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Tracing of single fibers of the nervus terminalis in the goldfish brain

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Summary. Central projections of the nervus terminalis (n.t.) in the goldfish were investigated using cobalt- and horseradish peroxidase-tracing techniques. Single n.t. fibers were identified after unilateral application of cobalt chloride-lysine to the rostral olfactory bulb. The central course and branching patterns of individual n.t. fibers were studied in serial sections. Eight types of n.t. fibers are differentiated according to pathways and projection patterns. Projection areas of the n.t. include the contralateral olfactory bulb, the ipsilateral periventricular preoptic nucleus, both retinae, the caudal zone of the periventricular hypothalamus bilaterally, and the rostral optic tectum bilaterally. N.t. fibers cross to contralateral targets in the anterior commissure, the optic chiasma, the horizontal commissure, the posterior commissure, and possibly the habenular commissure. We propose criteria that differentiate central n.t. fibers from those of the classical secondary olfactory projections. Branching patterns of eight n.t. fiber types are described. Mesencephalic projections of the n.t. and of secondary olfactory fibers are compared and discussed with regard to prior reports on the olfactory system of teleosts. Further fiber types for which the association with the n.t. could not be established with certainty were traced to the torus longitudinalis, the torus semicircularis, and to the superior reticular nucleus on the ipsilateral side.

Key words: Nervus terminalis – Telencephalon – Diencephalon – Tracer (cobalt, HRP) studies – Olfactory system – Goldfish

Since the discovery of the nervus terminalis (n.t.) during the last century the central projections of the "supernumerary" cranial nerve have been a subject of interest and research (for literature reviews, see Bonin 1941; Larsell 1950; Graziadei 1976). Various targets of the n.t. in the tel-, di- and mesencephalon were described in numerous studies (McKibben 1911; Holmgren 1918; Larsell 1918; Herrick 1948; von Bartheld and Meyer 1986a). However, the precise locations of termination, as well as the functional significance of the n.t. largely remain in the dark.

Recently, the n.t. has attracted attention for the following reasons: (1) N.t. fibers were revealed to be part of a luteinizing hormone-releasing hormone (LHRH) fiber system in the central nervous system (Schwanzel-Fukuda and Silverman 1980; Münz and Claas 1981; Münz et al. 1981, 1982; Stell et al. 1984). (2) N.t. projections to the retinae in teleosts (Tractus olfacto-opticus, Holmgren 1918) were confirmed by modern tracer studies (horseradish peroxidase: Ebbesson and Meyer 1981; Münz et al. 1981, 1982; Demski and Northcutt 1983; cobalt: Springer 1983). (3) Selective stimulation of the medial part of the medial olfactory tract (mMOT), which mediates secondary olfactory fibers and n.t. fibers, evoked sperm release in male goldfish (Demski and Northcutt 1983; Demski and Dulka 1984).

Investigations of central n.t. projections in certain fishes have to deal with one major technical problem: The proximity of the n.t. ganglion and the olfactory bulb (BO) proper, as well as the proximity of their central pathways, which render selective labeling a difficult task. Selective labeling of n.t. pathways thus seems possible only for those n.t. ganglion cells with peripheral processes into the olfactory mucosa (namely by injections into the mucosa) or with central processes to the retinae (namely by injections into the eyes). So far all other tel-, di- and mesencephalic n.t. projections could not be discerned with certainty from the classical secondary olfactory fibers (mitral cell axons). This problem seems to be partly responsible for the fact that we know considerably less about n.t. fibers projecting to the mesencephalon than about those that innervate the retinae (Münz et al. 1981, 1982; Bass 1981; Springer 1983; Demski and Northcutt 1983; Stell et al. 1984) or the rostral telencephalon (Bass 1981; Prasada Rao and Finger 1984; Levine and Dethier 1985).

The present study was performed to gain information on further caudally situated n.t. targets, and to approach an answer to questions regarding the functional role of this cranial nerve. On the basis of our findings we propose criteria that should help to differentiate central n.t. fibers from those of secondary olfactory projections. The branching patterns of individually traced central n.t. fibers are analyzed, thus providing evidence for collateral innervation of a variety of CNS structures.

The "standard" species for n.t. studies in lower vertebrates, the goldfish (*Carassius auratus*), was chosen for the present investigation, because the majority of recent studies dealing with the n.t. in fishes has used this species (Stacey and Kyle 1982, 1983; Demski and Northcutt 1983; Springer

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1983; Demski and Dulka 1984; Fernald and Finger 1984; Stell et al. 1984; Levine and Dethier 1985).

Materials and methods

In the present study 29 goldfish of 10 to 13 cm in body length were used. They were obtained from commercial sources and kept at a temperature of 21° C. Prior to all surgical procedures the animals were anesthetized with tricaine methane-sulfonate.

The main group of fishes was injected with cobalt chloride-lysine into the rostral part of the olfactory bulb (BO). Control groups were injected with either horseradish peroxidase (HRP) or cobalt into the caudal telencephalon; another group of specimens received an HRP injection into the rostral BO.

Main group. The left olfactory bulb was exposed and cobalt chloride-lysine was injected into the rostral part of the bulb using a glass microsyringe. Occasionally, we added 1% dimethyl sulfoxide (DMSO) to the cobalt chloride-lysine. Iontophoresis (1–5 μA) for 15–30 min was used to facilitate the outflow of cobalt ions. The survival time for the injected fish ranged between 18 and 25 h. Following anesthesia the animals were perfused through the conus arteriosus with phosphate-buffered saline (PBS), partly containing dissolved hydrogen sulfide. For fixation we used 2.5% glutaraldehyde (8–12 h). The fixed brains were transferred to a 30% sugar solution to avoid freezing artifacts in the later stages of the procedure. The brains were cut in a cryostat at 25 or 40 μm thickness in the frontal plane.

Sections were intensified according to the procedure of Gallyas (1979) for 8–10 min and counterstained with neutral red or cresyl violet. An intensification procedure similar to the one used in our study has recently been published (Bazer and Ebbesson 1984). Chartings of fiber courses in serial sections were carried out with the help of camera lucida-drawings. All sections containing more than one n.t. fiber to be individually traced were charted. Only cases in which the further course of the fiber could be determined without any doubt are described as "single fiber traced".

Control groups. One group of control specimens was injected with horseradish peroxidase (HRP) or cobalt chloride-lysine into the caudal telencephalon unilaterally after transection of the longitudinal trajectories. Some of these brains were cut in the sagittal plane at 25 or 40 μm, respectively; the other brains were cut in the frontal plane. Retrogradely filled central processes of n.t. ganglion cells were traced rostrally to their somata in the rostral olfactory bulb and the caudal olfactory nerve (compare Fig. 9 A–C). Another group of control specimens was injected with HRP into the rostral olfactory bulb in the proximity of the n.t. ganglion.

The HRP-injected fish were allowed to survive for four or five days. They were anesthetized and transcardially perfused with PBS and 2% glutaraldehyde. Frontal sections of the CNS and sagittal sections of the olfactory bulbs and tracts were cut at 25 μm in a cryostat. Alternate sections were mounted on two sets of slides and reacted with diaminobencidine (LaVail and LaVail 1972) or benzidine dihydrochloride (DeOlmos and Heimer 1977; Ebbesson et al. 1981a), respectively. Cobalt-injected specimens rendered better results with regard to morphological details of labeled nerve fibers. Favorable HRP cases show the precise sites of n.t. termination more clearly. The HRP material was helpful for understanding interpretations of previous studies solely relying on HRP techniques.

Sections of goldfish specimens injected with HRP into the olfactory bulb proper (von Bartheld et al. 1984) were used in order to compare the present findings with secondary olfactory pathways and projection areas. Abbreviations used in the text and the Figs. are listed in Table 1.

Results

I. Methodological considerations

The central connections of the nervus terminalis (n.t.) are suitable for cobalt-labeled single fiber tracing for the following reasons:

1. Sectioned n.t. fibers take up cobalt very well and are labeled intensively.
2. The n.t. ganglion in goldfish contains a restricted number of ganglion cells.
3. Fibers of the n.t. possess an axon diameter considerably larger than that of secondary or primary olfactory fibers; these morphological details are revealed in cobalt-labeled material.
4. N.t. fibers extend over a rather long distance in the central nervous system, thereby facilitating selective and individual fiber tracing.

Our findings provide information about eight different n.t. fiber types with regard to their central course, branching patterns and approximate area of termination in the tel-, di-, and mesencephalon. Fiber types of the n.t. are designated (1)–(8), reflecting the caudal extension of the fibers in the CNS and the areas of termination in a rostro-caudal sequence.

When continuous tracing of single n.t. fibers (from rostral telencephalic levels) was not possible, this is mentioned in the text and the corresponding fiber pathway is represented as a *dotted* line in the summarizing schemes (Figs. 1, 3, 5, 7). Dotted lines are also used to indicate the assumed n.t. fiber course in the medial olfactory tract.

In the present study we use the terminology for CNS nuclei and fiber tracts as suggested by the following authors: telencephalon: Northcutt and Braford (1980); Northcutt and Davis (1983); diencephalon: Braford and Northcutt (1983); optic tectum: Northcutt (1983); mesencephalon and rhombencephalon: Nieuwenhuys and Pouwels (1983). For the designation of sub-tracts of the olfactory tract we applied the terminology as used by Stacey and Kyle (1982, 1983); Demski and Northcutt (1983); von Bartheld et al. (1984) and Demski and Dulka (1984).

II. Experimental results

N.t. fiber type (1). Approaching the anterior commissure (AC) in the medial part of the medial olfactory tract (mMOT) the n.t. fiber runs ventrally on the lateral border of the ventral nucleus of the area ventralis telencephali (Vv). At the level of the AC the fiber turns medially (now located ventrally to the Vv nucleus) and enters the ventral portion of the AC. The fiber branches precisely in the midline of the AC (see Fig. 2A). Further tracing reveals a symmetrical distribution of the course of the branching fibers: One branch follows the fibers crossing in the AC to the contralateral hemisphere, the other one turns back on the ipsilateral side. Both fibers surround the Vv nucleus on their rostral course, first ventrally, then laterally, and again enter the mMOT, one branch ipsilaterally, the other contralaterally. Signs of further branching or termination could not be observed at levels of the AC or rostral telencephalon. The ipsilateral branch could be individually followed to levels of the rostral telencephalon only, because too many cobalt-labeled fibers are contained in the ipsilateral mMOT. Possibly the ipsilateral branch enters the ipsilateral olfactory tract and bulb. The contralateral branch could be individually traced through the rostral telencephalic hemisphere in the contralateral mMOT and through the peduncle of the mMOT. The fiber enters the contralateral olfactory bulb; the location of termination (in the bulb proper or n.t. ganglion?) could not be determined.

The course of the n.t. fiber type (1) is schematically summarized in Fig. 1. It seems unlikely that this n.t. fiber is labeled retrogradely rather than anterogradely for the following reason: One of the fiber branches coursing through the ipsilateral mMOT apparently possesses a larger diameter up to the branching point (Fig. 2A).

N.t. fiber type (2). This fiber enters the AC using a course similar to that described for n.t. fiber type (1). In the center of the AC the fiber heads dorsally and ramifies through the supracommissural nucleus of the area ventralis telencephali (Vs) (see Fig. 2B). Arborization of this n.t. fiber was not observed in Vs. Eventually the fiber runs through the contralateral Vs nucleus and follows the course of the fibers crossing in the horizontal and dorsal part of the AC. In a position just caudal to the level of the AC the n.t. fiber branches (see Fig. 2C). One collateral possesses a larger diameter; it enters the contralateral central nucleus and possibly the medial nucleus of the area dorsalis telencephali (Dc and Dm). The exact location of termination could not be observed.

The other collateral with the smaller caliber (see Fig. 2C) turns abruptly ventrocaudally and enters the (contralateral) lateral forebrain bundle. It could be traced into the diencephalon. At the level of the habenular nuclei the staining of this rather thin fiber completely vanishes in the area in between the contralateral nucleus preglomerulosus anterior (PGa) and the nucleus pretectalis superficialis, pars magnocellularis (PSm).

The course of n.t. fiber type (2) is schematically summarized in Fig. 1.

N.t. fiber types (3) and (4): Since olfacto-retinal projections have been described at length elsewhere (Münz et al. 1981, 1982; Bass 1981; Springer 1983; Demski and Northcutt 1983; Stell et al. 1984), we will concentrate on some new findings concerning the courses of single fibers.

N.t. fiber type (3). This fiber leaves the medial forebrain bundle in caudolateral direction. At the level of the ascending optic tract the n.t. fiber enters the layer of the medial optic tract fibers and courses within the optic tract in ventromedial direction. Until the optic chiasma is reached, the n.t. fiber remains in a medial, later dorsal position within the optic tract (see Fig. 2D). The fiber crosses with the decussating fibers of the optic chiasma to the (contralateral) optic nerve and apparently continues its course to the contralateral retina.

Possibly this fiber type possesses a collateral coursing from the optic chiasma to the area of the ipsilateral nucleus pretectalis superficialis, pars magnocellularis (PSm); however, this collateral pathway could not be proven in continuous single fiber tracings and may represent an independent fiber type.

The course of the n.t. fiber type (3) is schematically summarized in Fig. 1.

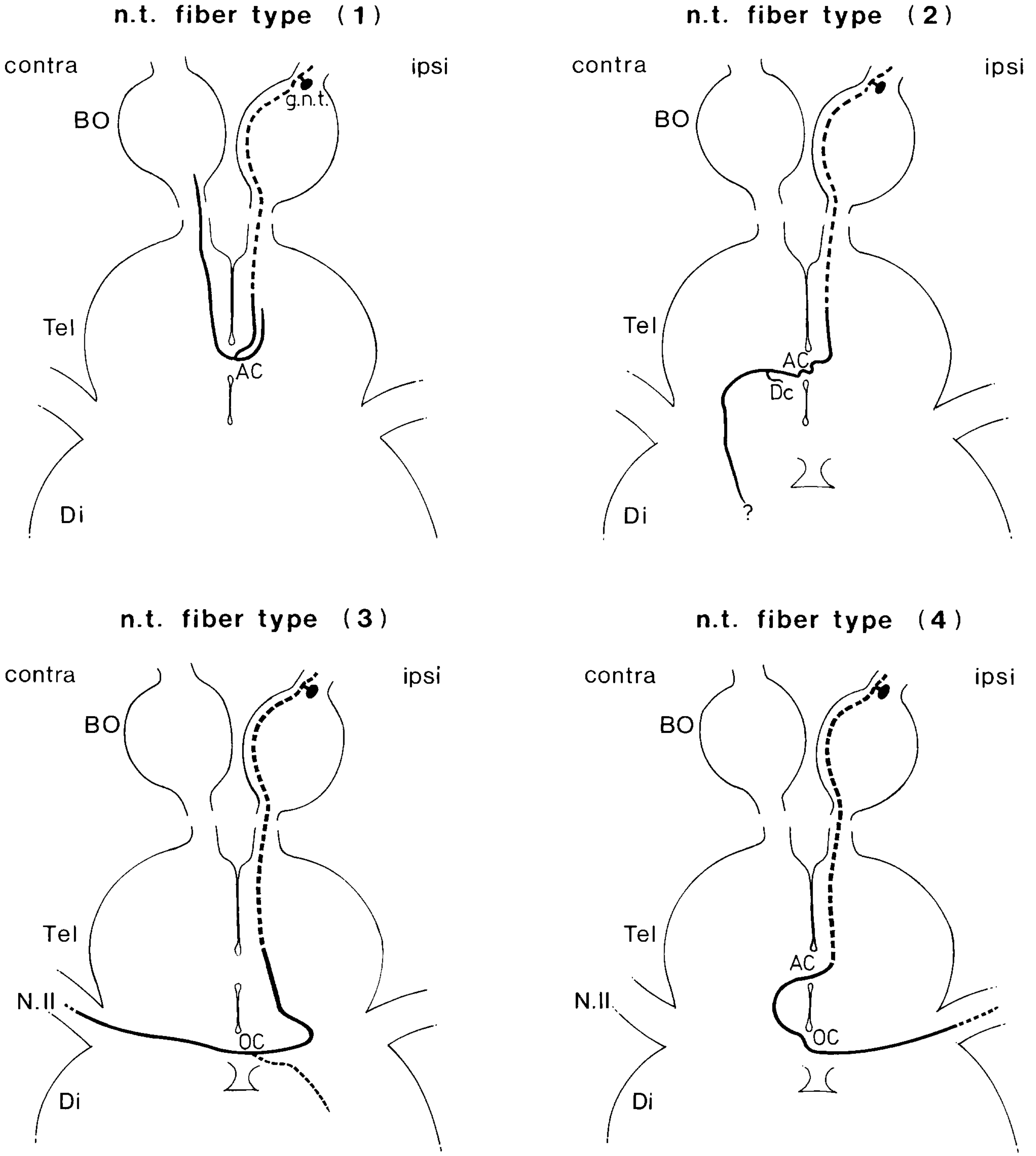
N.t. fiber type (4). The fiber crosses via the AC to the contralateral telencephalon. Whether this fiber possesses collaterals in the ipsilateral telencephalic hemisphere could not be established. The n.t. fiber heads caudally in the (contralateral) medial forebrain bundle; at the level of the ascending optic tracts it approaches the optic chiasma and enters the optic tract in a position near the midline. It intermingles with optic tract fibers close to the border of the ipsi- and contralateral optic tract (see Fig. 2D) and enters the ipsilateral optic nerve.

The course of the n.t. fiber type (4) is schematically summarized in Fig. 1.

There is some evidence in our material that the habenular commissure (HaC) is also involved in the olfacto-retinal projection. Possibly some n.t. fibers cross in the HaC, then enter the optic tract and follow the course of the optic tract fibers in centrifugal direction. This projection may concern both the ipsi- and contralateral innervation of the retina. However, this pathway could not be demonstrated

Table 1. *Abbreviations*

AC anterior commissure; *BO* olfactory bulb; *Cb* cerebellum; *CNS* central nervous system; *contra* contralateral to side of injection; *CZ* central zone of the optic tectum; *Dc* area dorsalis, pars centralis telencephali; *Di* diencephalon; *Dm* area dorsalis, pars medialis telencephali; *DMSO* dimethyl sulfoxide; *Dp* area dorsalis, pars posterior telencephali; *DWZ* deep white zone of the optic tectum; *g.n.t.* ganglion of the nervus terminalis; *Ha* habenular nucleus; *HaC* habenular commissure; *Hc* caudal zone of the periventricular hypothalamus; *HoC* horizontal commissure; *HRP* horseradish peroxidase; *ipsi* ipsilateral to side of injection; *LHRH* luteinizing hormone-releasing hormone; *LLF* lateral longitudinal fasciculus; *lMOT* lateral part of the medial olfactory tract; *LOT* lateral olfactory tract; *Mes* mesencephalon; *mMOT* medial part of the medial olfactory tract; *MO* olfactory mucosa; *MOT* medial olfactory tract; *N.II* optic nerve; *NPT* nucleus posterior tuberis; *n.t.* nervus terminalis; *n.t.-c* central fiber of the nervus terminalis; *n.t.-p* peripheral fiber of the nervus terminalis; *OC* optic chiasma; *ON* olfactory nerve; *OT* olfactory tract; *PBS* phosphate-buffered saline; *PC* nucleus pretectalis centralis; *PG* preglomerular nuclear complex; *PGa* nucleus preglomerulosus anterior; *PGZ* periventricular gray zone of the optic tectum; *PoC* posterior commissure; *PP* nucleus preopticus periventricularis; *PPd* nucleus pretectalis periventricularis, pars dorsalis; *PSm* nucleus pretectalis superficialis, pars magnocellularis; *PSp* nucleus pretectalis superficialis, pars parvocellularis; *RS* nucleus reticularis superior; *SC* nucleus suprachiasmaticus; *SWGZ* superficial white and gray zone of the optic tectum; *TBR* tractus tectobulbaris rectus; *Tel* telencephalon; *TeO* optic tectum; *TLo* torus longitudinalis; *tOT* tuberal olfactory tract; *TS* torus semicircularis; *Vs* area ventralis, pars supracommissuralis telencephali; *Vv* area ventralis, pars ventralis telencephali


n.t. fiber type (1)

n.t. fiber type (2)

n.t. fiber type (3)

n.t. fiber type (4)

Fig. 1. Schematic drawings of goldfish brains (horizontal plane) summarize our findings of four types (*types 1–4*) of nervus terminalis (*n.t.*) fibers. The right side of the brain is ipsilateral, the left side contralateral to the injected ganglion of the nervus terminalis (*g.n.t.*). *Dotted lines* represent fiber pathways that could not be traced as single fibers, *continuous lines* demonstrate courses of n.t. fibers that were traced individually. For abbreviations, see Table 1

in single fiber tracings; the precise trajectories and branching patterns of these fibers remain to be investigated.

N.t. fiber type (5). This fiber possesses a rather large diameter. It courses via the ipsilateral mMOT and the medial forebrain bundle apparently without giving rise to collaterals. In the ipsilateral diencephalon it is located somewhat ventral to the tuberal olfactory tract (tOT, compare Fig. 9D). Approaching the level of the horizontal commissure (HoC) the fiber sends one medium-sized collateral in ventral direction. One section further (40 μm) this collateral branches again (see Fig. 4B), sending one minor-caliber branch dorsally (to join the course of the major branch remaining in the ipsilateral diencephalon, see below).

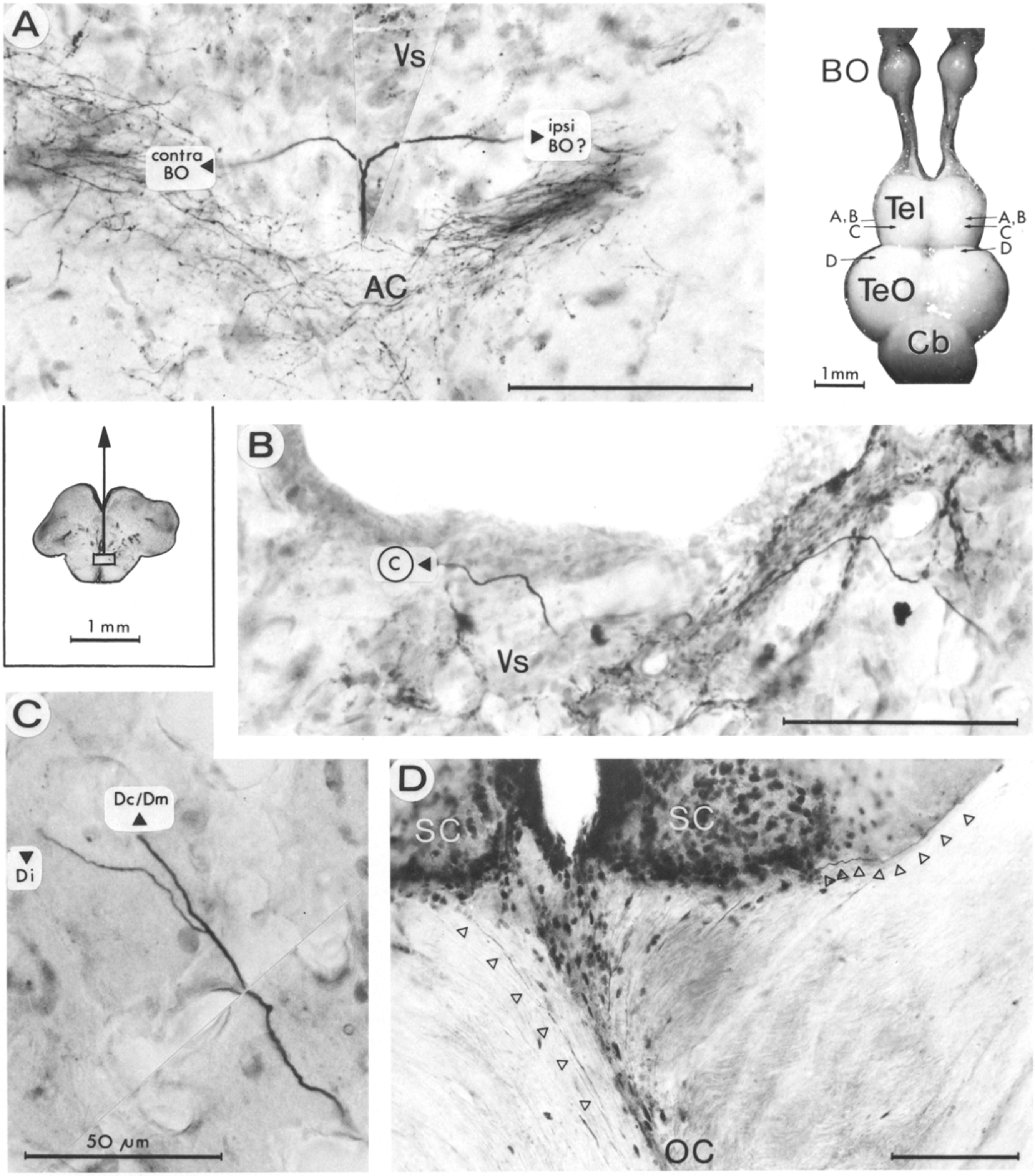


Fig. 2A–D. Frontal sections of goldfish brains after application of cobalt to the ganglion of the nervus terminalis (g.n.t.) located in the rostral olfactory bulb (*BO*). The level of section is indicated in the dorsal view (*upper right*). All overview sections (Figs. 2, 4, 6, 8, 9) are reproduced with the same magnification. **A–D** is from rostral to caudal. For abbreviations, see Table 1. *Bars* represent 0.1 mm, if not indicated otherwise. **A** Section through the anterior commissure (*AC*) shows symmetrical branching of n.t. fiber type (1). Lower magnification photomicrograph demonstrates position of the AC in the telencephalon. One fiber branch was traced to the contralateral BO. For summarizing scheme of n.t. fiber type (1), see Fig. 1. **B** Section through the supracommissural nucleus (*Vs*) shows two portions of the n.t. fiber type (2). The fiber courses through the ipsilateral Vs nucleus to the contralateral telencephalon. In **Fig. 2D**, the same fiber is shown 0.1 mm further caudally. For summarizing scheme of n.t. fiber type (2), see Fig. 1. **C** Section just caudal to the anterior commissure demonstrates branching of fiber type (2). Same fiber as shown in **Fig. 2B**. The larger caliber collateral was traced to the (contralateral) central nucleus in the dorsal telencephalon (*Dc*). The smaller caliber collateral enters the contralateral diencephalon. For summarizing scheme of n.t. fiber type (2), see Fig. 1. **D** Section through the optic chiasma (*OC*) shows one labeled fiber in the ipsilateral optic tract (*right side*) and another labeled fiber in the contralateral optic tract (*left side*). Both fibers are marked with *arrowheads*. N.t. fibers of the tractus olfacto-opticus (olfacto-retinalis) are schematically summarized in Fig. 1 [(and in Fig. 3; compare n.t. fiber type (5)]

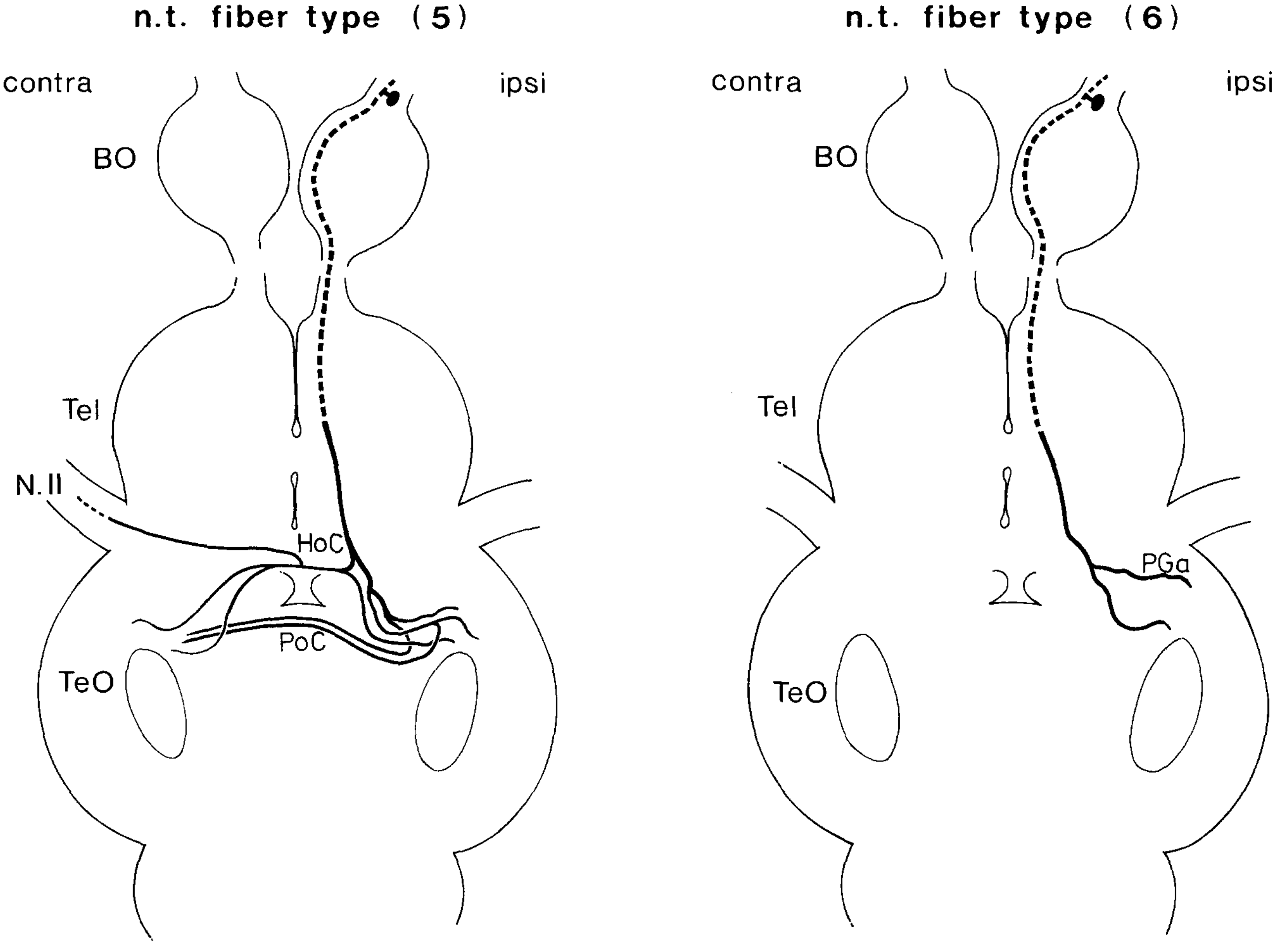


Fig. 3. Schematic drawings of goldfish brains (horizontal plane) summarize our findings of nervus terminalis (*n.t.*) fibers *types* (*5*) and (*6*). The right side of the brain is ipsilateral, the left side is contralateral to the injected ganglion of the nervus terminalis. *Dotted lines* represent fiber pathways that could not be traced as single fibers, *continuous lines* demonstrate courses of fibers that were traced individually. For abbreviations, see Table 1

The other branch continues its ventral course, then turns medially and rostrally thereby surrounding the suprachiasmatic nucleus (SC), and enters the horizontal commissure (HoC). Precisely in the midline of the HoC, on the ventral margin of the SC nucleus this fiber branches once again (see Fig. 4A). One process courses rostroventrally, enters the optic chiasma and is traced in the contralateral optic nerve. The other process surrounds the contralateral SC nucleus and divides again: The larger caliber branch runs in dorsolateral direction, penetrates the contralateral nucleus pretectalis superficialis, pars magnocellularis (PSm), apparently without termination in this nucleus, and ramifies in the area between the pretectal nuclei PSm and PC (nucleus pretectalis centralis) (see Fig. 4C). Termination probably occurs in the periventricular gray zone (PGZ) and possibly the deep white zone (DWZ) of the contralateral rostromedial optic tectum, as confirmed by our HRP material. The other, smaller diameter fiber takes a course through the contralateral diencephalon in dorsal direction. It bypasses the preoptic (laterally adjacent) and the thalamic (medially adjacent) nuclei and is traced to the area between the contralateral pretectal nucleus (PC) and the rostral optic tectum.

The further course of the main branch and the one collateral (5a) that remains in the ipsilateral diencephalon will now be described: At the level of the rostral PSm and the habenular nucleus the main branch further divides in a position just lateral to the tuberal olfactory tract (tOT). Both fibers (5b, 5c) possess about the same fiber caliber. All three fibers take a course in dorsocaudal direction; they pass through the area between the thalamic nuclei (medially adjacent) and the pretectal nuclei (laterally adjacent). Approaching the PSm nucleus fiber 5c divides once again. The collateral (fiber 5d) runs dorsally, penetrates the ipsilateral nucleus pretectalis periventricularis, pars dorsalis (PPd), turns medially first, then continues its course laterally on the dorsal margin of the pretectal nuclei toward the rostral optic tectum. Here fiber 5d runs rostrolaterally and cannot be traced further than the central zone (CZ) of the ipsilateral rostromedial optic tectum (see Fig. 4D). The other collateral, fiber 5c, also courses dorsally through the ipsilateral PPd nucleus and turns laterally into the most dorsal part of the pretectum. Fiber 5c makes a rostral turn on the border of the pretectum/rostral tectum (Fig. 4D) and eventually heads rostrally and medially in the most dorsal part of the pretectum. At the level of the habenular nucleus fiber 5c enters the posterior commissure (PoC) and crosses with the PoC fibers to the contralateral rostral optic tectum. Fiber (5c) could not be traced any further.

Fiber 5b does not penetrate the ipsilateral nucleus PPd

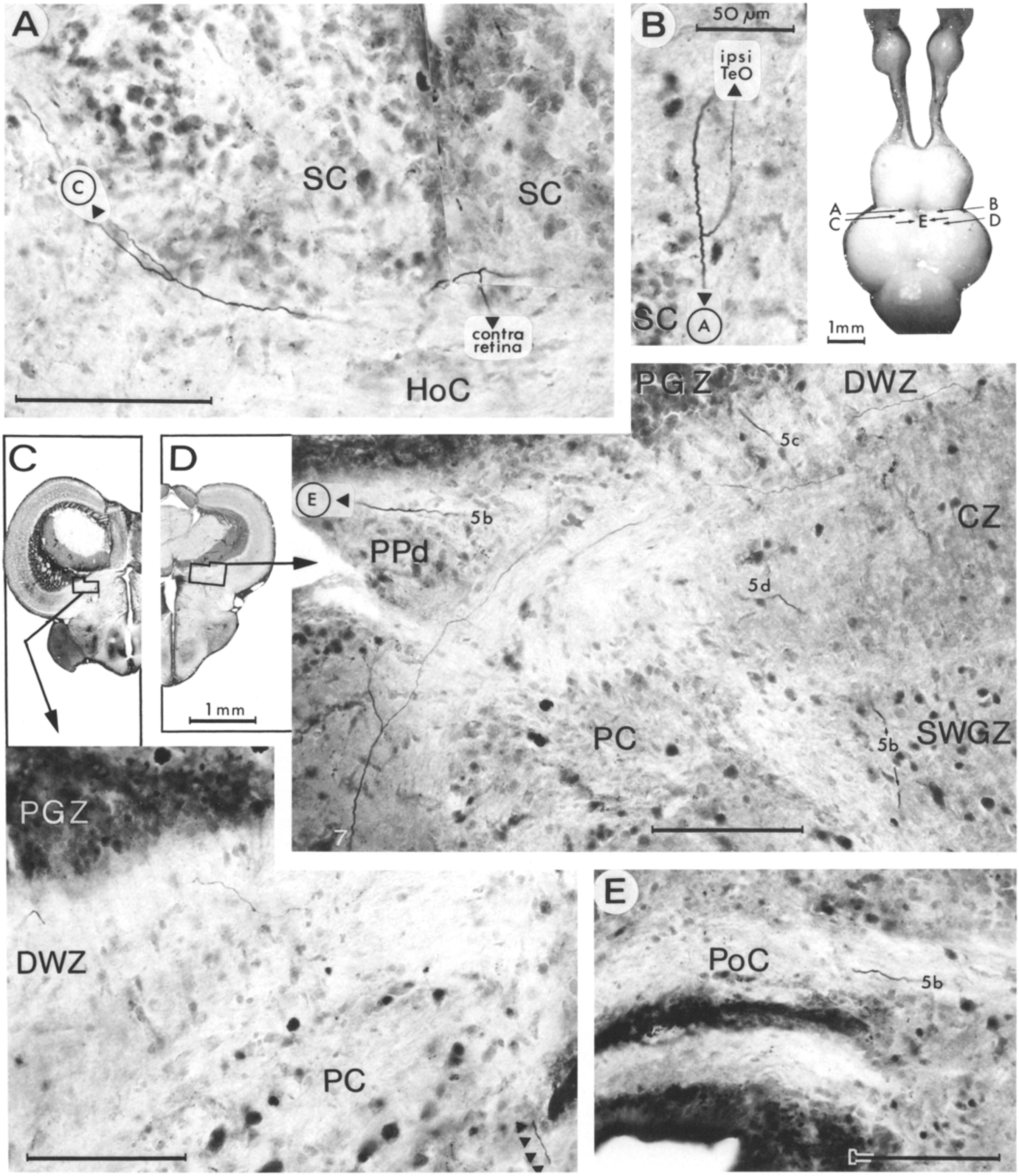


Fig. 4A–E. Frontal sections of one goldfish brain injected with cobalt into the ganglion of the nervus terminalis located in the rostral olfactory bulb. Levels of sections are indicated in dorsal view (*upper right*). *Bars* represent 0.1 mm, if not indicated otherwise. All sections are obtained from one specimen, showing two n.t. fiber types: type (5) and (7). For summarizing schemes, see Fig. 3 (type 5) and Fig. 5 (type 7). **A** Section through the horizontal commissure (*HoC*) shows one cobalt-labeled n.t. fiber of type (5) that branches twice. One collateral enters the contralateral optic nerve, the other two (with *arrowhead* pointing to encircled C) enter the contralateral rostral optic tectum (see **Fig. 4C**). **B** Section at level of the horizontal commissure demonstrates branching of n.t. fiber type (5). One collateral (marked with an *encircled A*) runs ventrally and enters the horizontal commissure (HoC), see **Fig. 4A.** The smaller caliber collateral (collateral 5a of n.t. fiber type 5; compare text) takes a dorsal course on its way to the ipsilateral optic tectum (TeO). **C** Section through the contralateral rostral optic tectum. *Arrow* indicates position of high magnification photomicrograph. Two labeled n.t. fibers course through the deep white zone (*DWZ*), another fiber (marked with *arrowheads*) approaches the central preoptic nucleus (*PC*). **D** Section through the ipsilateral rostral optic tectum. The position of the higher-magnification photomicrograph is indicated on the left side. Three collateral fibers of n.t. fiber type (5), collaterals *5b, 5c* and *5d*, and one branch of fiber type (*7*) are demonstrated in this section. The *arrowhead* with the encircled E indicates continuation of the fiber collateral 5b in **Fig. 4E. E** Section through the posterior commissure (*PoC*) shows one labeled n.t. fiber, the collateral *5b* of n.t. fiber type (5), entering the PoC on its way to the contralateral rostral optic tectum

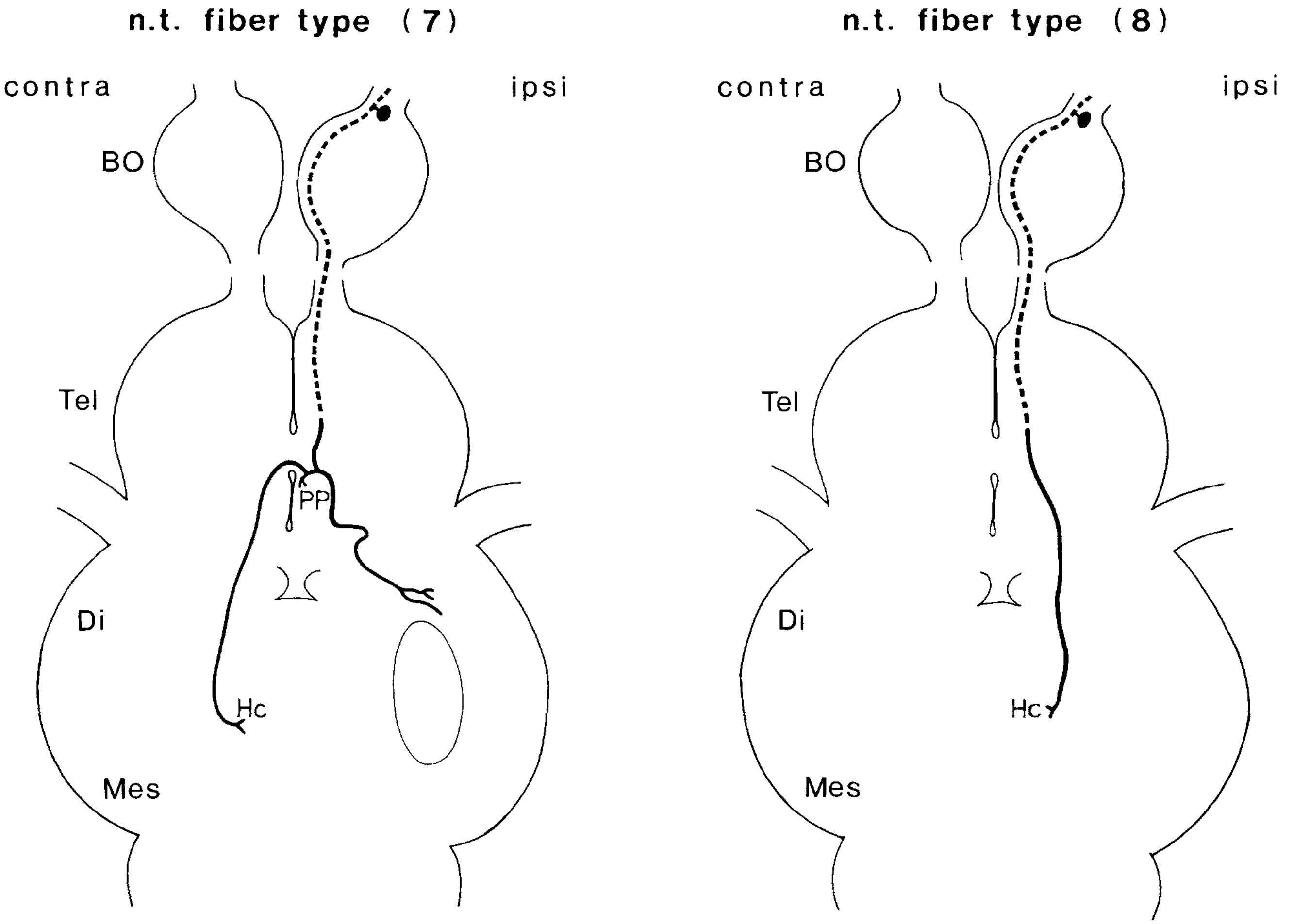


Fig. 5. Schematic drawings of goldfish brains (horizontal plane) summarize our findings of nervus terminalis (n.t.) fibers *types* (*7*) and (*8*). The right side of the brain is ipsilateral, the left side is contralateral to the injected ganglion of the nervus terminalis. *Dotted lines* represent fiber pathways that could not be traced as single fibers, *continuous lines* demonstrate courses of fibers that were traced individually. For abbreviations, see Table 1

on its dorsal course through the diencephalon, but turns laterally before it reaches the PPd nucleus and courses through the ipsilateral nucleus PC. Here it takes a rostral and lateral direction and approaches the area between the ipsilateral nucleus PC and the nucleus pretectalis superficialis, pars parvocellularis (PSp). In a position somewhat lateral to PSp, fiber 5b branches. One collateral turns rostrally and laterally into the rostral optic tectum, then heads dorsocaudally through the superficial white and gray zone (SWGZ) of the ipsilateral rostral optic tectum (see Fig. 4D). The fiber 5b-collateral seems to terminate in the deep white zone (DWZ) and/or central zone (CZ) of the ipsilateral rostral optic tectum. The remainder of fiber 5b takes a course dorsocaudally along the ipsilateral PC nucleus, reaches the ventral floor of the (ipsilateral) tectal ventricle (most dorsal part of the ipsilateral pretectum) (see Fig. 4D). Then it turns medially and rostrally and enters the PoC (see Fig. 4E). Via the PoC it courses to the contralateral diencephalon and can be traced through the contralateral pretectal nuclei to the contralateral rostral optic tectum. The precise location of termination could not be determined.

The further course of the fiber branch 5a remains to be described. It takes a caudal course in the ipsilateral diencephalon somewhat ventral to the other branches of n.t. fiber type (5). Fiber 5a approaches the ventrolateral border of the ipsilateral PPd nucleus, circumscribes this nucleus first medially, then dorsolaterally. On its caudal course it reaches the ventral floor of the tectal ventricle (most dorsal part of the ipsilateral pretectum). Here, fiber 5a turns ventrally and courses on the dorsolateral margin of the ipsilateral PC nucleus through the rostral optic tectum. The fiber was traced in the CZ of the ipsilateral optic tectum; the precise location of termination could not be determined.

The rather complicated course of the n.t. fiber type (5) is schematically summarized in Fig. 3.

N.t. fiber type (6). This fiber courses through the ipsilateral mMOT and medial forebrain bundle; no signs of collaterals were observed at these levels. At the level of the horizontal commissure (HoC) the fiber sends one thin axon collateral in ventral direction. This collateral bypasses the ipsilateral suprachiasmatic nucleus (SC) (medially adjacent) and then runs in ventrolateral direction. It enters the ipsilateral preglomerular nuclear complex (PG) (see Fig. 6B). Possibly, the collateral fiber terminates in the PG, or it may reach the nucleus diffusus of the inferior lobe, as indicated by some of our HRP cases.

The main fiber branch continues its dorsocaudal course through the ispilateral diencephalon. It takes a course similar to that described for the n.t. fiber type (5), collateral 5a. Approaching the ipsilateral nucleus PPd, the fiber sur-

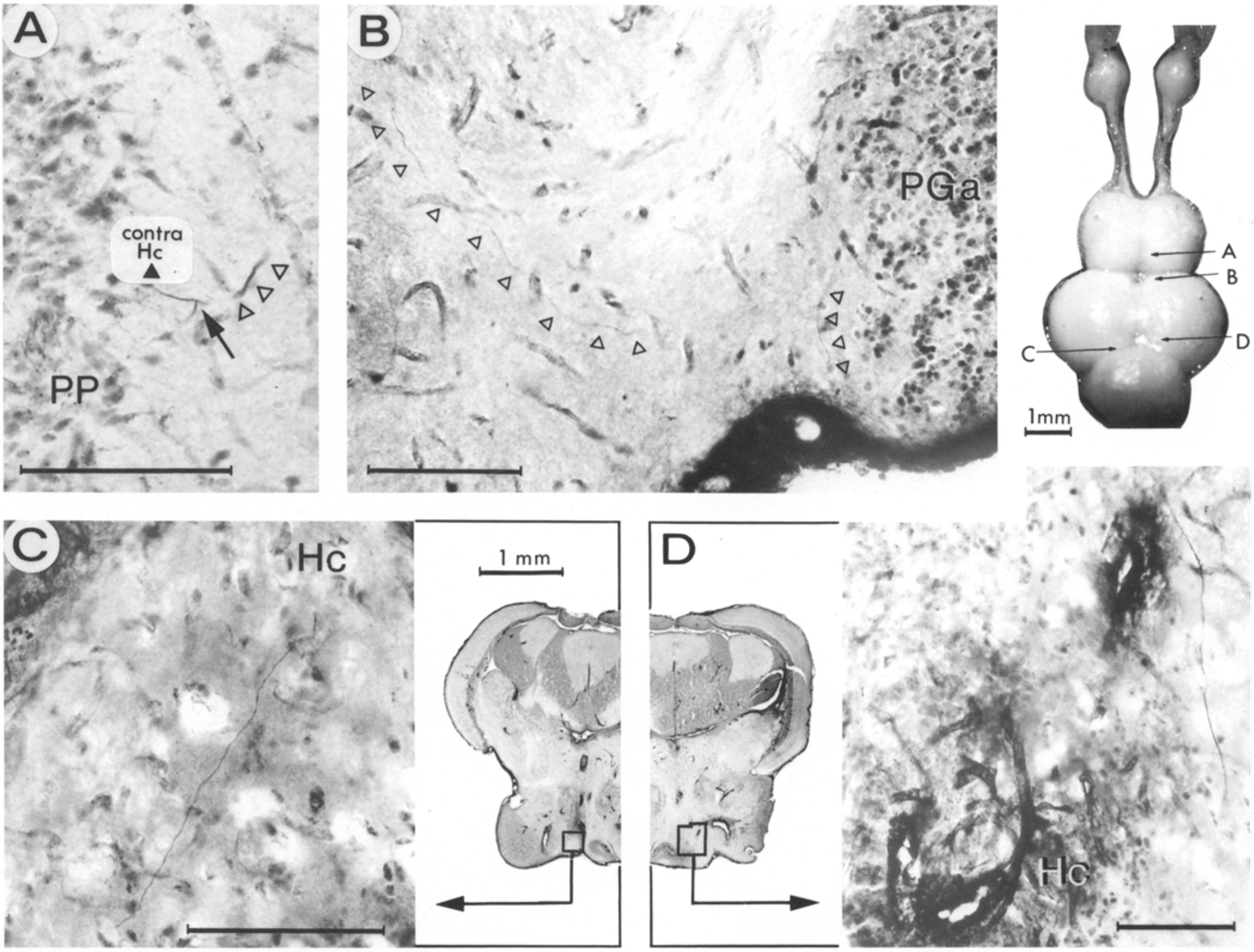


Fig. 6A–D. Frontal sections of goldfish brains after application of cobalt to the ganglion of the nervus terminalis located in the rostral olfactory bulb. The level of section is indicated in the dorsal view (*upper right*). *Bars* represent 0.1 mm, if not indicated otherwise. For abbreviations, see Table 1. **A** Section through the ipsilateral nucleus preopticus periventricularis (*PP*) just caudal to the anterior commissure demonstrates the main branch (*arrowheads*) of n.t. fiber type (7) and one collateral of the same fiber that branches (*arrow*) close to the PP nucleus. The dorsal branch was traced to the caudal zone of the (contralateral) periventricular hypothalamus (Hc), see **Fig. 6C**. The ventral branch apparently terminates in the ipsilateral PP. For summarizing scheme of n.t. fiber type (7), see Fig. 5. **B** Section through the ipsilateral nucleus preglomerulosus anterior (*PGa*) shows the course (*arrowheads*) of one collateral fiber of the n.t. fiber type (6). Possibly, the fiber terminates in the preglomerular nuclear complex. For summarizing scheme of n.t. fiber type (6), see Fig. 3. **C** Section through the caudal part of the contralateral hypothalamus. The position of the photomicrograph is shown at lower magnification (*right* of **Fig. 6C**). The course of the contralateral collateral of n.t. fiber type (7) on its way to the caudal zone of the (contralateral) periventricular hypothalamus (*Hc*) is demonstrated. The same fiber branch is marked with *arrowhead* "contra Hc" in **Fig. 6A**. For summarizing scheme of n.t. fiber type (7), see Fig. 5. **D** Section through the ipsilateral caudal hypothalamus shows the n.t. fiber type (8) approaching the caudal zone of the periventricular hypothalamus (Hc). The position of the photomicrograph is indicated in the lower magnification (*left* of **Fig. 6D**). For summarizing scheme of n.t. fiber type (8), see Fig. 5

rounds this nucleus first medially, then dorsally. At the most dorsal part of the pretectum the fiber, now decreasing in diameter, turns laterally and ramifies in the area of the rostral optic tectum adjacent to the ipsilateral pretectal nucleus (PC). Apparently the fiber terminates here, in the same area as shown for the fiber types (5) and (7) in Fig. 4D.

The course of the n.t. fiber type (6) is schematically summarized in Fig. 3.

N.t. fiber type (7). This fiber approaches the Ac in a way similar to that described for the n.t. fiber type (1). It surrounds the ipsilateral nucleus Vv, following the fibers of the rostral (ventral) part of the AC. Compared with the n.t. fiber type (1) this fiber takes a course somewhat further laterally. The fiber does not cross immediately via the AC, but bypasses the AC, thereby remaining on the ipsilateral side. At levels of the rostral nucleus preopticus periventricularis (PP) one medium-sized collateral branches off in a medial direction (see Fig. 6A). This fiber again branches close to the nucleus PP. One fiber branch apparently terminates in the ipsilateral PP, the other runs in rostrodorsal direction. It crosses in the ventral portion of the AC and then follows a caudal course in the contralateral hemisphere, similar to that of the main branch, which remains in the ipsilateral hemisphere.

The main branch heads caudally in between the ipsilat-

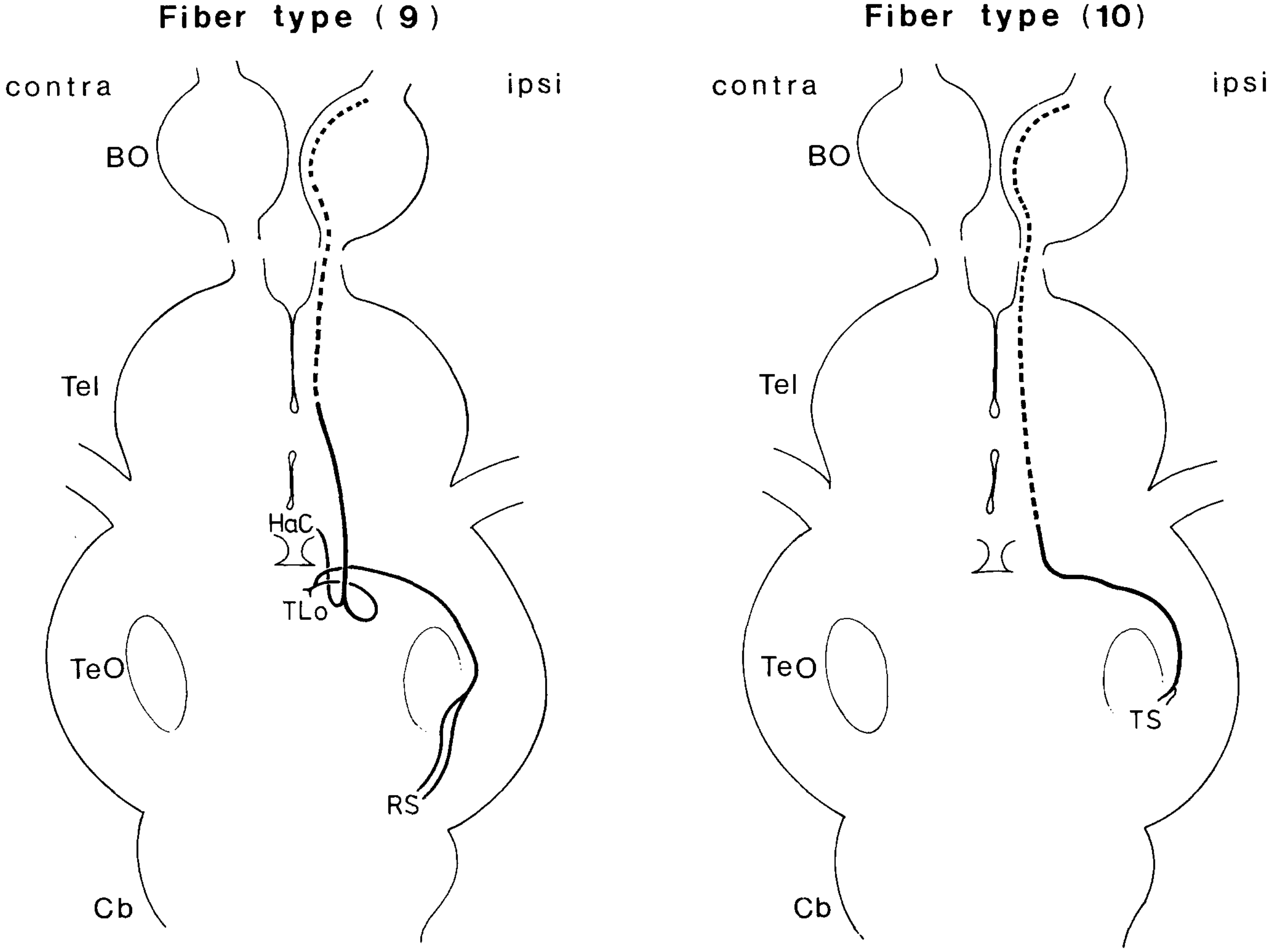
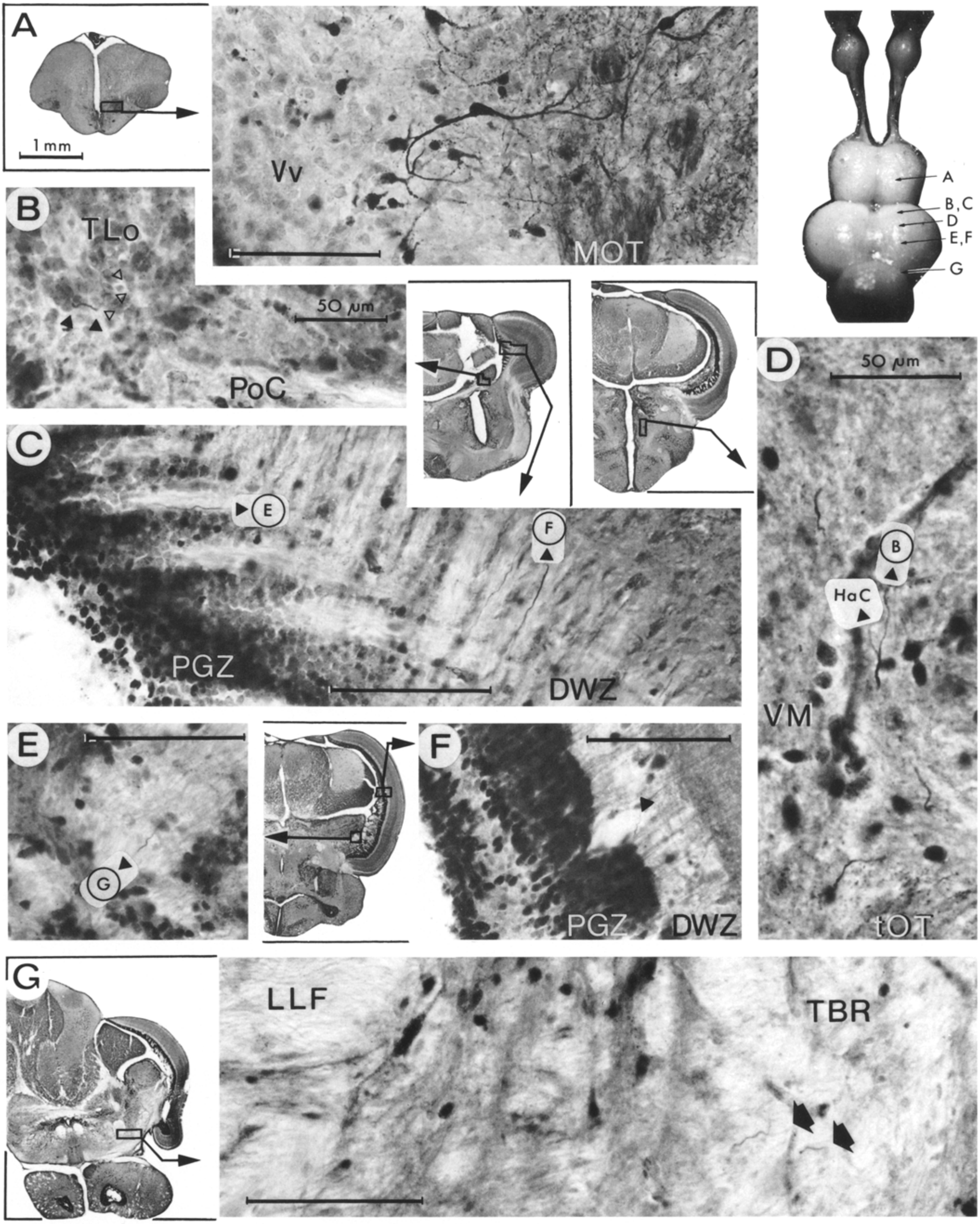


Fig. 7. Schematic drawings of goldfish brains (horizontal plane) summarize our findings of presumptive nervus terminalis fibers (possibly fibers of telencephalic neurons with processes extending into the olfactory bulb), fiber *type* (*9*) and (*10*). The right side of the brains is ipsilateral, the left side is contralateral to the injected ganglion of the nervus terminalis. *Dotted lines* represent fiber pathways that could not be traced as single fibers, *continuous lines* demonstrate courses of fibers that were traced individually. For abbreviations, see Table 1

eral PP nucleus and the entopeduncular nuclei, thus in a position somewhat ventral to the other n.t. fibers described so far. At the level of the horizontal commissure (HoC) the main branch approaches the ipsilateral ascending optic tract. It runs medially and adjacent to the optic tract fibers for a short distance (ca. 100 µm) and turns dorsomedially thus becoming located dorsal to the main bulk of n.t. fibers (compare fiber types 5, 6). The fiber passes dorsocaudally in between the ipsilateral thalamic nuclei (medially adjacent) and the PSm nucleus (laterally adjacent) toward the ipsilateral rostral optic tectum. While passing the ipsilateral pretectal nuclei the main branch further divides: Three collateral fibers apparently enter the DWZ of the ipsilateral rostroventral optic tectum (see Fig. 4D); fiber type (7) is marked with a "7". Further tracing of these fine-caliber collateral fibers was not possible.

The contralateral collateral (which crossed to the contralateral side via the AC) runs caudally through the telencephalon between the PP and the entopeduncular nuclei. It penetrates the contralateral ascending optic tract and continues its caudal and ventral course through the diencephalon. The fiber passes the anterior continuation of the

Fig. 8A–G. Frontal sections of a goldfish brain injected with cobalt into the rostral olfactory bulb (BO). Levels of sections are indicated in dorsal view (*upper right*). Positions of higher magnification photomicrographs (all ipsilateral) are shown in lower magnification micrographs. **A–G** is from rostral to caudal. *Bars* represent 0.1 mm, if not indicated otherwise. All sections are obtained from one specimen, showing two fiber types: type (9) and (10). These fibers are presumed nervus terminalis fibers, but possibly represent fibers of telencephalic neurons with processes extending into the BO. For abbreviations, see Table 1. For summarizing schemes of fiber types (9) and (10), see Fig. 7. **A** Section through the ventral nucleus of the area ventralis telencephali (*Vv*). Retrogradely labeled neurons possibly give rise to fibers that extend into the mesencephalon. **B** Section through the torus longitudinalis (*TLo*) shows branching and termination of one collateral of fiber type (9). **C** Section through the rostral optic tectum demonstrates two labeled fibers. One, fiber type (9), is marked with an *encircled E* (fiber continuation is shown in **Fig. 8E**), the other, fiber type (10), is marked with an *encircled F* (fiber continuation is shown in Fig. 8F). **D** Section through the thalamus demonstrates branching of fiber type (9) in a position close to the nucleus ventromedialis (*VM*). One collateral was traced to the habenular commissure (*HaC*), the course of



the other collateral is shown in Fig. 8B, C, E and G. **E** Section through the torus semicircularis shows one labeled fiber of fiber type (9), marked with *encircled G* (fiber continuation can be found in **Fig. 8G**). **F** Section through the optic tectum demonstrates one labeled fiber of type (10), marked with an *arrowhead*. The fiber is about to leave the deep white zone (*DWZ*) and to penetrate the periventricular gray zone (*PGZ*). **G** Section through the isthmic region shows two branches (*arrowheads*) of the fiber type (9) approaching the area in between the tractus tectobulbaris rectus (*TBR*) and the lateral longitudinal fasciculus (*LLF*).

dorsal zone of the hypothalamus (medially adjacent) and courses toward the ventral floor of the contralateral hypothalamus. The caudal course is continued in a position now ventral to the lateral nucleus of the ipsilateral hypothalamus. Here the fiber turns caudomedially to enter the area of the caudal zone of the ipsilateral hypothalamus (Hc). The fiber surrounds the lateral continuation of the Hc and then heads dorsally (see Fig. 6C). Apparently, it terminates in the midline portion of the ipsilateral dorsal Hc at a level just rostral to the corpora mammillaria.

The course of the n.t. fiber type (7) is schematically summarized in Fig. 5.

N.t. fiber type (8). This fiber courses via the ipsilateral mMOT and the medial forebrain bundle. The pathway through the telencephalon and the rostral diencephalon is similar to that of the fiber type (6). While most other n.t. fibers (so far described) head dorsocaudally (to the ipsilateral optic tectum) or rostroventrally (to the contralateral optic tectum via the horizontal commissure, HoC), this fiber turns *ventro*caudally at levels caudal to the HoC. It remains in a position ventral to the tuberal olfactory tract (tOT). On its caudal and ventral course the fiber bypasses the nucleus of the zona limitans diencephali and the anterior continuation of the dorsal zone of the ipsilateral hypothalamus (medially adjacent), the ipsilateral nucleus tuberis anterior and the lateral extension of the dorsal zone of the ipsilateral hypothalamus. The fiber is traced to the caudal zone of the ipsilateral hypothalamus (Hc, see Fig. 6D). Termination seems to occur along periventricular cells of the most caudal part of the caudal zone of the ipsilateral hypothalamus (approximately 150 µm further caudal to the level of the section shown in Fig. 6D).

The course of the n.t. fiber type (8) is schematically summarized in Fig. 5.

III. Further observations

Fiber types (9) and (10). It could not be established with certainty that the fibers representing fiber types (9) and (10) belong to the n.t.; this failure is due to rather extensive labeling of the lateral part of the medial olfactory tract (l MOT) which mediates almost exclusively centrifugal fibers (von Bartheld et al. 1984). Because of intensive staining of neurons and their processes in the telencephalic nuclei Vv (see Fig. 8A) and Dc, it could not be excluded in these cases that the fibers to be described have their origin in retrogradely labeled telencephalic neurons.

Fiber type (9). Caudal to levels of the Vv nucleus this fiber courses through the mMOT and the medial forebrain bundle. No collaterals were observed at these rostral levels. At the level of the horizontal commissure (HoC) the fiber joins the fibers of the tuberal olfactory tract (tOT). It leaves this pathway in dorsal direction at the level shown in Fig. 8D. Approaching the thalamic nucleus ventromedialis (VM) the fiber branches (see Fig. 8D); both collateral fibers run in dorsal direction: One collateral first takes a dorso*rostral* course and approaches the habenular nucleus to enter the habenular commissure (HaC). This collateral could not be traced further.

The other collateral fiber takes a dorso*caudal* course. It runs through the ipsilateral thalamic nuclei and approaches the ipsilateral nucleus pretectalis periventricularis, pars ventralis (PPv). Here, the fiber turns rostrally and medially, remaining in the most dorsal part of the ipsilateral pretectum. The rostral course is continued to the point of attachment of the torus longitudinalis (TLo) to the pretectum. Here, the fiber leaves the caudal part of the posterior commissure (PoC) in dorsal direction and enters the ipsilateral TLo. The fiber now branches and one collateral arborizes and apparently terminates in the rostroventral part of the TLo (see Fig. 8B). The main branch continues its course in dorsal direction through the ipsilateral TLo to the point of attachment of the TLo to the rostral optic tectum. Here, the fiber turns laterally and enters the rostral (ipsilateral) optic tectum as shown in Fig. 8C. The fiber continues its course in caudal direction in one trajectory of the deep white zone (DWZ) of the ipsilateral optic tectum. The fiber is traced in this layer through the rostral half of the optic tectum (see Fig. 8E). It leaves the DWZ at the level of the rostral part of the nucleus of the medial longitudinal fasciculus. Now, the fiber turns medially and ventrally, penetrates the periventricular gray zone (PGZ) of the ipsilateral optic tectum and continues its caudal course. In the lateral part of the ipsilateral torus semicircularis (TS) the fiber branches. Two branches of the fiber are traced caudally in a position lateral to the fasciculus longitudinalis lateralis (LLF). Further caudally the fibers join the (ipsilateral) tractus tectobulbaris rectus (TBR) and then turn medially to approach the nucleus reticularis superior (RS). The further course and precise location of termination could not be determined.

The course of the fiber type (9) is schematically summarized in Fig. 7.

Fiber type (10). The course through the telencephalon could not be followed in continuous tracings. In the ipsilateral diencephalon the fiber takes a dorsal course through the pretectum and enters the rostral optic tectum in a dorsally directed trajectory of the deep white zone (DWZ) (see Fig. 8C). While coursing caudally the fiber remains in this fiber bundle of the DWZ in the (ipsilateral) dorsolateral optic tectum. At the level of the torus semicircularis (TS) the fiber leaves the DWZ (see Fig. 8F), runs ventromedially, penetrates the periventricular gray zone (PGZ) and enters the fiber bundles attached to the PGZ of the ipsilateral optic tectum. Still coursing caudally the fiber can be traced to its entrance into the ipsilateral TS. Here, it divides into two branches with rather thin diameters. These fibers could not be traced any further. The question remains whether the fibers terminate in the ipsilateral TS or continue their ventral and caudal course to the tegmentum or isthmic area.

The course of the fiber type (10) is schematically summarized in Fig. 7.

Discussion

It has been known for some time (Tiedemann 1816) that fishes have evolved two different types of forebrains: One type has the olfactory bulbs (BO) attached to the telencephalon, the other possesses long, pedunculated olfactory tracts. In fishes with the latter type (Ostariophysi: Cypriniformes, Siluriformes) ganglion cells of the nervus terminalis were discovered in the caudal part of the olfactory nerves (Sheldon 1909, 1912; Sheldon and Brookover 1909; Rossi and Basile 1968; Fujita et al. 1985). These fishes provide some technical advantages for the investigator, because the

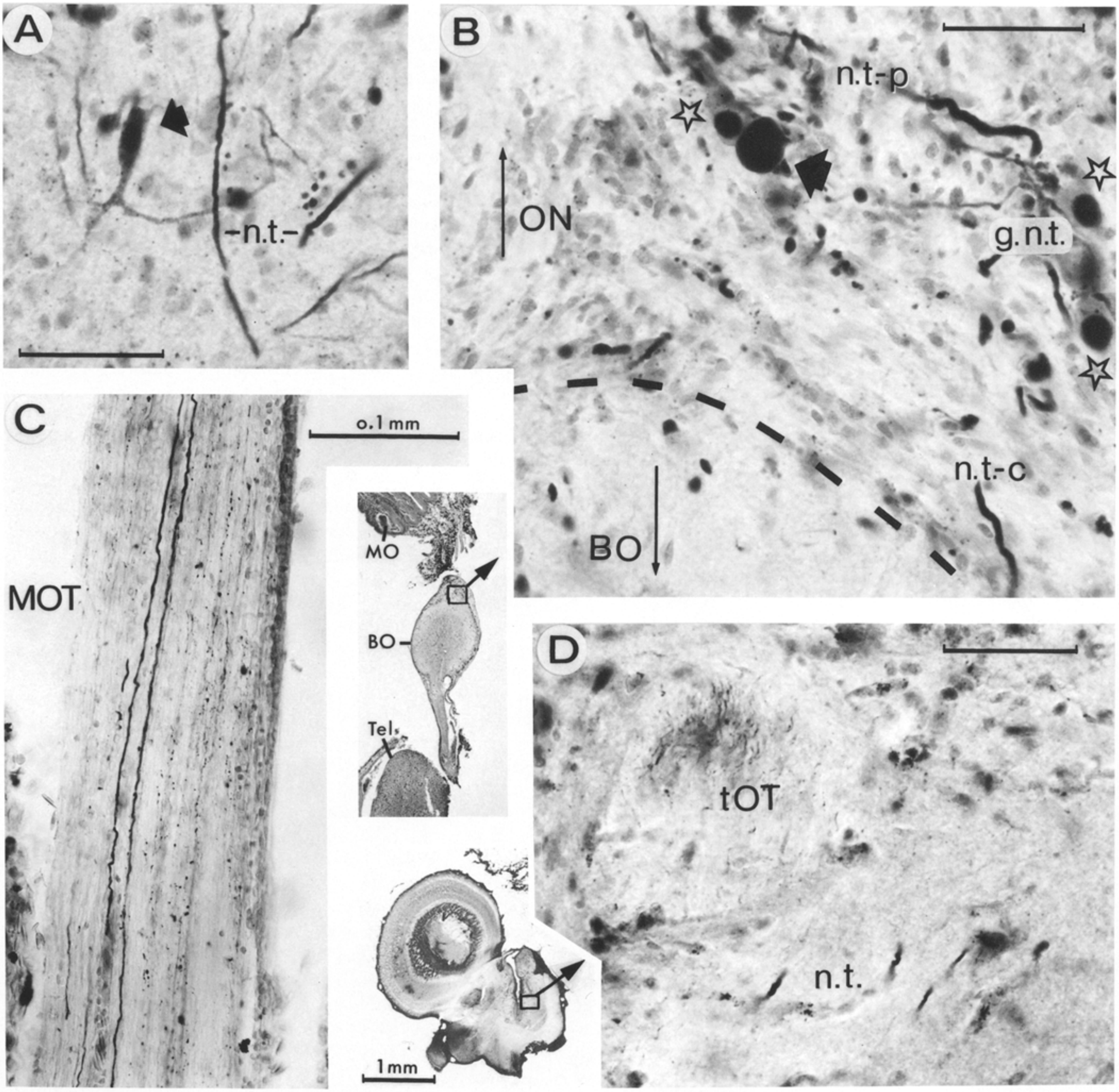


Fig. 9A–D. Sagittal sections (A–C) through the forebrain of one goldfish after application of cobalt to the ipsilateral caudal telencephalon (control group). *Bars* represent 50 μm, if not indicated otherwise. **A** Section through the olfactory bulb proper shows one retrogradely labeled mitral cell (*arrow*) and two labeled nervus terminalis (*n.t.*) fibers. Note the difference in fiber diameters and staining quality. **B** Section through the rostral olfactory bulb (*BO*), caudal olfactory nerve (*ON*) and ganglion of the nervus terminalis (*g.n.t.*). Ventral is to the right, dorsal is to the left. The position of the higher-magnification photomicrograph is indicated at the bottom of **Fig. 9B**. *Arrowhead* indicates retrogradely labeled n.t. ganglion cell. The central process of this labeled cell is marked *n.t.-c*, the peripheral process is marked *n.t.-p*. Three other n.t. ganglion cells (*asterisks*) can be recognized, because their cell nuclei (only!) show positive reaction in the cobalt intensification procedure. **C** Section through the medial olfactory tract (*MOT*) demonstrates two labeled n.t. fibers. **D** Frontal section through the diencephalon of a goldfish after application of cobalt to the ipsilateral rostral olfactory bulb and nervus terminalis ganglion. High magnification photomicrograph shows labeled secondary olfactory fibers in the tuberal olfactory tract (*tOT*) and labeled nervus terminalis fibers (*n.t.*). Note the difference of fiber diameters and staining qualities

BOs and nervus terminalis ganglia are positioned "outside" the telencephalon proper. However, due to the proximity and joint pathways of the n.t. and the classical secondary olfactory fibers it is difficult to distinguish these two systems among labeled fibers.

Differentiation of n.t. fibers and secondary olfactory fibers

One important result of the present investigation is that cobalt labeling provides criteria that allow to distinguish central n.t. fibers from secondary olfactory fibers. Our arguments for the validity of this differentiation are based on the following observations:

(1) Fiber courses of types (2)–(10) never were found in a series of 44 cases of HRP injections into the goldfish BO proper (von Bartheld et al. 1984), but only in cases in which the injections were made into the rostral part of the BO, i.e., in the proximity of the g.n.t. (see Fig. 9B).

(2) Because of their staining qualities (intensity, contrast, sharpness) these cobalt-labeled fibers can easily be distinguished, as shown in Fig. 9A, B, D.

(3) The fiber diameters of n.t. fibers are considerably larger than those of secondary olfactory fibers (see Fig. 9A, D); compare Fujita et al. (1985). The caliber cannot be used as a criterium to differentiate n.t. fibers from those of telencephalic neurons projecting to the BO (located, e.g., in the Vv and Dc nuclei). In goldfish, centrifugal fibers are mediated almost entirely via the lateral part of the medial olfactory tract (lMOT), (von Bartheld et al. 1984), and not via the mMOT, as goldfish n.t. fibers. Furthermore, our material from which the fiber types (1)–(8) are derived does not contain any or only very little retrograde labeling of telencephalic neurons. These cases clearly demonstrate that the fibers in question do not represent central processes having their origin in telencephalic neurons (with the possible exception of fiber types (9) and (10), as noted above).

(4) Consistently a small number of n.t. fibers (ca 2–11) is labeled in our successful cases. This rather small number is in accordance with the number of n.t. ganglion cells, rather than with the number of mitral cells in the BO: A total of 65 (Springer 1983) and 62 (Stell et al. 1984) g.n.t. cells in the goldfish olfactory bulb and nerve was reported.

(5) In goldfish, n.t. fibers seem to course exclusively via the mMOT, whereas mitral cell axons are mainly mediated via the LOT.

(6) In very few cases only (interbulbar connection; nuclei Vv, Vs and PP) does the termination area of n.t. fibers overlap with that of secondary olfactory fibers. In our material no n.t. fibers were observed or suspected to project to the main olfactory terminal field, the posterior nucleus of the area dorsalis telencephali (Dp).

(7) The areas of termination of single traced fibers correspond with those of earlier reports on targets of the n.t. (compare McKibben 1911; Holmgren 1918; von Bartheld and Meyer 1986a).

(8) Large-diameter fibers were traced rostrally through and beyond the BO to n.t. ganglion cell somata in control specimens (telencephalic injections) (compare Fig. 9A–C).

(9) One presumed n.t. fiber was found to project to the contralateral retina as well as bilaterally to the optic tectum. Retrograde labeling of optic fibers never revealed any projection from the BO mitral cells; this finding supports the assumption that the fibers in question belong to the nervus terminalis.

Following the proposed arguments recent reports on BO connections in teleosts should be reexamined.

Olfactory projections to the mesencephalon

Modern tracing techniques have confirmed findings of earlier workers that secondary olfactory projections extend beyond the telencephalon in teleosts (Finger 1974; Bass 1981; Ebbesson et al. 1981b; Murakami et al. 1983; von Bartheld et al. 1984). A bilateral olfactory projection to the area of the nucleus posterior tuberis (NPT) was consistently observed. Recently, a study of BO connections in goldfish (Levine and Dethier 1985) seemed to have revealed the existence of BO projections to the mesencephalon in addition to the area of the NPT (and the nucleus of the saccus vasculosus). Levine and Dethier describe olfactory fibers in the following CNS areas: 1) in the hypothalamus, distinct from the tuberal olfactory tract (tOT), their para-olfactotuberal tract (POT) and ventral descending bundle (VDB); 2) fibers coursing towards the optic tectum; 3) one fiber branching in the torus longitudinalis (TLo), only shown in one of their Figs.; 4) fibers in the posterior commissure (PoC), (only shown in one of their Figs.); 5) fibers mediated via the medial olfactory tract (MOT) in the habenular commissure (HaC).

It has been known for some decades that the n.t. projects to the di- and mesencephalon in lower vertebrates (McKibben 1911; Holmgren 1918; Herrick 1948; Kuhlenbeck 1977). Recently Münz et al. (1982) observed n.t. collaterals in the diencephalon (thalamus) in the cichlid fish *Cichlasoma biocellatum*. Surprisingly, Levine and Dethier (1985) do not consider that labeled BO efferents (described above) may represent fibers of the n.t. Our cobalt-labeled material provides evidence that the above-noted "olfactory" projections actually represent n.t. fibers that do not have their origin in mitral cells of the BO, but in n.t. ganglion cells located rostral to the BO proper. These fibers may have been labeled in the material of Levine and Dethier (1985), because these workers applied HRP to the entire (olfactory and n.t.) tract. This would also explain the peculiar finding of Levine and Dethier (1985) that fibers of the MOT, and not of the LOT, project via the HaC, a finding in contrast to all other studies of teleosts that describe a projection of the *LOT* via the HaC, and not of the MOT (Holmgren 1920; Bannister 1973; Finger 1974; Bass 1981; von Bartheld et al. 1984).

It is of interest to note that Levine and Dethier (1985) describe fibers in the same di- and mesencephalic areas in which cobalt-labeled n.t. fibers were observed in our material; the similarity of fiber courses (see below) may indicate some consistent mechanism in the process of branching of certain n.t. fiber types. The fibers described by Levine and Dethier (1985) could correspond to the fiber types described in the present study as follows: Fibers in TeO: type (5), (6), (7); in Cb?: type (9), (10); in Di: type (7), (8); in PoC: type (5); in TLo: type (9); in HaC: tpye (3?), (4?), (9).

Differentiation of n.t. fibers and fibers afferent to the BO

We are able to distinguish clearly centrifugal and centripetal fibers in the di- and mesencephalon of our cobalt-labeled material. The failure to differentiate centrifugal and centripetal pathways in their HRP material caused some confusion in recent reports (Prasada Rao and Finger 1984; Levine and Dethier 1985) and restricted the interpretation of their data. In the present contribution we describe the courses of labeled centripetal fibers only; single fiber tracing of neurons projecting to the ganglion of the nervus terminalis are described elsewhere (von Bartheld et al. 1986).

Branching patterns of single n.t. fibers

The introduction of the cobalt technique offers significant advantages in the analysis of neuronal connectivity. Complicated (double-labeling) procedures seemed to be required for answers to questions concerning collateral innervation of different CNS structures by one single n.t. neuron (e.g., Demski and Northcutt 1983). Concerning central projections of the n.t. these questions can be answered with the help of cobalt-tracing techniques.

Continuous fiber tracing of the n.t. reveals that single n.t. ganglion cells innervate distinct areas in the CNS. This finding concerns many, if not all n.t. ganglion cells. In our material collateral innervation was proven in six out of ten individually traced n.t. fibers. Whether the presence of collateral innervation of rather distinct CNS structures is a characteristic of the n.t. remains to be established.

Naturally, the n.t. fibers observed in our material represent just a sample, and other fiber types may exist in addition. However, a comparison of n.t. branching patterns shows the following peculiarities. Some, but not the majority of n.t. fiber types project bilaterally to symmetrical areas in the CNS, type (5) and possibly type (1). It is remarkable that branching of n.t. fibers often takes place *in* (type 1, 5) or *in the proximity* of commissures (type 2, 3?, 5, 7, 9). "Double crossing" of one fiber, i.e., crossing back to the same hemisphere, was never observed, with the exception of the ipsilateral olfacto-retinal projection. However, the optic chiasma may not be regarded a true commissure, and thus the fiber course – via the anterior commissure to the contralateral telencephalon, then via the contralateral optic tract to the ipsilateral optic nerve – may be regarded a pathway using *one* commissure only.

The number of commissures involved in the course of one n.t. fiber varies between 0 and 2. The following commissures were found to mediate n.t. fibers: the anterior commissure (AC), the horizontal commissure (HoC), the optic chiasma (OC), the posterior commissure (PoC), and possibly the habenular commissure (HaC).

It should be mentioned that different combinations of collateral n.t. innervation occur: the contralateral BO and the ipsilateral BO (?); contralateral Dc and some area in the contralateral diencephalon; the contralateral retina and both rostral optic tecti; the ipsilateral PP and optic tectum and the contralateral caudal hypothalamus; the ipsilateral PGa (?) and the ipsilateral optic tectum; the ipsilateral torus longitudinalis and the nucleus reticularis superior (?). Based on Golgi-stained material Holmgren (1918) reported branching of single n.t. fibers in *Osmerus* sp. with ipsi- and contralateral collateral innervation of the PP nucleus and the hypothalamus. It seems likely that n.t. ganglion cells of different types serve different functions.

At present, questions concerning inter- and intraspecific variability of n.t. branching patterns cannot be answered. Our HRP material (control group) and observations of other studies (Levine and Dethier 1985) indicate that certain branching patterns are consistently present in goldfish. However, the precise branching pattern of a given n.t. type possibly differs from specimen to specimen.

Comparison of projection areas of the n.t. and of secondary olfactory fibers

N.t. and olfactory pathways have been compared in *Osmerus* sp. (Holmgren 1918) and *Polypterus palmas* (von Bartheld and Meyer 1986a, b). Holmgren noted an overlap of secondary olfactory and n.t. fibers and described glomerular-like terminations of the n.t.; for these reasons he regarded the n.t. as a special sort of olfactory projection. However, in our *Polypterus palmas* material we observed a clear distinction of projection areas of the n.t. and targets of secondary olfactory fibers. Furthermore, we noted that nearly all n.t. terminations are located in periventricular nulcei. Our findings of caudal targets of the n.t. in goldfish partly support our hypothesis that n.t. fibers prefer to terminate on periventricular cells. In addition to the long-known periventricular n.t. targets Vv, Vs and PP we found projections to the periventricular gray zone (PGZ) of the rostral optic tectum bilaterally, to the caudal zone of the periventricular hypothalamus (Hc) bilaterally, and a n.t.(?) projection to the ipsilateral torus longitudinalis (TLo). Unfortunately, we could not determine precisely the location of other caudal n.t. targets (PGa, TS, RS). For this reason it is not certain whether these fibers terminate in periventricular areas. Nevertheless, our data support the hypothesis that the n.t. serves a function distinct from that of the classical olfactory system.

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