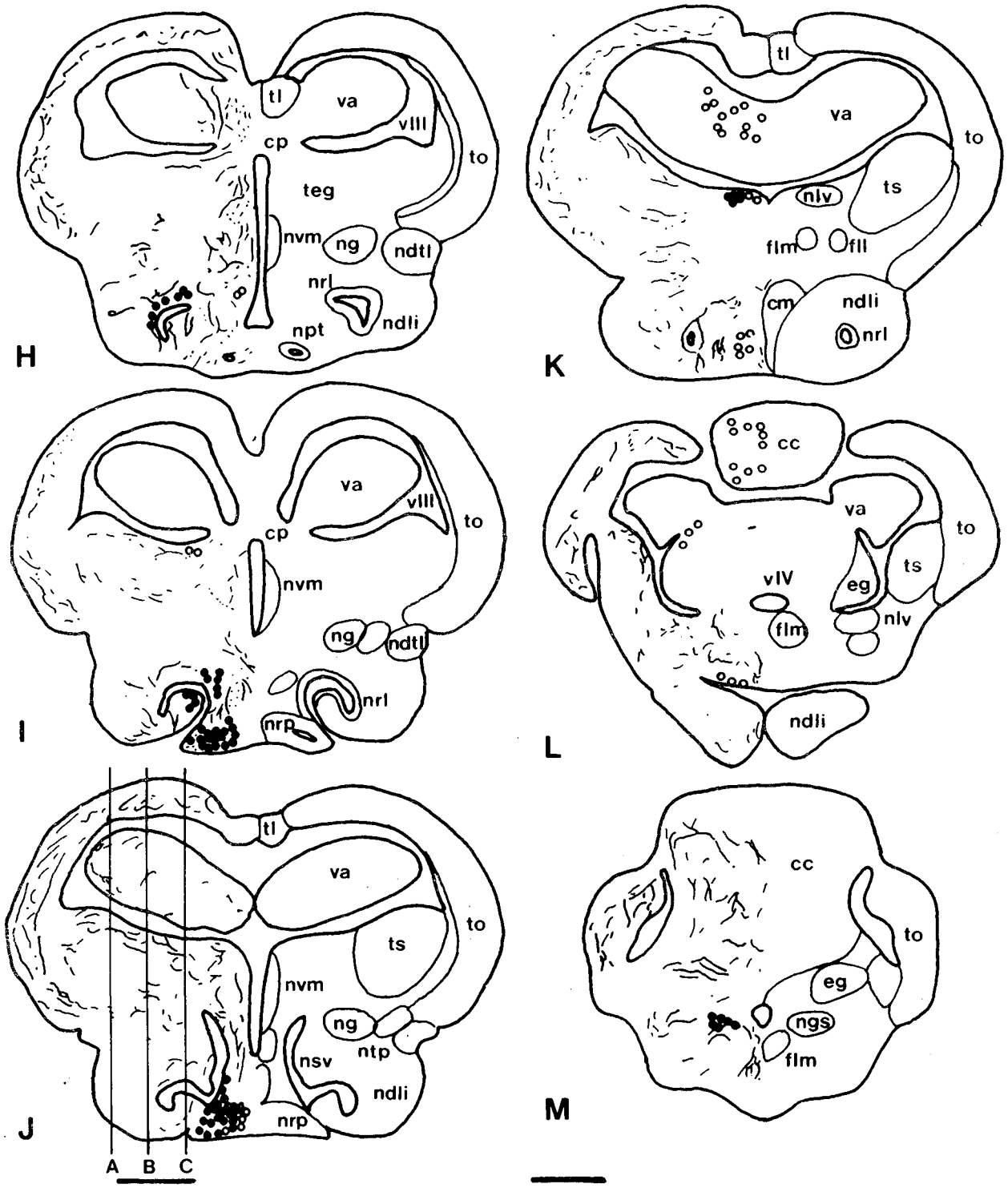

Fig. 2H—M

In the control animals no MA-specific fluorescence was observed. The distribution of the MA-containing neurons is described in rostro-caudal order. The nomenclature of the telencephalon and the forebrain nuclei is based upon that used by SCHNITZLEIN (1964) and PETER and GILL (1975). For other structures, HERRICK (1905), ARIENS KAPPERS et al. (1960), TUGE (1934, 1935), TUGE et al. (1968) and LUITEN (1975) were consulted.

1. Telencephalon

No green-fluorescent perikarya were found in the telencephalon (tel.). Some fluorescent fibres were scattered over the whole olfactory bulb. These were connected to the strongly innervated frontal part of the goldfish telencephalon (Figs 2A; 4B). There was a massive MA-innervation containing many varicose MA-fibres in the most rostral part of the area dorsalis

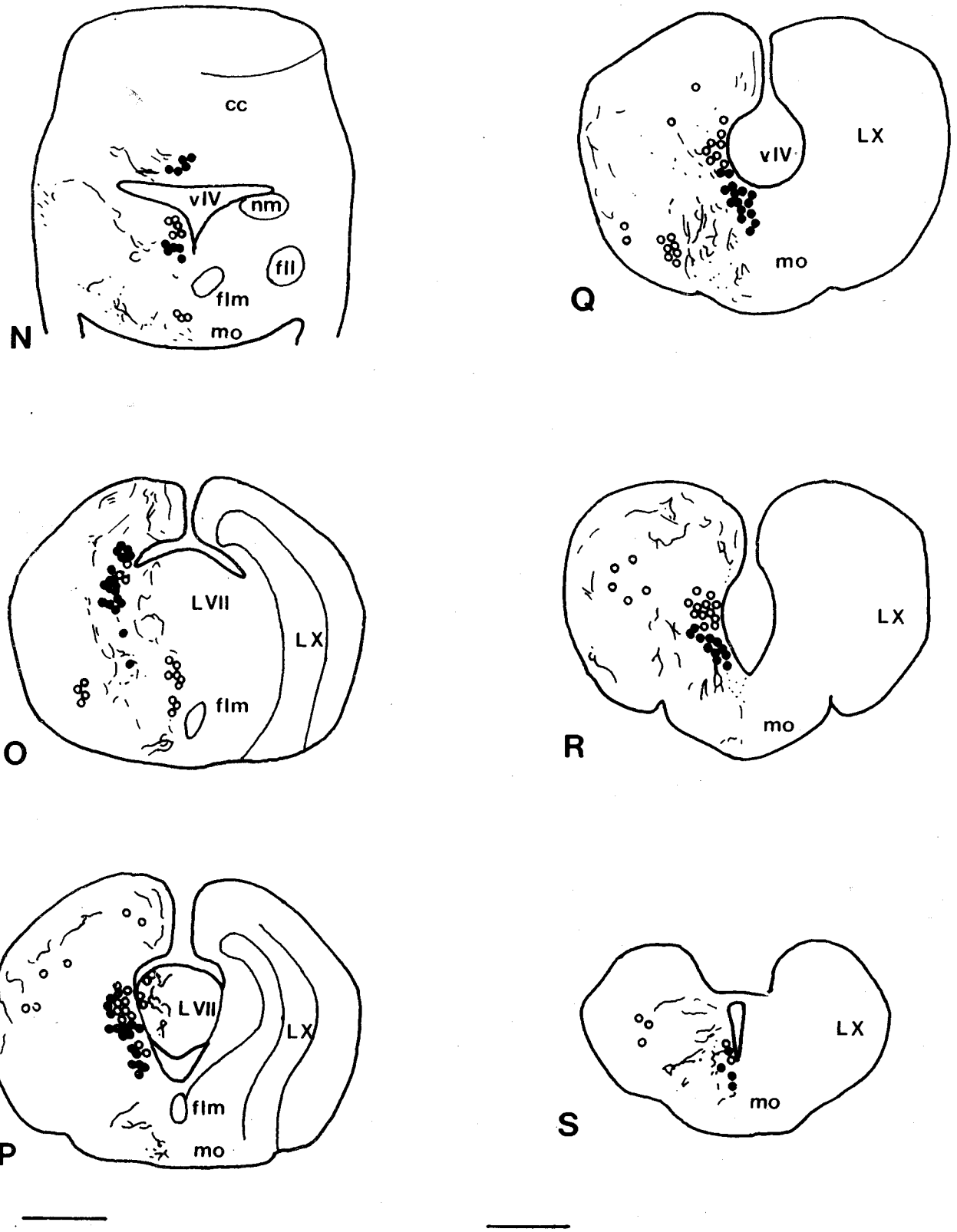
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Fig. 2N–S

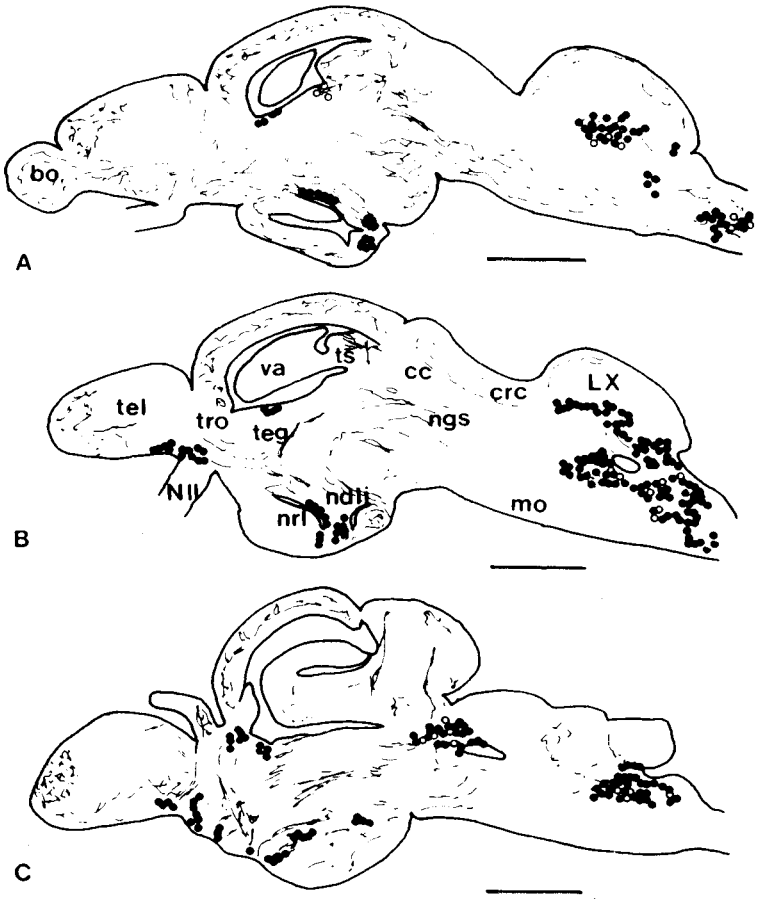

Fig. 3. Distribution of MA-containing neurons in three sagittal sections of the goldfish brain, from lateral (A) to medial (C) sagittal level. The positions of these sagittal sections are indicated in Fig. 2 J; Bar = 0.5 mm.

Abbreviations bo bulbus olfactorius; cc corpus cerebelli; crc crista cerebellaris; L X lobus vagi; mo medulla oblongata; ndli nucleus diffusus lobi inferioris; ngs nuc. gustatorius secundus; nrl nuc. recessus lateralis; N II nervus opticus; va valvula cerebelli; teg tegmentum; tel telencephalon; tro tractus opticus; ts torus semicircularis.

telencephali pars medialis (dm) and lateralis (dl). More caudally, most MA-fibres occurred in the pars medialis (dm) and the area ventralis telencephali pars dorsalis (vd) and lateralis (vl). Fewer MA-fibres were observed in the dorsolateral (dl) region and none in the dorsocentral (dc) area. In some preparations, some yellow-fluorescent perikarya were observed at the ventro-frontal side of the area dorsalis telencephali pars lateralis (dl), in most cases near blood vessels. MA-fibres were also present in the ventral parts of the area ventralis telencephali pars ventralis (vv) and lateralis (vl) and massively in the dl and dd (Fig. 4D, F). The fibres described above connect the caudal tel with the diencephalon (di) via the tractus olfactorius medialis (tom), others via the tr. olfact. lateralis (tol). In the caudal tel, anterior to the chiasma opticum (co), the medial, ventral and ventro-lateral areas were strongly MA-innervated. The number of the MA-fibres was still greater in the ventral area of the telencephalon at the level of the optic chiasm (Fig. 2C).

2. Diencephalon

MA-perikarya. In the frontal diencephalon (di), some perikarya of the nucleus praeopticus (npo) were weakly fluorescent, but surrounded by many MA-fibres. This nucleus belongs to the PRO (VIGH and VIGH-TEICHMANN 1973). The neurons were extremely weak yellow-fluorescent with a diameter of about 25 μm; they belong to the pars magnocellularis of the npo. The fluorescence of these cells was only seen after prolonged (2-hour) gassing with formaldehyde vapour which visualized the well-known peptidergic content of these neurons in UV light. None of these cells showed fluorescence in ungassed control brains.

The first green-fluorescent nerve cell bodies were found in the dorsally situated nucleus habenularis (nh, Fig. 2E). MA-neurons also occurred in the nucleus dorsolateralis (ndl) and ventromedialis (nvm) next to the 3rd ventricle (Figs 2G; 5F). These cell groups were connected to the medially situated part of the nucleus posterioris periventricularis (nppv) which consisted of strongly fluorescent nerve cell perikarya (Fig. 5G, H, I, K). These perikarya were situated along the lateral recess of the 3rd ventricle to the nucleus recessus lateralis (nrl, Fig. 2G, H, 5M). At a level between those represented by Figs 2H and I, the nrl contained many green-fluorescent neurons. Some of them formed club-like protrusions into the lumen of the ventricle (Fig. 5M) and belong to the liquor contact neurons. The two nuclei were interconnected by a small band of cells.

In the caudoventral part of the goldfish di, the nucleus recessus posterior (nrp) also contained intensely fluorescent MA-neurons (Fig. 2 I).

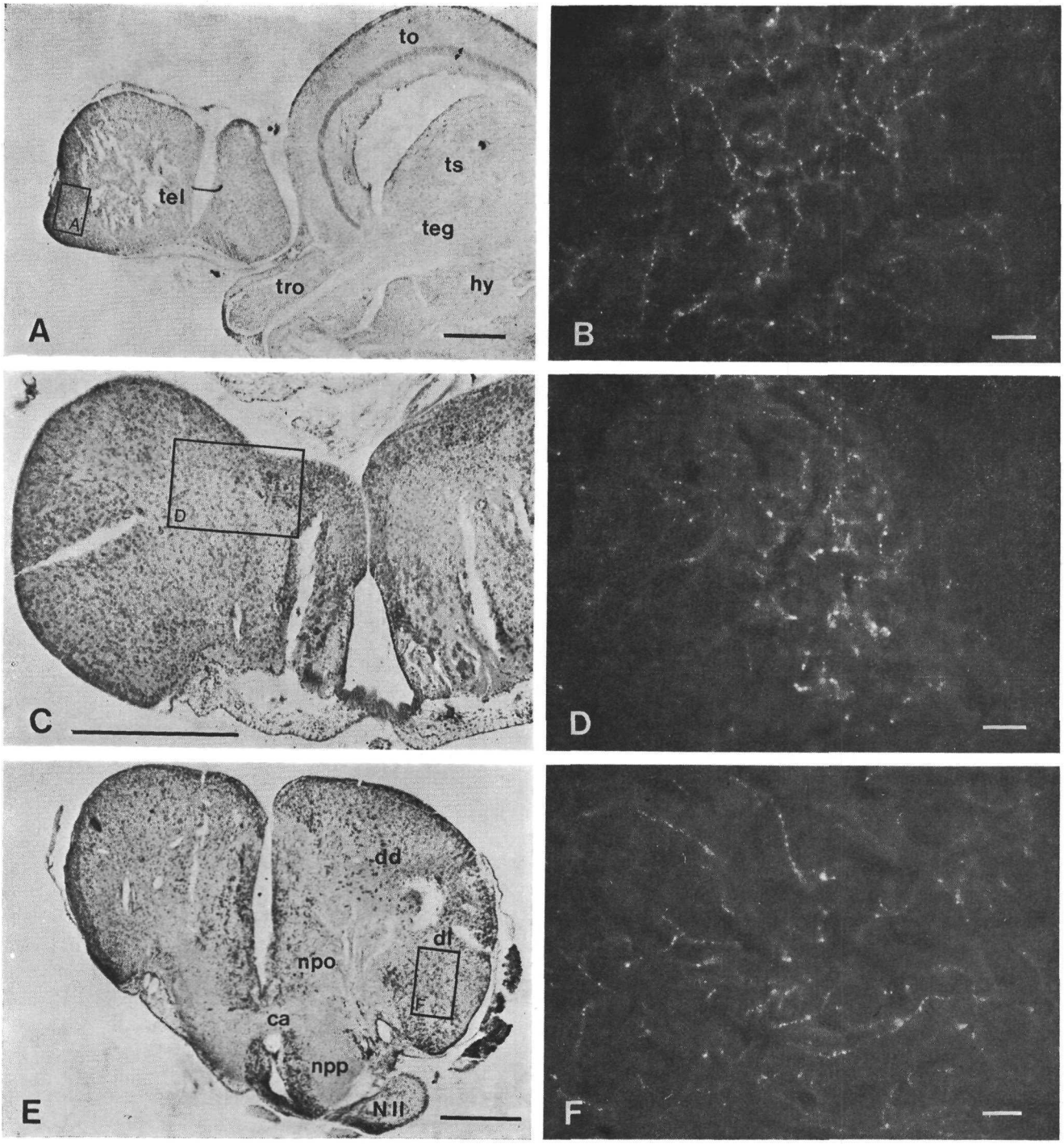

Fig. 4. Monoaminergic fluorescence in the telencephalon of *Carassius*. The position of the fluorescent structures is indicated in the photographs of Nissl-stained sections on the left side (A, C, E).

Abbreviations ca commissura anterior; dd area dorsalis telencephali pars dorsalis; dl a. dors. tel. pars lateralis; hy hypothalamus; N II nervus opticus; npp nucleus posterior periventricularis; npo nuc. praeopticus; teg tegmentum; to tectum opticum; tro tractus opticus; ts torus semicircularis.

A. Sagittal section of the forebrain of *Carassius*; Nissl-staining; bar = 500 μm.
B. MA-fibres in the frontal telencephalon of *Carassius*, as indicated in Fig. 4 A); FIF, sagittal section, bar = 50 μm.
C. Frontal section of the goldfish telencephalon; Nissl-staining; bar = 500 μm.
D. MA-fibres in the mediodorsal telencephalic area, as indicated in Fig. 4C); FIF; bar = 50 μm.
E. Transverse section of the forebrain of *Carassius* at the level of the commissura anterior (ca); Nissl-staining; bar = 500 μm.
F. Fluorescent fibres in the ventrolateral part (= dl) of the telencephalon; FIF; bar = 50 μm.

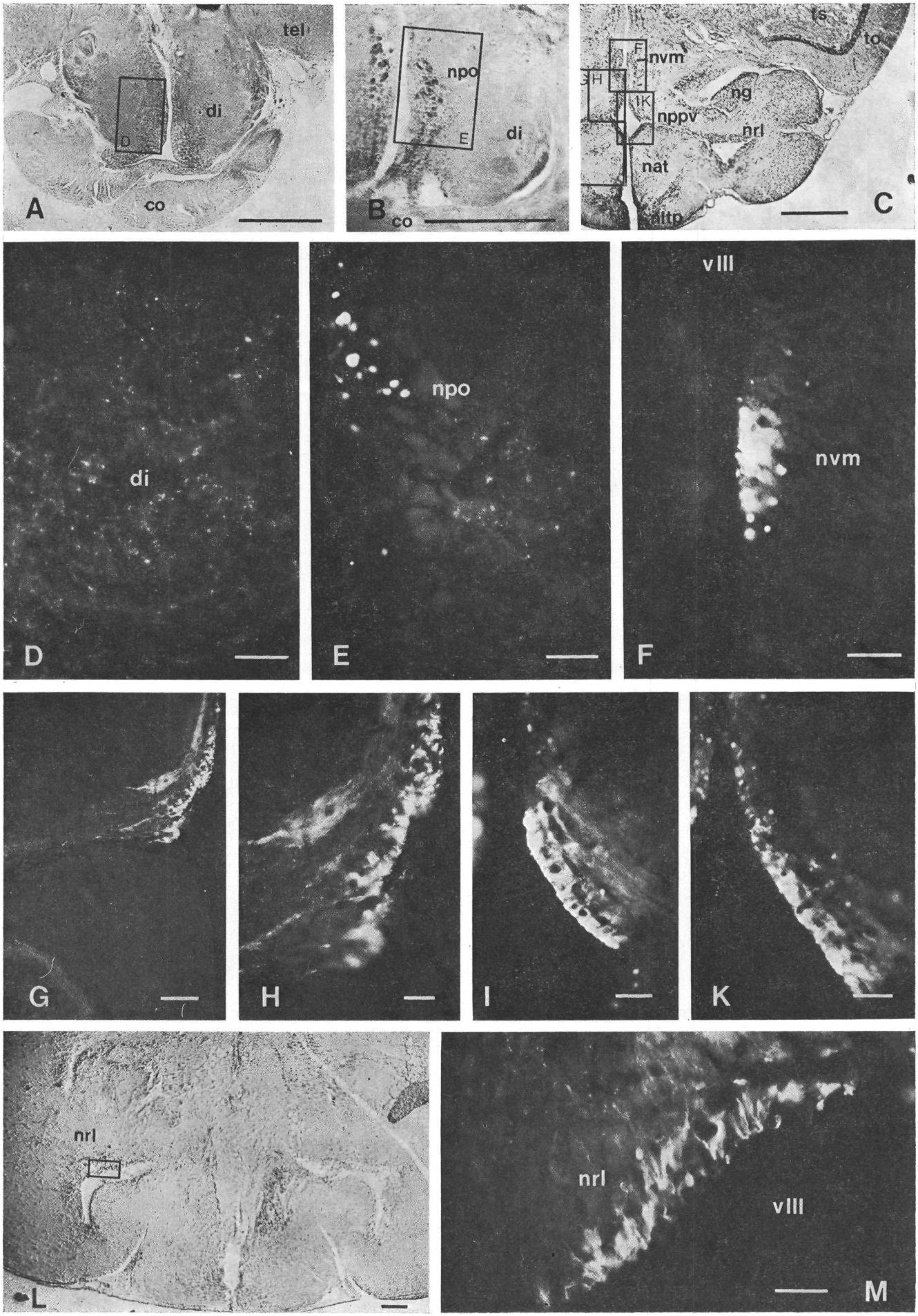
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Fig. 5

Yellow-fluorescent cells were recognized near the tractus olfactorius lateralis (tol, Fig. 2C) and in the region of the corpus mamillare (cm, Fig. 2J, K, L). Only few such cells were observed in ungassed sections.

MA-fibres. In the frontal diencephalon, many MA-containing fibres occurred in dorsoventral as well as in longitudinal extension (Fig. 2 C, D). In transverse sections they often appeared in V-shaped orientation with the highest densities of fibres and nerve endings in the medioventral diencephalon (Fig. 5D). No fluorescence was found in the commissura posterior (cp).

Caudal to the cp, fluorescent fibres connected the nppv-neurons with the nrl and ndtl (Fig. 5G). At the same level, MA-fibres occurred throughout most of the mediodorsal and ventral parts of the frontal and medial hypothalamus, except for the nucleus anterior hypothalami (nah, Fig. 2E). The fibres were arranged in mediodorsal orientation. In the frontal parts of the nucleus diffusus lobi inferioris (ndli, Fig. 2G), some MA-fibres could also be found while many fibres appeared dorsally and laterally to both the nucleus anterior tuberis (nat) and nucleus glomerulosus (ng, Fig. 2G). These were concentrated near the ventral part of the nucleus ventromedialis (nvm) and near the nrp and nrl in the ventral and lateral diencephalon, respectively. More caudally, fibres also ran dorsally, parallel to the midline and through the area of the nucleus posterior tuberis (npt). Such fibres were also present in the region of the nucleus glomerulosus (ng), its medial (npgm) and lateral parts (npgl) and in the nucleus posterioris thalami (ntp). Fibre projections ran between the nrl-neurons and the mesencephalic tegmentum (teg, Figs 2H, 6). Some MA-fibres could still be seen in the dorsal part of the ndli, in the ndtl (Fig. 2K) and in the ventromedially situated corpus mamillare (cm, Fig. 2K). In the frontal part of the ndli, some fibres appeared in the medial part, then scattering over the whole caudal area.

In the sagittal sections of the goldfish diencephalon, five populations of MA-fibre tracts could be recognized:

a) a laterally running fibre tract to or from the telencephalon dividing into a dorsal and a ventral branch. The ventral part was arranged in a V-shaped fashion

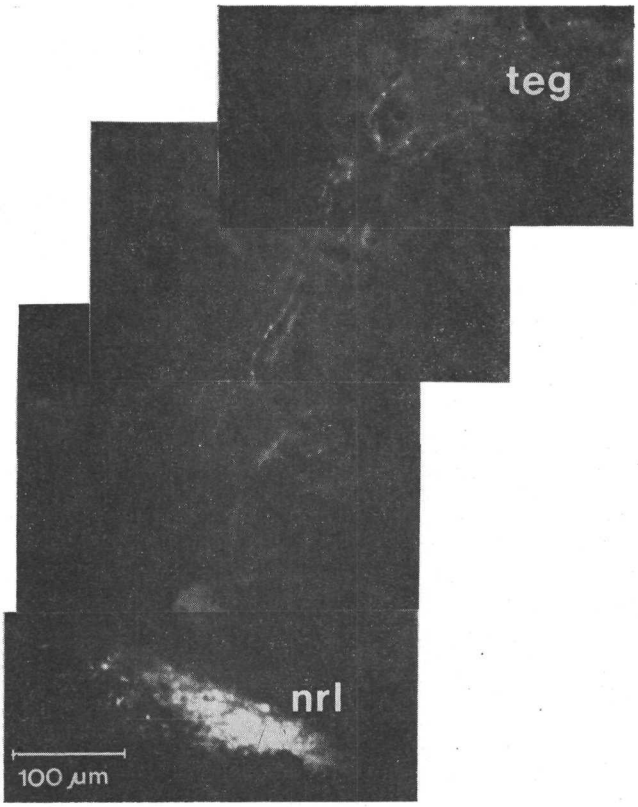

Fig. 6. Photomontage of sagittal sections of the dorsal hypothalamus and tegmentum of *Carassius*, showing brightly fluorescent perikarya in the nrl and MA-fibres projections to the tegmentum (teg); FIF; bar = 100 µm.

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Fig. 5. Monoaminergic fluorescence in the diencephalon of *Carassius*. Abbreviations co chiasma opticum; di diencephalon; nat nuc. anterior tuberis; ng nuc. glomerulosus; nltp nuc. lateralis tuberis pars posterior; npo nuc. praeopticus; nppv nuc. posterior periventricularis; nrl nuc. recessus lateralis; nvm nucleus ventromedialis; tel telencephalon; to tectum opticum; ts torus semicircularis; v III ventriculus tertius.

A. Transverse section of the frontal diencephalon; Nissl-staining; bar = 500 µm.

B. Transverse section, 340 µm more caudal to A), showing the small and big cells of the nucleus praeopticus (npo) in the frontal diencephalon; Nissl-staining; bar = 500 µm.

C. Transverse section of the ventral diencephalon; Nissl-staining; bar = 500 µm.

D. MA-containing fibres in the ventral diencephalon, as indicated in Fig. 5A); FIF; bar = 50 µm.

E. MA-perikarya at the border of the third ventricle, as indicated in Fig. 5B). Note the weakly fluorescent large perikarya which belong to the pars magnocellularis of the npo; bar = 50 µm.

F. Transversal section of the diencephalon at the level of the nucleus ventromedialis (nvm) as indicated in Fig. 5C); bar = 50 µm.

G. MA-containing perikarya and lateral fibre projections in the left side nucleus posterior periventricularis (nppv), as indicated in Fig. 5C).; FIF; bar = 100 µm.

H. Magnification of the fluorescent nppv of Fig. 5I); bar = 50 µm.

I., K. Magnification of the right side nppv, as indicated in Fig. 5 C). The level of K) is 40 µm more caudal to the level of I); FIF; bar = 50 µm.

L. Transversal section of the ventral diencephalon; Nissl-staining; bar = 100 µm.

M. MA-perikarya in the dorsal part of the nucleus recessus lateralis (nrl), as indicated in Fig. 5L); FIF; bar = 10 µm.

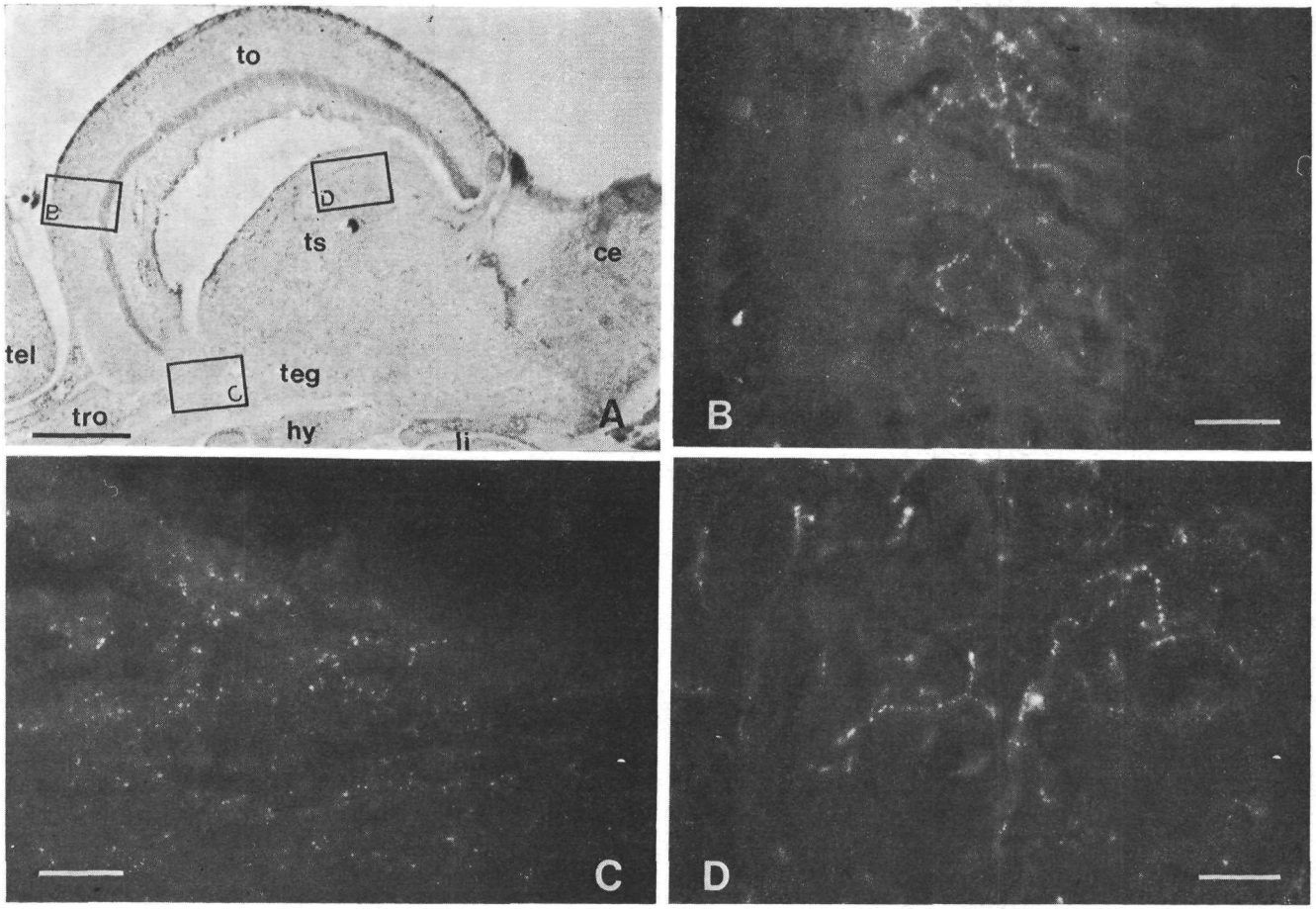

Fig. 7. Monoaminergic fluorescence in the mesencephalon of *Carassius*. Abbreviations ce cerebellum; hy hypothalamus; li lobus inferior; ng nucleus glomerulosus; nrl nuc. recessus lateralis; teg tegmentum; tel telencephalon; to tectum opticum; tro tractus opticus; ts torus semicircularis.

A. Sagittal section of the midbrain; paraplast; Nissl-staining; bar = 500 μm.
B. Sagittal section of the tectum opticum (to), as indicated in Fig. 7A); FIF; bar = 50 μm.
C. Sagittal section of the tegmentum with fluorescent fibres and nerve endings, as indicated in Fig. 7A); FIF; bar = 50 μm.
D. MA-fibres in the goldfish torus semicircularis (ts), as indicated in Fig. 7A); FIF; bar = 50 μm.

in the ventral hypothalamus, connecting the nucleus anterior tuberis (nat) and nucleus recessus lateralis (nrl). The other branch led horizontally to the ndli.

b) a medially running tract, probably the tractus olfactorius medialis (tom), led to the medulla oblongata (mo).

c) a MA-fibre tract between the caudoventral diencephalon and the frontal myelencephalic areas, probably with the nucleus gustatorius secundus (ngs).

d) a dorsal projection forming a connection between the nrl and the tegmentum.

e) a fibre tract first running dorsally to the teg, then turning horizontally to the medulla oblongata (mo).

3. Mesencephalon

MA-perikarya. A group of MA-containing neurons occurred at the ventral border of the third ventricle, e.g. in the dorsal part of the midbrain tegmentum, in the nucleus lateralis valvulae (nlv) (Fig. 2K). No further MA-containing cells were found in the goldfish mesencephalon.

MA-fibres. In the most rostral part of the tectum opticum (to), the fibres were evenly distributed throughout the periphery (Fig. 2F). Together with fibres occurring in the dorsal tectal areas and layers, a remarkable MA-innervation was seen in the mesencephalic tegmentum (Fig. 7C). The number of these fibres decreased in subsequent sections. Throughout the tectum, fibres were mainly found in the stratum fibrosum et griseum centrale and in the stratum fibrosum centrale, although some occurred in other layers (Fig. 7B).

The torus semicircularis (ts) was another MA-containing area in the brain of *Carassius* (Figs 2I, J, K; 7D) in which MA-fibres running parallel to the border of the ventricle in mediolateral orientation appeared. The MA-innervation was only visible in the

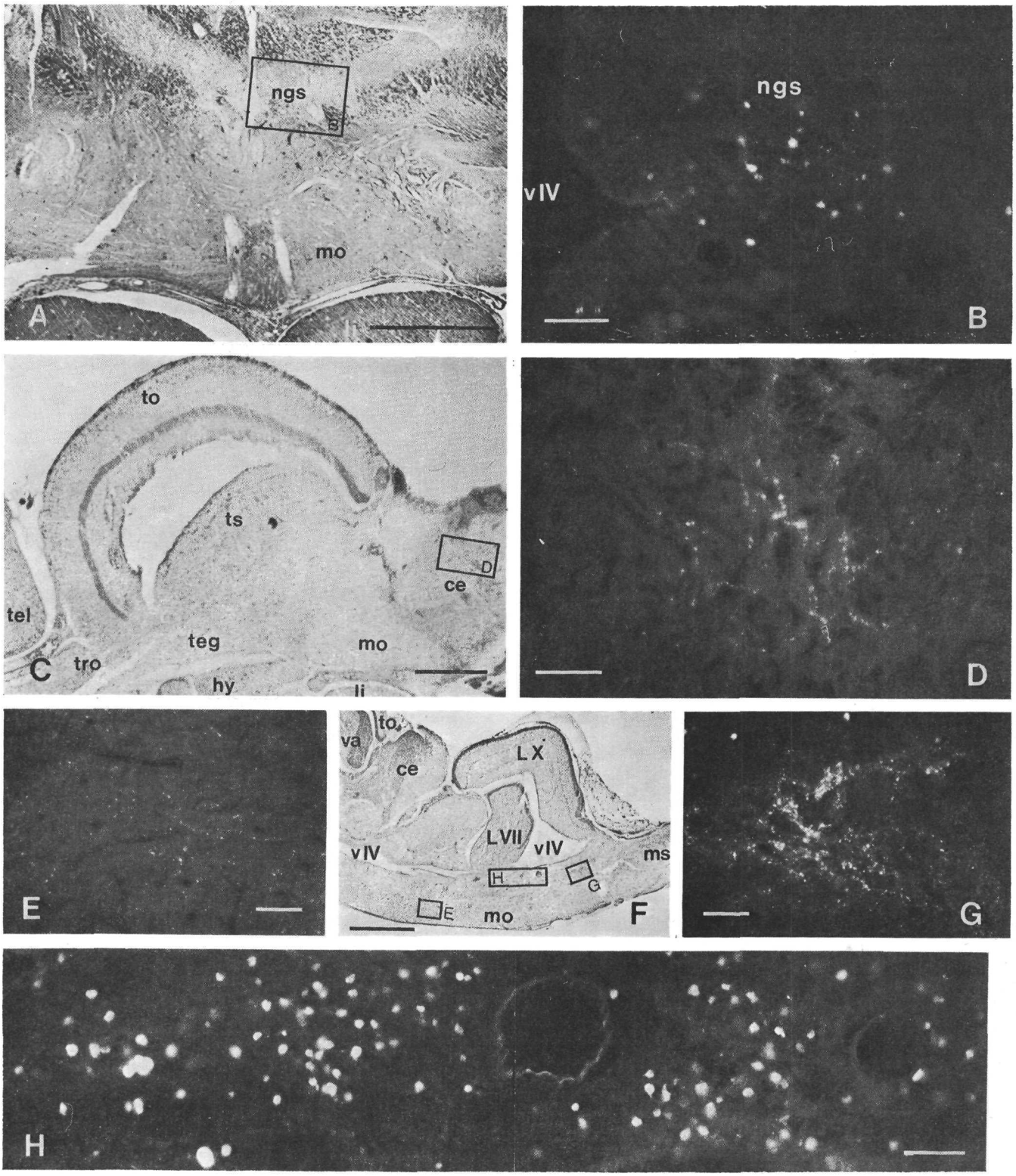

Fig. 8. Monoaminergic neurons in the rhombencephalon of *Carassius*. Abbreviations ce cerebellum; hy hypothalamus; li lobus inferior; L X lobus vagi; mo medulla oblongata; ms medulla spinalis; ngs nucleus gustatorius secundus; teg tegmentum; tel telencephalon; to tectum opticum; tro tractus olfactorius; ts torus semicircularis; va valvula cerebelli; v IV ventriculus quartus.

A. Transversal section of the rostral goldfish hindbrain, at the level of the nuc. gustatorius secundus and the caudal half of the lobus inferior; paraplast; Nissl-staining; bar = 500 μm.
B. MA-perikarya in the nuc. gustatorius secundus (ngs) as indicated in Fig. 8A); transverse section; FIF; bar = 50 μm.
C. Sagittal section of the goldfish hindbrain; paraplast; Nissl-staining; bar = 500 μm.
D. Fluorescent nerve-endings and fibres in the cerebellum (ce) of *Carassius* (as indicated in Fig. 8C); FIF; bar = 50 μm.
E. MA-fibres in the ventral medulla oblongata (mo), as indicated in Fig. 8F); FIF; bar = 50 μm.
F. Sagittal section of the goldfish hindbrain; paraplast; Nissl-staining; bar = 1 mm.
G. MA-fibres and nerve-endings in the dorsal medulla oblongata (mo), as indicated in Fig. 8F); FIF; bar = 50 μm.
H. Photomontage of MA-perikarya in sagittal sections of the goldfish hindbrain, near the border of the fourth ventricle, as indicated in Fig. 8F.; FIF; bar = 50 μm.

exterior part of the ts, whereas in its centre no fluorescent axons or nerve terminals were seen. Below this region, a bundle of MA-fibres was observed in longitudinal extent (Fig. 3B).

4. Rhombencephalon

a) Metencephalon

MA-perikarya. Except for some green-fluorescent perikarya in the nucleus gustatorius secundus (ngs) (Figs 2M, N; 3C; 8B) and a few yellow-fluorescent perikarya in the mediodorsal area of the valvula cerebelli (va) and in the frontal parts of the corpus cerebelli (cc; Fig. 2L), no further fluorescent cells were seen in the goldfish metencephalon.

MA-fibres. In the va, only few fibres occurred (Figs 2G—K). This was also true in the cc (Fig. 8D), where some MA-fibres appeared in ventral orientation (Fig. 3).

b) Myelencephalon.

MA-perikarya. Fluorescent cells always appeared throughout most of the lateral border of the 4th ventricle (Figs 2O—S; 3A—C; 8H). At the caudal end of the lobus facialis (L VII), the number of the green-fluorescent perikarya increased continuously, most of them lying in the ependymal wall of the hindbrain ventricle (v IV), dorsally to the fasciculus longitudinalis medialis (flm) which was completely non-fluorescent. Green-fluorescent cells appeared at the ventricular border of the vagal lobe (Figs 2O—R). Among this very large area of green-fluorescent MA-cells, relatively few neuronal somata showed yellow fluorescence. The number of these perikarya was greatly increased after nialamide administration, but markedly reduced following reserpine treatment. In untreated brains the number of yellow-fluorescent cells was rather small.

MA-fibres. A strong MA-innervation was observed in the ventral and dorsomedial parts of the medulla oblongata (mo; Figs 2M—S; 3A; 8E—G). These fibres were oriented mediolaterally (Fig. 2N) as well as longitudinally (Fig. 3A). They seem to connect the mid- and hindbrain areas in the goldfish brain (Fig. 3). At the level of the cc and caudally, the MA-innervation of the medulla was concentrated in the ventral and dorsomedial areas (Figs 3A; 8G). In most sections, the fibres were concentrated in two bundles in the ventral medulla (Fig. 3D). There was also a remarkably dense MA-innervation in the vagal (L X) and facial (L VII) lobes (Fig. 2P). In caudal direction, fascial MA-innervation increased and fluorescent fibres were observed running in vertical orientation.

5. MA-concentrations in the brain of Carassius

The MA-concentrations were determined by HPLC (Table 1). Adrenaline was below the limit of detection (about 4 ng/g wet weight).

Noradrenaline (NA) concentrations were significantly greater than dopamine (DA) concentrations in all brains and brain parts ($p < 0.01$; Wilcoxon matched-pairs signed-ranks test). The NA-concentrations of the tel and di-mes were markedly higher than those of the hindbrain ($0.02 < p < 0.05$).

The DA-concentration in the tel was significantly lower than those of the other brain regions ($p < 0.02$). The DA-concentration of the combined di-mes was significantly higher compared with the hindbrain parts. There was no difference in the DA-concentrations of the two hindbrain areas. The NA- over DA-concentration ratios of the telencephalon as well as those of the di- and mesencephalon differed significantly from the NA- over DA-concentration ratios of the caudal brain parts. There was no difference between the NA/DA-ratios of the cerebellum (including the frontal medulla oblongata) and the myelencephalon.

Tabelle 1. Mean values and standard deviations (SD), of weight (W), brain weight (BW), and the concentrations of the catecholamines noradrenaline (NA) and dopamine (DA), and the indoleamine 5-hydroxytryptamine (5-HT). Na/DA = mean concentration ratio of NA over DA (N = 27 except * where N = 19; pi = pituitary; n.d. = not detected).

brain part	W (g)	BW (mg)	NA (ng/g wet weight)	DA (ng/g wet weight)	5-HT (ng/g wet weight)	NA/DA
total	4.00	63.7	640	130	88*	5.5
(SD)	0.22	2.4	25	9	19	
telencephalon	(N = 7)		781 ± 149	40 ± 6	n.d.	20
di-/mesencephalon	(N = 7)		772 ± 60	120 ± 12	n.d.	6.7
cc/mo	(N = 7)		404 ± 39	78 ± 6	n.d.	5.2
myelencephalon	(N = 7)		422 ± 48	83 ± 9	n.d.	5.2
pituitary	(N = 3)		53 ± 18	107 ± 13	n.d.	0.5

The mean 5-HT-concentration of the whole brain of *Carassius* was about 75% of the DA-concentration. Because of technical difficulties the 5-HT-concentrations was not determined for individual brain parts.

6. Microspectrofluorometry

The emission spectra of green and yellow-fluorescent perikarya and axons of the nuc. praeopticus, nuc. post. periventricularis, nuc. rec. lateralis, nuc. rec. post, lobus inferior, tectum opticum, torus semicir-

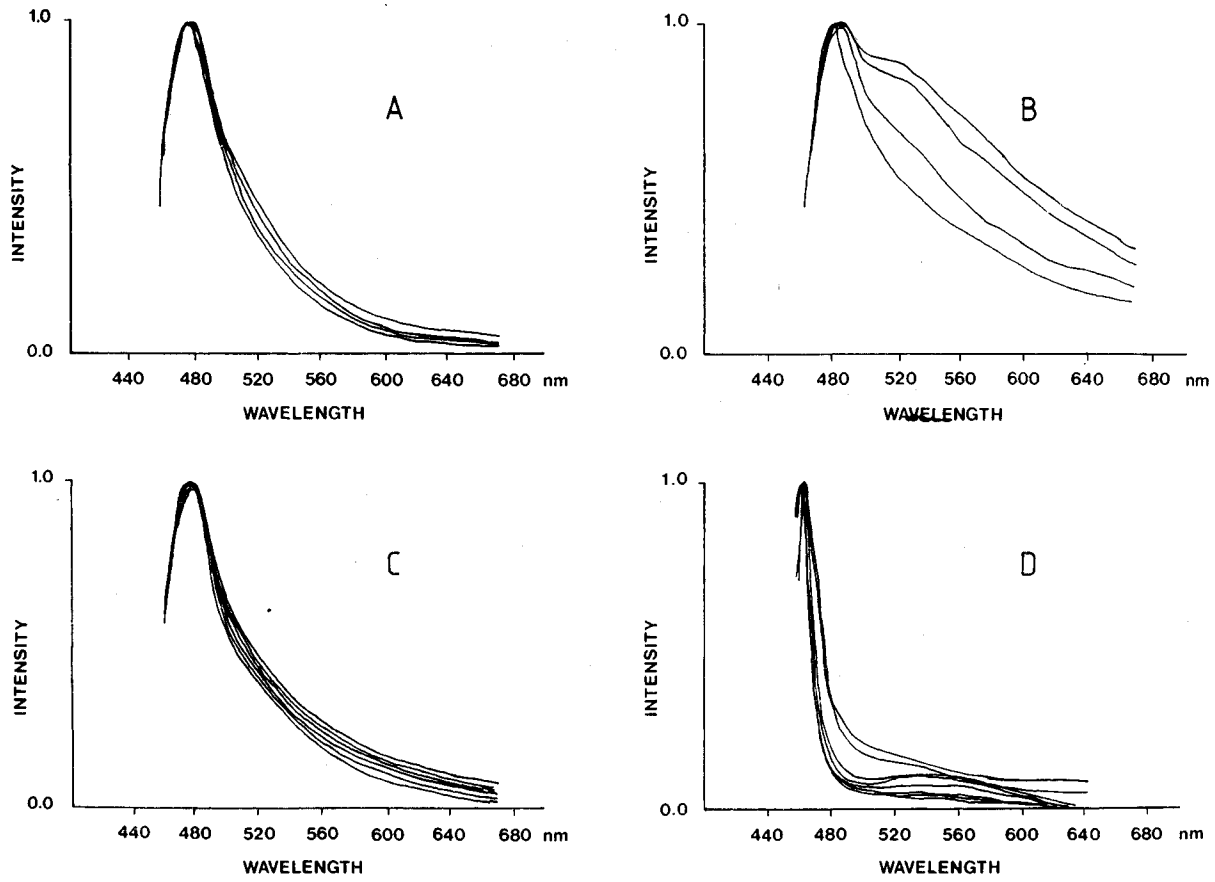

Fig. 9. Emission spectra of fluorescent neurons in the brain of *Carassius*.

A. Green fluorescent perikarya with emission maxima of 475—480 nm.

B. Yellow-fluorescent perikarya with similar emission maxima as in A); note the different spectral intensity above 500 nm.

C. Varicose fluorescent fibres.

D. Unspecific fluorescent neurons from formaldehyde-treated material and from ungassed controls.

cularis, valvula cerebelli, corpus cerebelli, nuc. gust. secundus, medulla oblongata, lobus vagi, and periventricular myelencephalic cells were measured by microspectrofluorometry (MSF).

All green-fluorescent and most of the yellow-fluorescent neurons and fibres (Fig. 9A, B, C) had emission maxima of about 470—480 nm, and therefore are regarded as MA-specific. Some of the fluorescent cells and fibres, however, had emission maxima of about 460 nm with a steep decline of fluorescence at greater wavelengths, indicating unspecific fluorescence (non-aminergic content; Fig. 9D). The emission spectra of the green-fluorescent cells (Fig. 9A) are almost identical to the spectra of the green- to yellow-fluorescent fibres (Fig. 9C), while the spectra of the yellow- or yellowish-green-fluorescent neurons show more intense fluorescence above 520 nm. This may be due to either high CA-concentrations (BJÖRKLUND et al. 1975) or — in addition — to small amounts of faded 5-HT which normally has its emission maximum at about 520 nm.

Discussion

The fluorescence of Falck-Hillarp-treated MA-containing tissue may vary from green or yellowish-green to pure yellow. These subtle differences in colour tones are difficult to interpret because of the change of spectral sensitivity of the eye with increasing light intensity (JONSSON 1967). Therefore, corrected spectra were used in microspectrofluorometry. These show maxima at 470—480 nm, which all primary and secondary catecholamines have in common.

The similarity of the corrected emission spectra of green-fluorescent perikarya and varicose fibres suggests the presence of the same transmitter. This is in agreement with FALCK (1962) who considered varicose fluorescent fibres as noradrenergic, and with the present findings of a high noradrenaline content and the exclusive presence of MA-specific varicose fibres in the telencephalon.

The similarity of the emission maxima of MA-specific green- and yellow-fluorescent cells suggests

a concentration-dependent shift of the subjectively observed fluorescence (BJÖRKLUND et al. 1975).

After nialamide- and reserpine-administration the number of yellow-fluorescent cells increased and decreased, respectively. Both drugs influence the amount of CA and 5-HT of neurons (BJÖRKLUND et al. 1972, 1975). The fact that the fluorescence of these cells did not fade rapidly during UV-illumination should exclude 5-HT/5-HTP. However, the possibility that both CA and 5-HT (or related compunds) occur in the same neuron cannot be excluded (BOER et al. 1984).

Although the brains which were perfused and treated according to the modification of LOREN et al. (1976) contained more intensely fluorescent neurons than those treated with the standard FIF technique, the enhancement observed was weaker than obtained by these authors. The reasons for this are still not clear. However, no difference could be noticed in the intensity and occurrence of the fluorescence between the perfused brains and those pretreated with MAO-blocking agents or CA-precursors. Thus the results of the standard FIF-technique were confirmed by the modified perfusion technique.

Fluorescent fibres in the olfactory bulb were also described in *Lepomis* (PARENT et al. 1978), *Myoxocephalus* (WATSON 1980) and *Blennius* (KOTRSCHAL and ADAM 1983) where they seemed to be located primarily in the dorsal parts. The presence of MA-fluorescence in the telencephalon corresponds well with the findings in other fish.

Although some green-fluorescent cells have been described in a nucleus of the posterior tel of *Anguilla* (LEFRANC et al. 1969), this was not confirmed by FREMBERG et al. (1977), and nothing similar has been reported for other teleosts or lower vertebrates. Except for some yellow-fluorescent cells (presumably mast cells) no other fluorescent cell bodies were found in the telencephalon of *Carassius*. A further indication of this MA-innervation is the high MAO-activity in the goldfish telencephalon (KUSUNOKI and MASAI 1966).

Telencephalic MA-fibres are present in several lower vertrebrates. They have also been described in *Salmo* and *Anguilla*, and a strong MA-innervation is known from *Lepomis* and *Myoxocephalus*. Lateral and medial to the area dd of *Myoxocephalus*, green-fluorescent fibres have been described to form a network, similar to that found in the dm and dl of *Carassius*.

Strong MA-innervation in the rostral hypothalamic area was also reported by BAUMGARTEN and BRAAK (1967). Similar results were found in *Salmo* (BERTLER et al. 1963), in *Lepomis* (PARENT et al. 1978), *Myoxocephalus* (WATSON 1980) and *Blennius* (KOTRSCHAL and ADAM 1983). MA-containing perikarya were also observed in *Carassius*, while in *Salmo* (TERLOU et al. 1978), a network of fluorescent fibres was found in the preoptic recess. In both species, the anterior commissure (ca) is completely non-fluorescent while high densities of specific fluorescence occurred in the area preoptica. This was also found in *Lepomis* (PARENT et al. 1978), in *Blennius* (KOTRSCHAL and ADAM 1983), in *Rana* (BRAAK 1970), in *Ambystoma* (SIMS 1977) and in *Lacerta* (MARSCHALL 1980). Except for *Anguilla* (FREMBERG et al. 1977), no other study has reported fluorescent habenular regions in lower vertebrate brains.

Strong fluorescence in the nucleus recessus posterior (nrp) and lateralis (nrl) of *Carassius* (BAUMGARTEN and BRAAK 1967) was confirmed in the present study. Similar results had been obtained in *Salmo* (TERLOU et al. 1978; BERTLER et al. 1963), in *Leuciscus* (EKENGREN 1975), in *Anguilla* (FREMBERG et al. 1977), in *Blennius* (KOTRSCHAL and ADAM 1983) and in *Lampreta* (BAUMGARTEN 1972; KONSTANTINOVA 1973). These fluorescent cell groups occurred in similar configuration in the brains of *Lebistes reticularius, Hemichromis bimaculatus, Ictalurus nebulosus* (BONN, unpublished), as well as in *Eigenmannia lineata* (BONN and KRAMER, submitted). They seem to be typical of teleost brains and the previously reported contact of these cells with the ventricle has also been found in the present study. Secretion of neurotransmitters into the cerebrospinal fluid is considered unlikely, but certain sensory and/or control functions have been proposed (KONSTANTINOVA 1973; TERLOU et al. 1978; VIGH-TEICHMANN and VIGH 1983). The 'hypothalamic feeding area' (DEMSKI 1981; PETER 1979) also contained monoaminergic neurons. The MA-fibres which most likely connect the nrp and nrl with the ndli are typical of *Carassius, Lepomis, Myoxocephalus* and *Leuciscus*. The MA-fibre tract connecting the ventrolateral hypothalamus and the midbrain could be part of HERRICK's gustatory fibre tract III, to the fibres coursing through the caudal tegmentum in *Lepomis*, and to the ventromedially running fibre system in this brain region of *Myoxocephalus*.

The presence of cell bodies in the region of the nucleus lateralis valvulae have not been reported previously for *Carassius* or any other teleost brain. Although such cells appeared near blood vessels and therefore could be confused with histamine-containing mast cells, the low sensitivity of the FALCK-HILLARP-technique for histamine makes this interpretation unlikely.

In the tectum opticum of *Carassius*, fluorescent fibres always appeared in the stratum fibrosum et griseum superficiale and in the stratum fibrosum centrale. Strong MAO-activity in this region was reported by KUSUNOKI and MASAI (1966). A similar pattern of MA-fluorescence was found in the to of

Myoxocephalus, Lepomis, Lampreta, Rana and *Lacerta*, while KOTRSCHAL and ADAM (1983) reported a uniform MA-fibre distribution in the tectum of *Blennius*. The present findings of fibres in the torus semicircularis are similar to those in *Lepomis* and *Myoxocephalus*.

The yellow-fluorescent cells demonstrated in the present study have not been observed in the rhombencephalon before. The yellow-fluorescent cell bodies dorsolateral to the fasciculus longitudinalis regions of *Lepomis* and *Myoxocephalus* were not observed in *Carassius*.

In the valvula cerebelli (va) of the goldfish (present study), as well as in *Lepomis* (PARENT et al. 1978), only few fibres were observed while in *Myoxocephalus* neither the va nor the corpus cerebelli (cc) contained fluorescent fibres. The weak fluorescence in the cc of *Carassius* may account for the faint MAO-activity in that region (KUSUNOKI and MASAI 1966). Although CA-perikarya also occurred in the myelencephalon of *Lepomis* and *Myoxocephalus*, the number of MA-cells in the hindbrain of *Carassius* was much greater.

In addition to its fast fading, the absence of specifically fluorescent yellow neurons in the raphe-region of the *Carassius* brain may be due to 1) the low sensitivity to 5-HT/5-HTP (about 30% of the sensitivity for CA; BJÖRKLUND et al. 1975) of the FIF technique. 2) the relatively low 5-HT content in the brain of *Carassius*, 3) to diurnal changes in the 5-HT-concentrations in the teleost brain (MARGOLIS-KAZAN et al. 1985).

According to EVANS (1934), the structure of the hindbrain lobes reflects the feeding habits in teleost fishes. Neither in *Lepomis* nor in *Myoxocephalus* are the myelencephalic lobes so strongly developed as in *Carassius* which has well developed gustatory and olfactory systems. The gustatory and olfactory fibres system in the brain of the carp brain (HERRICK 1905) is very similar to the MA-fibres decribed here. Because of the close relationship of the carp and the goldfish, both belonging to the Cyprininae, and their similar brain structures and feeding habits, this similarity could be expected (for literature concerning olfaction and taste in fishes, see FINGER 1975, 1978).

Abbreviations

CA: catecholamines; DA: dopamine; FIF: formaldehyde induced fluorescence; HPLC: high performance liquid chromatography; MA: monoamines; MAO: monoamineoxidase; MSF: microspectrofluorometry; NA: noradrenaline; S. E.: standard error; 5-HT: 5-hydroxytryptamine (serotonin); 5-HTP: 5-hydroxytryptophane.

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