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Central connections of the olfactory bulb in the weakly electric fish, *Gnathonemus petersii*

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Summary. We have investigated the central connections of the "classical" olfactory system in the weakly electric fish *Gnathonemus petersii* using HRP and cobalt labelling techniques. The olfactory bulb projects bilaterally via the medial and lateral olfactory tracts to restricted areas of the telencephalon, namely to its rostromedial, lateral and posterior medial parts. The most extensive telencephalic target is the posterior terminal field, which arcs around the lateral forebrain bundle at levels posterior to the anterior commissure. Projections to the contralateral hemisphere cross in the ventral telencephalon rostral to the anterior commissure and via the posterior dorsal part of the anterior commissure; endings are also present within the anterior commissure. Bilateral projections to the preoptic area, to the nucleus posterior tuberis and to an area in the thalamus are apparent. In all cases, contralateral projections are less extensive than those on the side ipsilateral to the injected bulb. A projection via the medial olfactory tract can be followed to the contralateral bulb. Following injections into the olfactory bulb, retrogradely labelled neurons are found in the contralateral bulb and in six telencephalic areas; they are also present in the periventricular diencephalon and in an area lateral to the nucleus posterior tuberis. The present results support the suggestion that a reduction in olfactory input to the telencephalon occurs together with increased telencephalic differentiation in actinopterygian fishes.

Key words: Olfactory system – Electric fish – HRP/Cobalt labelling – *Gnathonemus petersii* (Teleostei)

The structure and development of the actinopterygian telencephalon differ fundamentally from those found in other vertebrates. During embryological development, the neural tube undergoes eversion (bends outwards), rather than inversion followed by an evagination, as is evident in other vertebrates (Gage 1893; Nieuwenhuys 1962a). The degree of eversion and extent of telencephalic differentiation differs between and within the actinopterygian superorders (Nieuwenhuys 1962a, b, 1963; Northcutt and Braford 1980). A progressive increase in telencephalic differentiation is evident during the transition from the polypteriform to the teleostean grades of organization. Previous studies suggest that a reduction in olfactory input to the telencephalon accompanies increased telencephalic differentiation (Nieuwenhuys 1963; Northcutt and Braford 1980). This trend is evident not only between the actinopterygian superorders but also within the teleost lineage (Northcutt and Braford 1980).

Recent neuroanatomical investigations of the primary and secondary olfactory projections in the teleost species have, with the exception of a preliminary report on the olfactory projections in *Xenomystis nigri* (Nieuwenhuys and Verijdt 1983), an early study by Scalia and Ebbesson (1971) on the central olfactory projections in moray eel (*Gymnothorax funebris*), and a recent combined immunocytochemical and HRP study by Grober et al. (1987) on the terminal nerve in *Anguilla rostrata*, exclusively employed species of the euteleost lineage (Table 1). In the present study, we have examined the central projections of the "classical" olfactory system of the weakly electric fish *Gnathonemus petersii* (Mormyridae), which like *Xenomystis* belongs to the most primitive group of living teleosts, the Osteoglossomorpha. The Mormyridae possess one of the most highly differentiated teleost telencephalons (Weston 1937; Meader 1939; Nieuwenhuys 1962b, 1963). However, modern neuroanatomical techniques have not previously been employed to investigate telencephalic connections in this species.

Materials and methods

Forty-three specimens of *Gnathonemus petersii* (8–15 cm in length) were used in the present investigation. Prior to surgery, anaesthesia was induced by immersion of the fish in a solution containing 100 mg/l tricain methane sulphonate (MS 222). The fish were then fixed on an operating block. During surgery, the animals were artifically respired by circulating water containing MS 222 at a concentration of 90 mg/l. HRP injections into the olfactory bulbs and nerves were made by opening a 3 mm observation hole just posterior to the nares.

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Table 1. Teleost species in which the olfactory bulb projections have been studied with modern neuroanatomical techniques. *Abbrevations* for methods: *A* autoradiography; *C* cobalt lysine; *D* degeneration; *H* horseradish peroxidase; *I* immunocytochemistry; *TB* true blue; *P* primuline

Lineage	Order, family	Genera, species	References	Method
Osteoglossomorpha				
	Osteoglossiformes			
	Mormyridae	*Gnathonemus petersii*	Current paper	C, H
	Notopteridae	*Xenomystis nigri*	Nieuwenhuys and Verijdt 1983	D, H
Elopomorpha				
	Anguilliformes			
	Muranidae	*Gymnothorax funebris*	Scalia and Ebbesson 1971	D
	Anguillidae	*Anguilla rostrata*	Grober et al. 1987	H, I
Euteleostei (Ostariophysi)	Characiformes			
	Serrasalamidae	*Serrasalmus nattereri*	Ebbesson et al. 1981	D, H
	Cypriniformes			
	Cyprinidae	*Carassius auratus*	Ichikawa 1975	D
			Oka 1980	D, P
			Levine and Dethier 1985	H
			von Bartheld et al. 1984	H
			von Bartheld and Meyer 1986	C, H
			von Bartheld et al. 1986	C, H, TB
			Demski and Northcutt 1983	H
			Stell et al. 1984	I
		Cyprinus carpio	Ito 1973	D
	Siluriformes			
	Ictaluridae	*Ictalurus nebulosus*	Finger 1975	D
		Ictalurus punctatus	Bass 1981a, b	A, H, D
(Salmonidae)	Salmoniformes			
	Salmonidae	*Salmo gairdneri*	Northcutt and Davis 1983	D
		Oncorhynchus tshawytscha	Bazer et al. 1987	C
(Percomorpha)	Scorpaeniformes			
	Scorpaenidae	*Sebastiscus marmoratus*	Murakami et al. 1983	H, D
	Perciformes			
	Belontiidae	*Macropodus opercularis*	Davis et al. 1983	A, D
	Centrarchidae	*Lepomis cyanellus*	Northcutt and Davis 1983	D
	Pleuronectiformes			
	Pleuronectidae	*Pseudopleuronectes americanus*	Prasada Rao and Finger 1984	D, H

HRP labelling

In 30 specimens, the ipsilateral bulb was cut and either a pledget of gelfoam saturated with 30% HRP (Sigma type VI) or a small drop of HRP paste was inserted in the bulb. In 5 fish, HRP (30% in 0.9% NaCl) was iontophoretically (4 μA for 2×15 min) injected into the bulb. Three animals had a small piece of gelfoam saturated with 30% HRP placed on the central stump of the cut olfactory nerve in an attempt to label the nervus terminalis system. Following HRP implantation, the wound was packed with gelfoam and sealed with tissue glue.

After post-operative survival times of 2–9 days, the fish were reanaesthetized with MS 222 and perfused trans-cardially with teleost Ringer's solution (Wolf 1963) followed by 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed and allowed to postfix for several hours in a phosphate-buffered solution of 4% glutaraldehyde and 30% sucrose. They were then transferred to a 30% sucrose-containing buffered solution overnight. The following day, the brains were embedded in a sucrose/gelatin solution (30% sucrose 10% gelatin), and the blocked brains were fixed for several hours in the glutaraldehyde/sucrose buffer solution. Serial sections (40 μm thick) were cut on a cryomicrotome in the frontal, sagittal, or horizontal planes. The sections were processed either according to a modified Hanker-Yates protocol (Bell et al. 1981) or using the diaminobenzidine (DAB) method (La Vail and La Vail 1972). Sections were mounted and counterstained with cresyl violet.

Cobalt-filling

In 5 subjects, a small piece of gelfoam saturated with cobalt-lysine complex was inserted into the olfactory bulb. Following a survival time of 18 h, the fish were killed in a bath of MS 222 (200 mg/l). The brains were removed and processed according to the method of Lazar et al. (1983). Serial sections (60 μm thick) were cut in the transverse plane and intensified with Gallyas solution (Gallyas 1979). The sections were counterstained with nuclear fast red.

Results

Since the telencephalon of *Gnathonemus petersii* has not previously been the subject of systematic investigation, a

general description of the telencephalon will be presented. The nomenclature we employ in describing the principal subdivisions within the telencephalon of *Gnathonemus petersii* (Fig. 1a–e) is in general derived from Nieuwenhuys (1963).

General aspects of the telencephalon

As in other teleosts, the telencephalic hemispheres of *Gnathonemus petersii* can be divided into two main areas, the ventral area (*area ventralis*, V) and the dorsal area (*area dorsalis*, D).

Area ventralis. Seven major zones can be recognized in area V. At pre-anterior commissural (AC) levels, 4 cell groups are present, the ventral (Vv), dorsal (Vd), lateral (Vl) and medial (Vm) nuclei of area V (Fig. 1b). The darkly staining Vv and Vd nuclei occupy a periventricular position, whereas Vl is situated between the sulcus endorhinalis (E) and Vv and Vd; the cells of Vl are widely scattered compared with those of Vv and Vd, and the nucleus is not as clearly delimited as in other teleost species. The darkly staining cell group (Vm) is located lateral and slightly dorsal to Vd; this cell group disappears at levels close to the AC (Fig. 1b). More caudally at AC levels (Fig. 1c) the supracommissural (Vs) nucleus of area V can be recognized lying dorsal to the AC. The cells in this nucleus are slightly larger than those of Vd. At the postcommissural levels (Fig. 1d), two cell masses, the posterior (Vp) and intermediate (Vi) nuclei of area V, are present. The medially situated Vp nucleus lies dorsal to the area praeoptica (PO) and is less distinct than the laterally situated cell group Vi, which surrounds the dorsal margin of the lateral forebrain bundle (lfb). Ventral to the Vi, the darkly staining small cells of the entopeduncular nucleus (NE) are juxtaposed to the medial aspect of the lfb (Fig. 1c, d).

Area dorsalis. As in most teleosts, four major zones can be recognized in the area dorsalis of the telencephalon of *Gnathonemus petersii*. Three are longitudinal, the dorsalmedial (Dm), dorsal (Dd) and lateral (Dl) zones; they surround a central zone of scattered large neurons (Dc), (Nieuwenhuys 1962b, 1963). In addition, a number of distinct subfields can be identified within these four principal zones.

Dm, the medial part of area dorsalis, extends from the rostral to the caudal pole of the telencephalon (Fig. 1a–d). The Dm at posterior telencephalic levels fills the entire dorsalmedial lobe. In some teleosts, a cell-free zone, the zona limitans (ZL), demarcates the border between Dm and Vd; however, in our material no clear ZL was apparent (Fig. 1b). Several authors (Bannister 1973; Northcutt and Braford 1980; Murakami et al. 1983; Diez et al. 1987) have subdivided the Dm area into a number of smaller subfields; whereas subfields may be present in Dm in *Gnathonemus petersii*, they are not immediately obvious and we await further information on the fibre connections of Dm before subdividing this area.

Dd, the dorsal part of area dorsalis, is separated from Dm by the sulcus ypsiliformis (Y, in Fig. 1b). Dd is a clearly delimited, darkly staining curved plate of small cells, which appear at mid-rostral telencephalic levels (Fig. 1a) and disappear caudal to the AC. A cell-free zone separates the Dd from the more ventrally situated Dl zone (Fig. 1b).

Dl, the lateral part of area dorsalis, shows a high degree of differentiation in *Gnathonemus petersii* as in other mormyrid species (Weston 1937; Meader 1939; Nieuwenhuys 1962b, 1963, 1982). Three sharply delimited cell masses can be recognized along the ventrolateral wall of the mid-rostral telencephalon; to conform with previous observations (Weston 1937; Nieuwenhuys 1962b, 1963, 1982), we have named these nuclei Dla, Dlb and Dlc (Fig. 1a–c). In our material, a fourth darkly staining subfield can be recognized at mid and post AC levels. We have provisionally named this nucleus – the medial nucleus of the lateral zone of the area dorsalis telencephali (Dlm) (Fig. 1c). Dlm may however represent a medially migrated portion of Dlb; future studies may clarify this point. The Dla or Dlb nuclei do not extend as far caudally as Dlc or Dlm. Posterior to the AC, the Dlm and Dlc nuclei are replaced by the medial part of the posterior lateral area dorsalis (mDlp) (Fig. 1c, d). The Dlp area, which consists of a number of poorly defined cell groups, occupies the entire posterior ventrolateral lobe of the telencephalon.

A darkly staining pear-shaped group of small cells, the nucleus taenia (NT) appears between the Dlc nucleus and the sulcus endorhinalis (E) at mid-anterior commissural levels. Further caudally, Dlc is replaced by mDlp and a distinct cell free zone (arrows, Fig. 1d), separates NT from mDlp.

Dc, the central part of the area dorsalis is composed of a number of scattered groups of large cells. In *Gnathonemus petersii*, the Dc area can be subdivided into two major subfields, which Nieuwenhuys (1962b, 1963) named Dcm and Dcd because of their association with the overlying Dm and Dd zones (Fig. 1c).

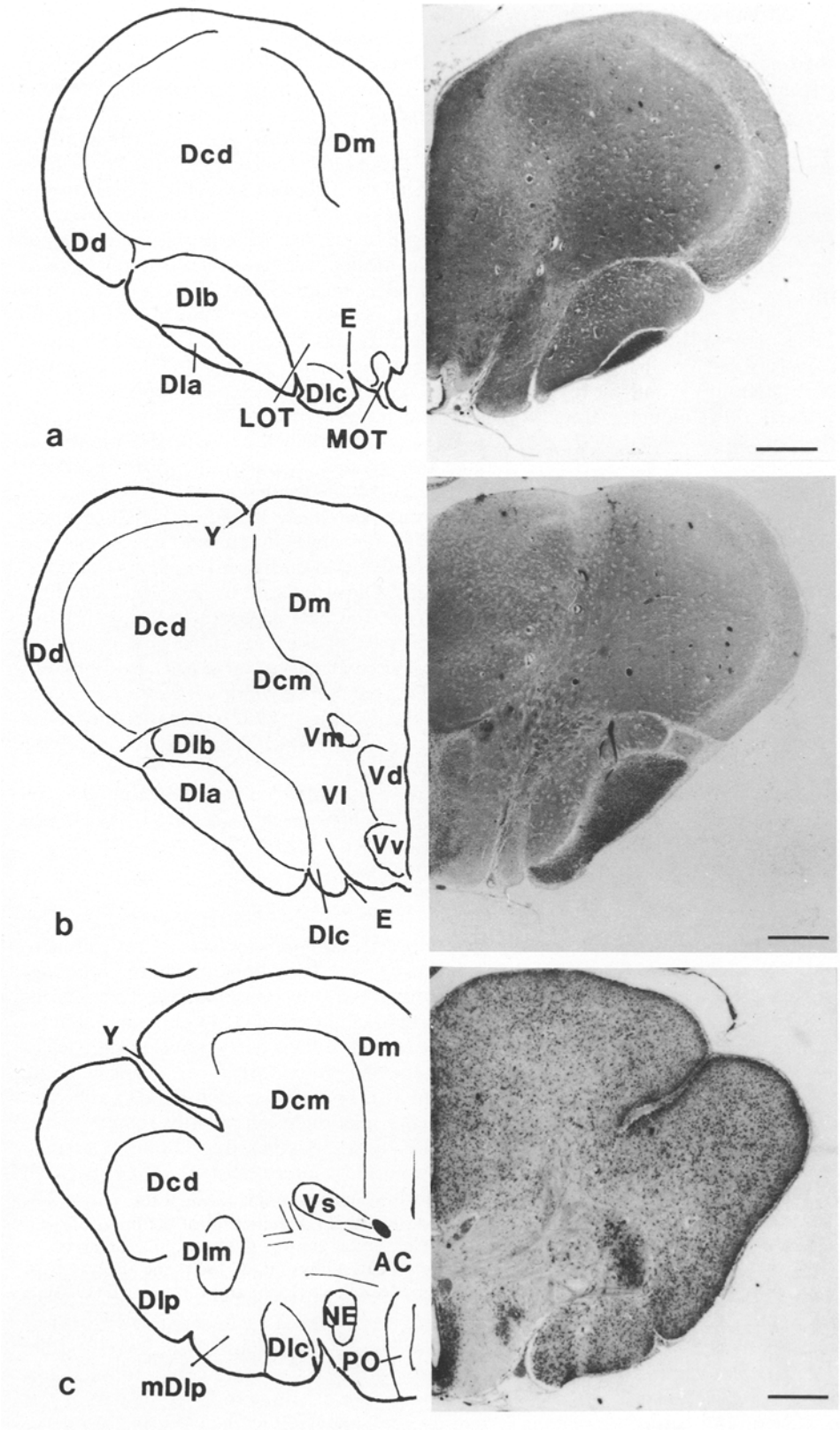
The nomenclature we employ for the diencephalic cell groups (Fig. 1e) is adopted from Lazar et al. (1984) and Meek et al. (1986).

General aspects of the olfactory system

The olfactory bulbs in *Gnathonemus petersii* are pedunculated and connected to the telencephalon by the olfactory tracts, 5–8 mm long in the subjects employed. Connections with the olfactory epithelium are via (2–3 mm long) olfactory nerves. Slightly rostral to their entry point into the telencephalon, each olfactory tract forms two main separate bundlets, a lateral (LOT) and a medial (MOT) subtract. The LOT enters the telencephalon slightly rostral to the MOT entry point (Figs. 2A, 3a). In addition, a small number of fascicles form an intermediate tract (IT) between the two major fibre systems in the entry zone (Figs. 2A, 3a). Further caudally the IT intermingles within the fascicles of the LOT.

Attempts to label separately the lateral and medial bundlets of the olfactory tract proved unsuccessful because the only position where they could easily be separated lay below the rostral pole of the telencephalon; the different fibre projections at posterior levels therefore could not be determined with certainty. Our attempts to label the nervus terminalis system by applying HRP to the cut olfactory nerve also proved unsuccessful. This problem appears to be associated with poor HRP transport from the cut nerve.

Efferent projections. The olfactory bulb projects bilaterally via the MOT and LOT to restricted areas of the telencephalon, preoptic area and diencephalon, the ipsilateral projections being more extensive than those on the contralateral



Abbreviations: *CP* posterior commissure; *C1* lobulus C1 corporis cerebelli; *dap* nucleus dorsalis anterior pretectalis; *FR* fasciculus retroflexus; *Hb* nucleus habenulae; *HYP* hypothalamus; *LT* lobus transitorius cerebelli; *Nel* nucleus exterolateralis of the torus semicircularis; *Nl* nucleus lateralis; *NPC* nucleus of the posterior commissure; *NR* nucleus rotundus; *nt* nervus terminalis; *OT* optic tectum; *Pn* pretectal nucleus; *pchc* postchiasmatic commissure; *SCN* suprachiasmatic nucleus; *TEL* telencephalon; *TH* thalamus; *TL* torus longitudinalis; *tm* tectum marklage; *tmca* tractus mesencephalocerebellaris anterior; *Valv* valvula; *VMC* ventral mesencephalic commissure

side. The contralateral bulb and AC also receive an input (Fig. 4).

(a) Ipsilateral projections of the MOT. The MOT enters the midventral rostral telencephalon (see Fig. 1, level a) as a compact bundle of fibres close to the midline (Fig. 3a). As the MOT runs caudally, it separates into a compact dorsal (dMOT) and a more diffuse ventral (vMOT) subdivision (Fig. 2C).

The dMOT rises along the lateral surface of areas Vv and Vd. As it courses caudally, some fibres terminate along the lateral edges of both areas (Fig. 2C). A dense terminal

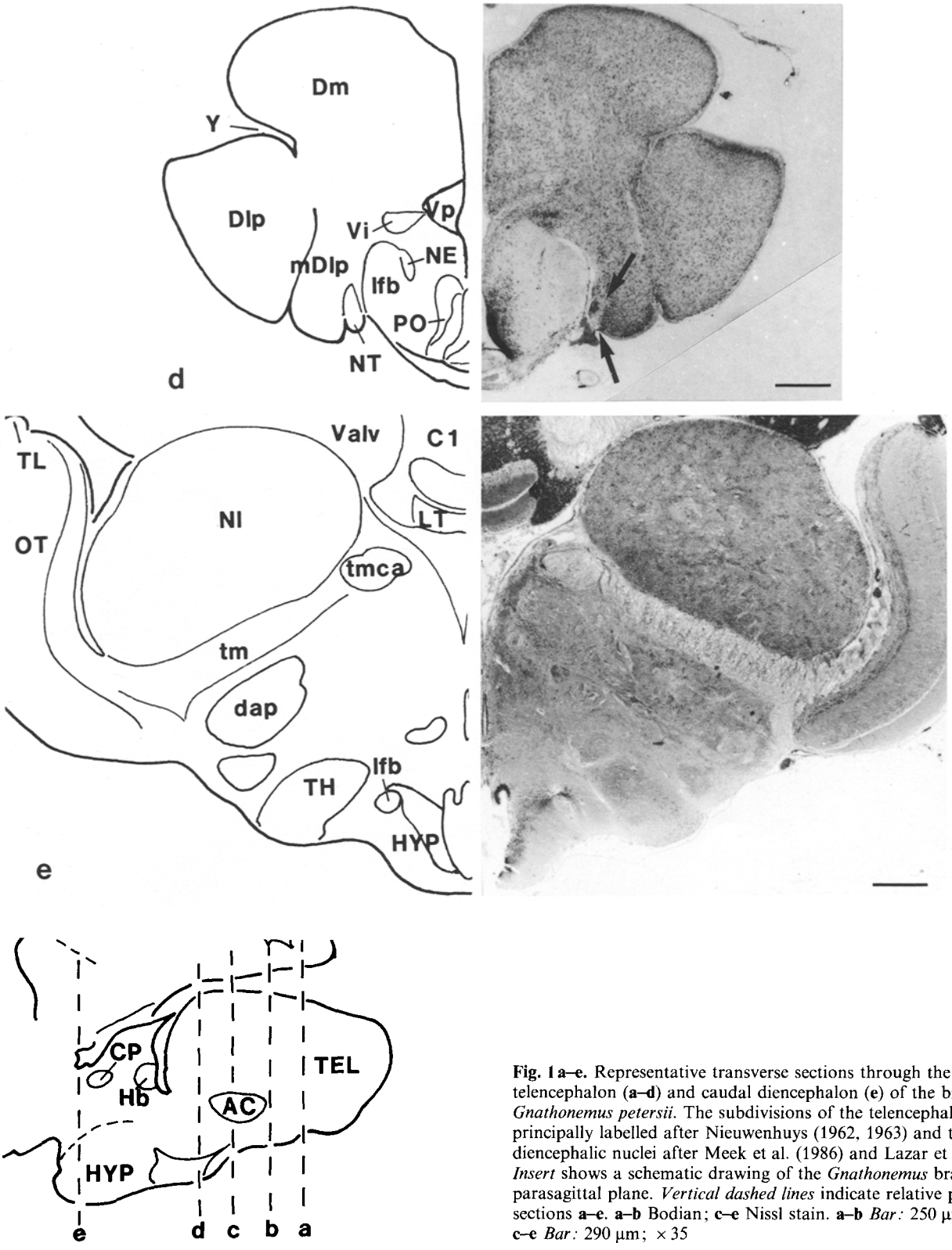


Fig. 1 a–e. Representative transverse sections through the telencephalon (**a–d**) and caudal diencephalon (**e**) of the brain of *Gnathonemus petersii*. The subdivisions of the telencephalon are principally labelled after Nieuwenhuys (1962, 1963) and the diencephalic nuclei after Meek et al. (1986) and Lazar et al. (1984). *Insert* shows a schematic drawing of the *Gnathonemus* brain in a parasagittal plane. *Vertical dashed lines* indicate relative positions of sections **a–e**. **a–b** Bodian; **c–e** Nissl stain. **a–b** *Bar:* 250 μm; ×40. **c–e** *Bar:* 290 μm; ×35

cluster covers the dorsal lateral part of the Vd area (Fig. 5b). In its caudally oriented course, the dMOT is joined by medial components of the dLOT and some rising axons from the vMOT; together, they form an extensive terminal field over Vs. (Figs. 2D, 3d, 5a). Some fibres of the dMOT turn medially at the level of the posterior part of the AC, whereas others continue caudally to separate to the Vp, Vi and medial portions of the Dlp area (Fig. 2G), thus forming part of the posterior telencephalic terminal field (PTF) (Fig. 6a, c).

The vMOT containing both thick and thin elements, projects to areas Vv and Vl in the rostral telencephalon.

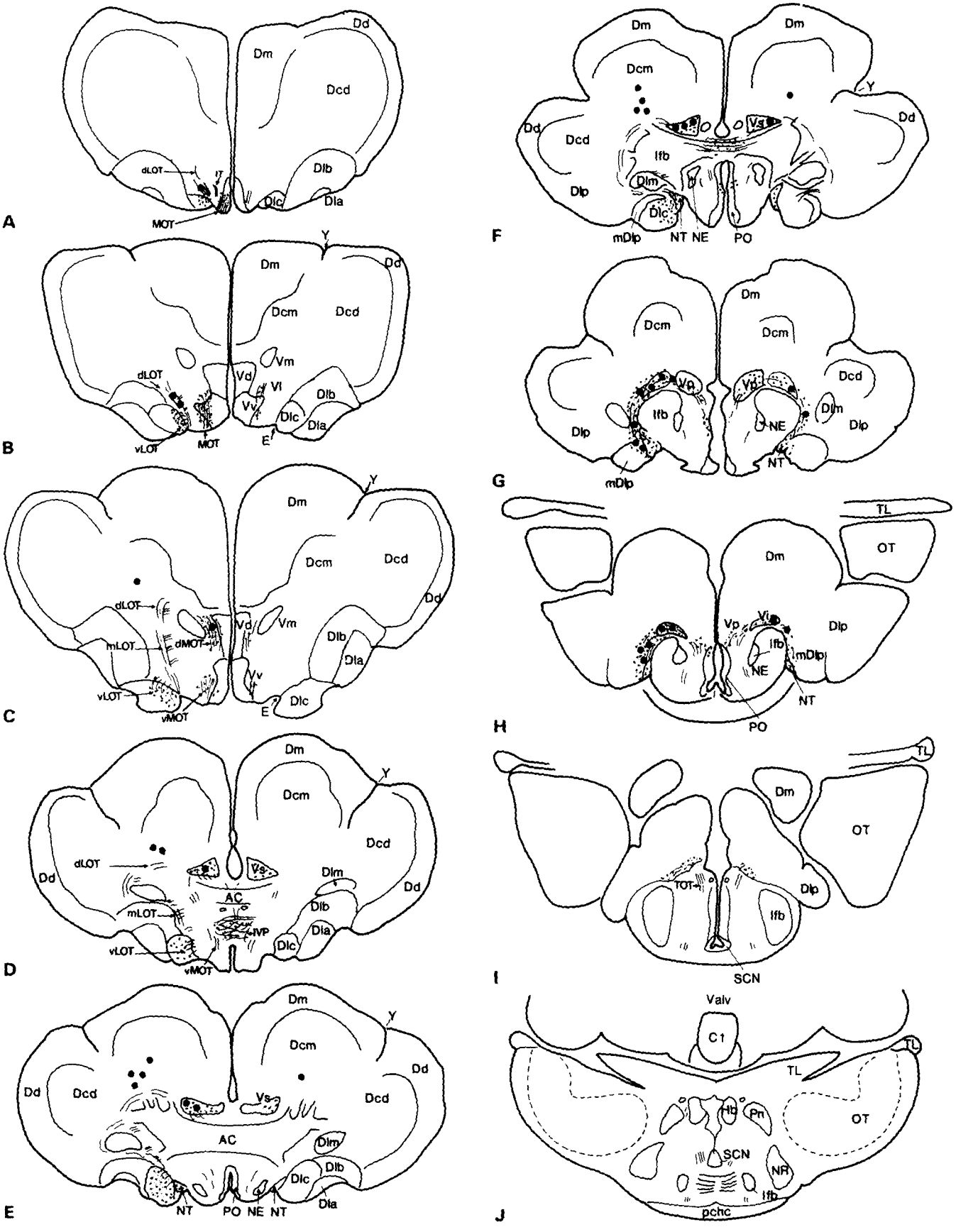
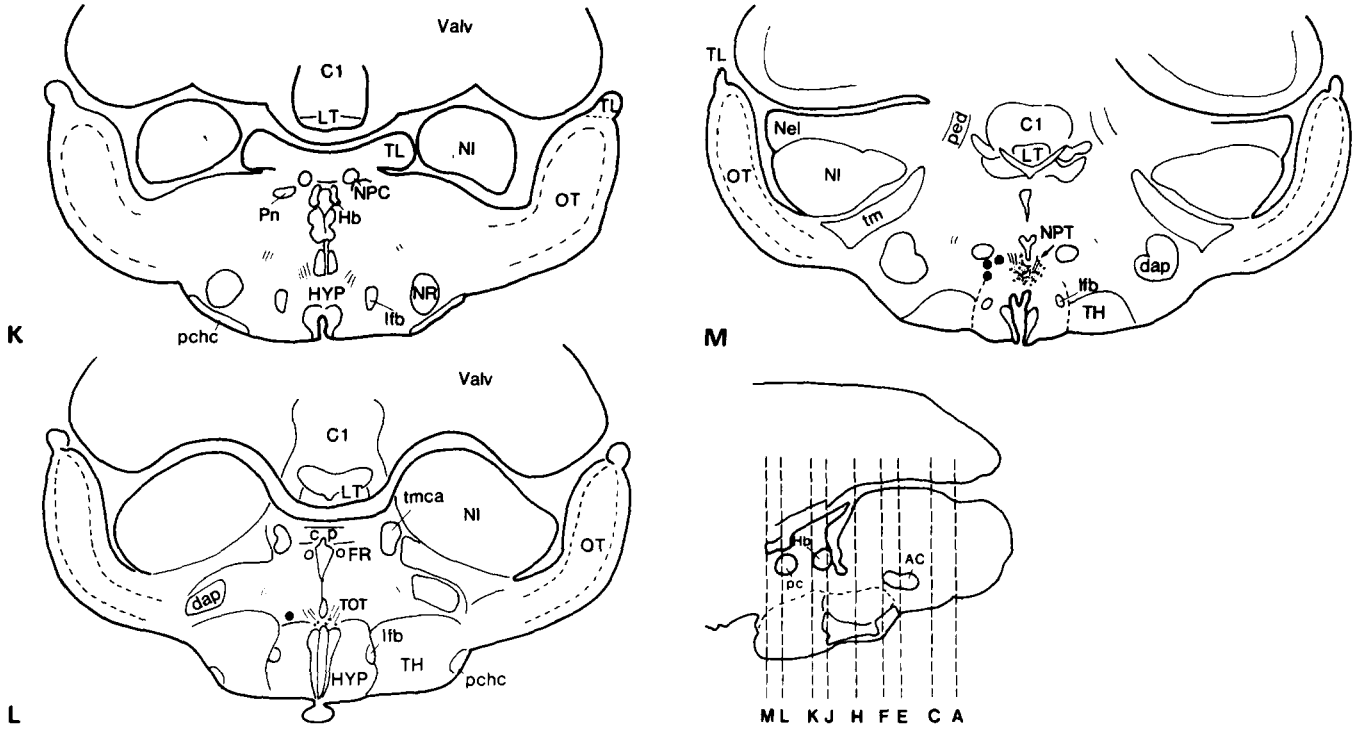


Fig. 2A–M. Schematic camera lucida drawings of transverse sections through the telencephalon and diencephalon of the brain of *Gnathonemus petersii* showing the position and extent of secondary olfactory projections demonstrated by the application of HRP or cobalt to the left olfactory bulb. Rostral to caudal (*Insert:* levels from which the sections were taken). Labelled fibres are represented by *broken thin lines*, terminal areas by *small dots* and retrogradely labelled neurons by *solid circles*



More caudally, a diffuse area of terminal aborizations is present in the midline area where the two hemispheres join the inter-ventral plexus (IVP) (Figs. 2D, 3c). Some fibres, after passing initially caudally in a ventromedial plane, rise to join the dMOT at precommissural levels.

(b) Ipsilateral projections of the LOT. The lateral olfactory tract enters the rostral telencephalon as a dense sheet of fibres that curve around the dorsal and lateral edges of the sulcus endorhinalis (Fig. 2A). As the tract moves caudally, it splits into a number of fibre bundlets (Fig. 3b), which we have provisionally grouped into dorsolateral (dLOT), medial (mLOT) and ventral (vLOT) fascicles (Fig. 2C).

The dLOT fascicle separates from the main trunk bundle shortly after entering the telencephalon and ascends along the border between the telencephalic areas Vl and Dlb to enter the ventral Dcd area (Figs. 2B, C, 3a). Some fibres branch off slightly rostral to the anterior commissure and course medially to join the dorsolateral component of the MOT in the Vs area (Fig. 2D). Further caudally, other fibres of the dLOT turn medially and project to the posterior and dorsal part of the AC. The remaining fascicles of the dorsolateral division proceed to the posterior telencephalic areas Vp, Vi and dorsomedial components of Dlp (Fig. 5c).

The vLOT and mLOT, situated along the transitional zone between the ventrolateral and dorsolateral telencephalic areas, presented a banded appearance rostral to the AC, when viewed in the transverse plane (Figs. 2C, 3b). The vLOT coursed caudally along the medial surface of the Dlc and terminal labelling was observed throughout the length of the Dlc; at rostral levels, terminals appeared to be restricted to the medial edge of the nucleus (Fig. 2C), whereas further caudally, at the level of the AC, the entire Dlc was covered by dense terminal aborizations (Figs. 2D, 3b). Apart from its connections with the Dlc, the vLOT also projected to a medial zone of the caudoventral Dlp (Fig. 5c). The major ipsilateral targets of the mLOT are the NT and the most ventral aspect of the medial Dlp, dorsal to the NT. Some dorsal fascicles of the mLOT pass medial to the Dlm nucleus and project towards the AC (Fig. 2E).

(c) Contralateral projections to the telencephalon. Olfactory projections to the contralateral hemisphere cross in the preanterior commissural area ventralis (Figs. 2D, 3c) and via the posterior dorsal part or the anterior commissure (Figs. 2F, 3d). The vMOT appears to be the sole olfactory fibre tract that contributes to the precommissural interventral area and that crosses rostral to the AC. This projection, which may correspond to the commissure of Goldstein in other teleost species, appears however in *Gnathonemus petersii* as a diffuse web of fibres (IVP) (Figs. 2D, 3c) rather than a compact commissural system. The largest part of the intertelencephalic pathway is located in the posterior dorsal part of the AC (Fig. 3d). Fascicles from the MOT, dLOT and mLOT contribute to the decussation. The terminal fields in the contralateral hemisphere are similar to those on the ipsilateral side except for the contralateral Dlc nucleus rostral to the AC. They are, however, less extensive. The origin of terminations within the AC could not be determined.

(d) Extratelencephalic projections. The olfactory bulb projects not only to the telencephalon, but also to the contralateral olfactory bulb, the preoptic area, the diencephalon and the thalamus. The interbulbar connections appear to travel exclusively via the MOT.

The most substantial descending olfactory projection passes via the olfactory tuberal tract (TOT; Fig. 2L) to

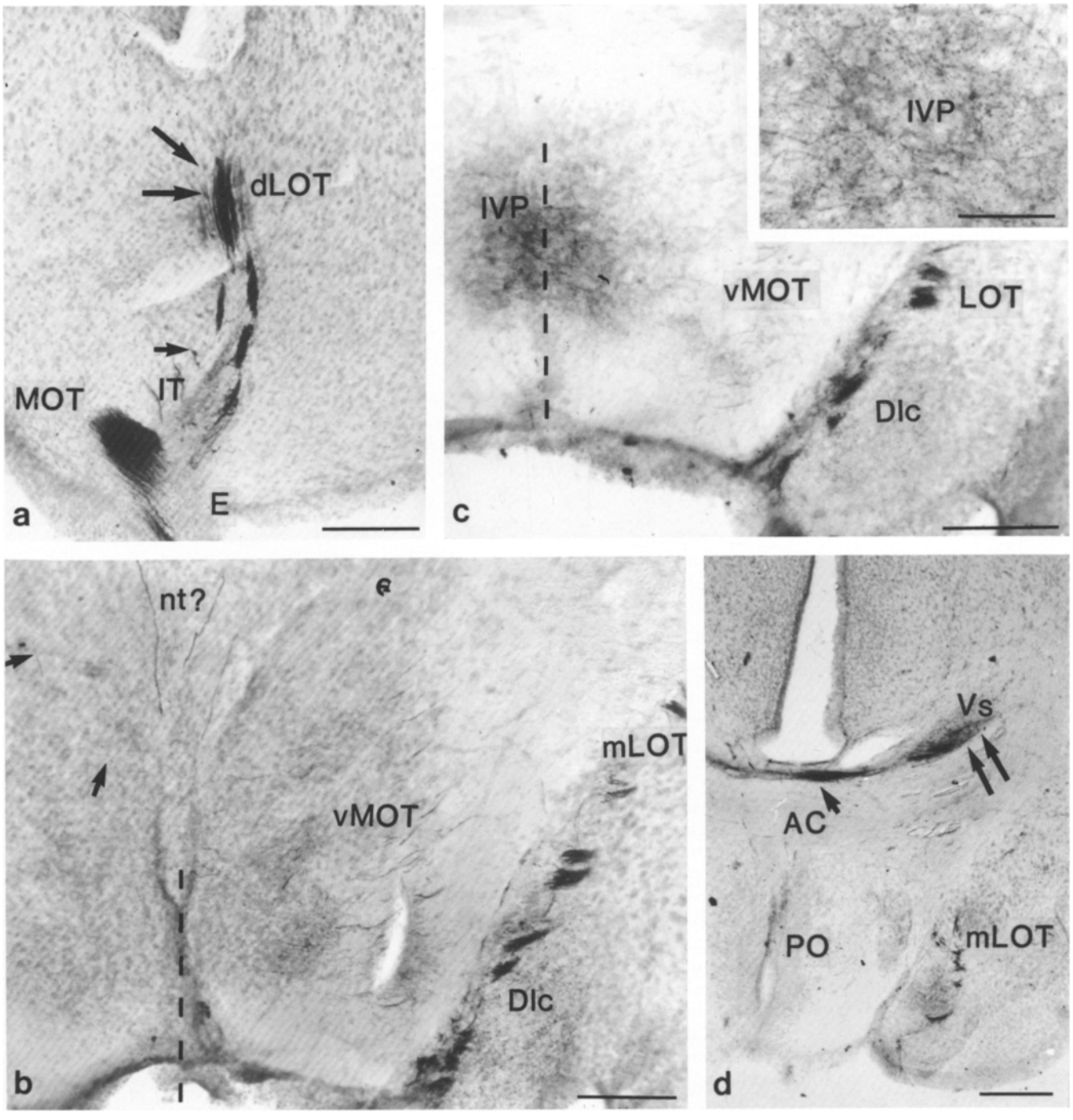


Fig. 3. **a** Transverse section through the rostral telencephalon showing the entry points of the *MOT* and *LOT*; the fascicules of the *IT* lie between the two principal bundlets. The position of retrogradely filled cells (*arrows*) along the medial edge of the *LOT* is also apparent. *Bar:* 100 μm; × 160. **b** Extensive differentiation of the *LOT* into fascicles. The position of possible *nervus terminalis* fibres (*nt?*) and fine labelled fibres (*arrows*) in the contralateral hemisphere is evident. The *dashed line* represents the midine. *Bar:* 100 μm; × 160. **c** Photomicrograph of a transverse section slightly rostral to the anterior commissure showing the extensive interventral plexus (*IVP*). *Dashed line* midline. *Bar:* 100 μm; × 190. *Insert: IVP* (from **c**) and its extensive terminal network shown at higher magnification. Cobalt preparation. *Bar:* 50 μm; × 320. **d** Transverse section through the telencephalon at the level of the posterior part of the *AC*, showing decussating olfactory fibres (*arrow*) and a terminal area (*double arrow*) over the Vs nucleus (compare **2F**). *Bar:* 200 μm; × 60

the nucleus posterior tuberis (NPT) in the diencephalon (Fig. 6a). The olfactory tuberal tract descends bilaterally from the posterior telencephalon and courses medially through the diencephalon to form an extensive terminal field over the nucleus posterior tuberis (Figs. 2M, 6b).

A second, less substantial, bilateral tract was observed descending lateral to the olfactory-tuberal tract (Fig. 2H); this tract, which was composed of very fine fibres, could be followed to an area of the thalamus caudal to the posterior commissure and lateral to the NPT terminal field. A

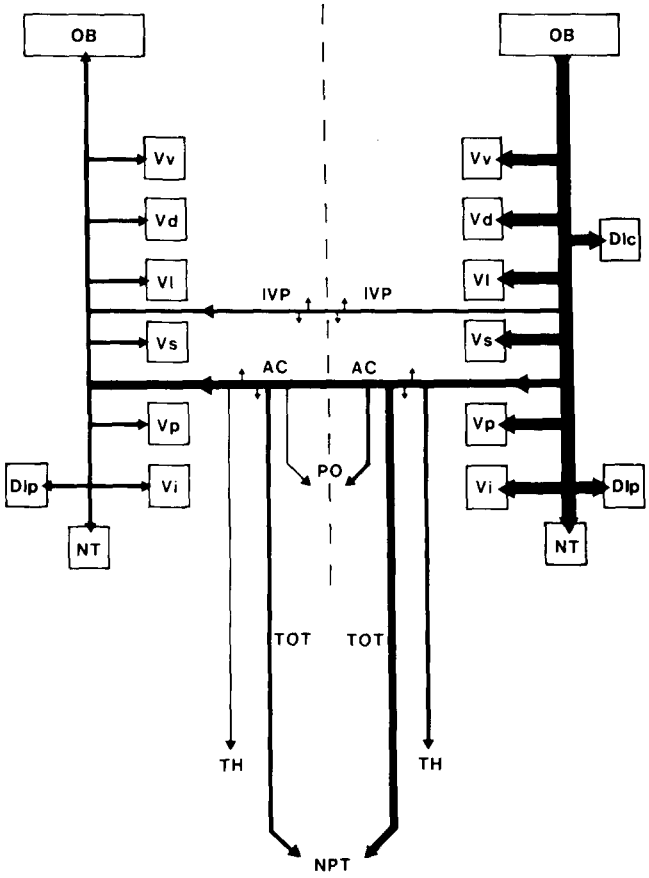


Fig. 4. Simplified schematic drawing of the efferent fibre connections of the olfactory bulb in *Gnathonemus petersii*

few olfactory fibres terminating bilaterally in the medial region of the preoptic area were also identified.

Afferent projections

Following unilateral injections of HRP or cobalt into the olfactory bulb, retrogradely filled cells were observed in the telencephalon, diencephalon and contralateral olfactory bulb.

In the telencephalon, retrogradely labelled neurons were found bilaterally in the Vi and Dlp areas of the posterior telencephalon (Fig. 6c) and in the Dcm and Vs areas at AC levels. The cells in the Vi and Dlp areas were fusiform or oval-shaped, their long axis measuring 10–18 μm. Labelled neurons were present along the lateral edge of the NT, but not in the nucleus itself (Fig. 6c). The number of labelled cells on the contralateral side was small and they were predominantly located in the medial Dlp area. The labelled neurons in the Vs area were fusiform and had a long axis of 12–18 μm (Fig. 5a). A plate of cells (10–12 μm) was bilaterally labelled in the Dcm area in the rostral telencephalon; only a few cells were labelled on the contralateral side.

Retrogradely labelled neurons were found only ipsilaterally within the dorsal Vd area of the rostral telencephalon (Fig. 5b, insert). These neurons were small (8–10 μm long axis) and oval shaped. A small group of fusiform cells (20 μm long axis), were filled just caudal to the entry point of the LOT (Fig. 3a, arrows), ipsilaterally to the injected bulb.

In the diencephalon, HRP-filled cells were observed lateral to the terminal field of the tuberal olfactory tract (Fig. 6d). Most of these cells were on the side ipsilateral to the injection site where they appear to arch around the rostral hypothalamus. Occasionally, a number of large neurons were labelled bilaterally in the periventricular diencephalon, medial to the descending tuberal olfactory tract and in an area dorsal to the NPT terminal field (Fig. 5d).

Retrograde labelling was present in a small number of mitral cells in the contralateral olfactory bulb.

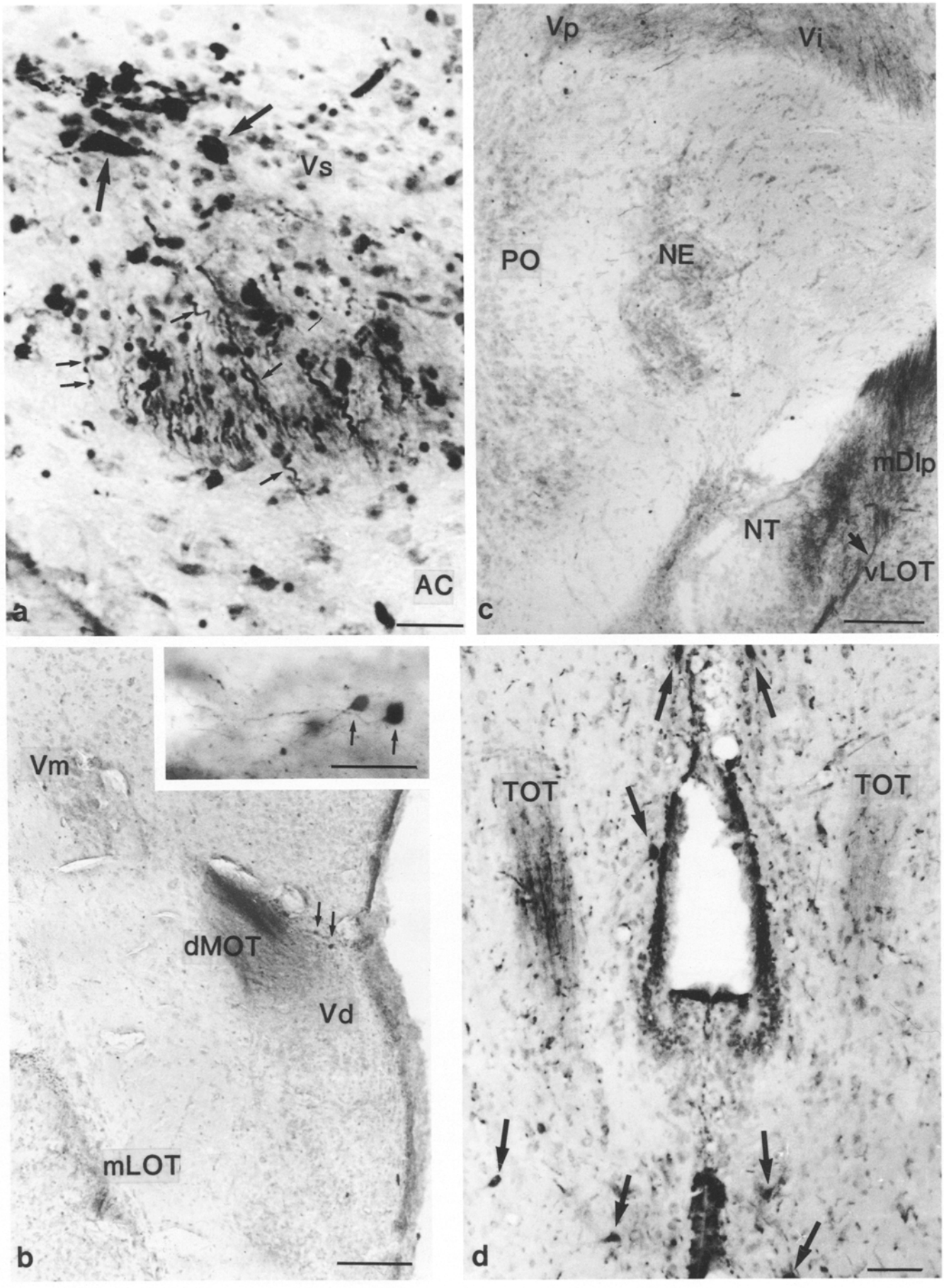
Discussion

The present findings concerning the secondary olfactory projections in *Gnathonemus petersii* are in close agreement with previous reports (Table 1). However, although a considerable degree of similarity has become apparent with respect to the areas receiving olfactory input, there remain notable interspecies differences, particularly in relation to (a) the degree of differentiation of the olfactory tracts; (b) the number of crossing fibre pathways; (c) the location and number of groups of cells projecting to the bulb; and (d) the extent of olfactory input to the telencephalon.

(a) Olfactory tracts

Although we were unable to label the different divisions of the olfactory tract in *Gnathonemus petersii* selectively, as was possible in *Ictalurus* (Finger 1975; Bass 1981a) and *Carassius* (von Bartheld et al. 1984; Levine and Dethier 1985), we could identify the different olfactory subtracts in the rostral telencephalon. The separation of the MOT into distinct dorsal and ventral components appears to be a relatively constant condition in most of the teleost species studied to date. In *Salmo* (Northcutt and Davis 1983), where the separation is not distinct, the dorsal component of the MOT is reported to be more extensive than the ventral part.

The degree of differentiation recognized in the LOT varies greatly between the species studied. The lateral olfactory tract in *Gymnothorax* (Scalia and Ebbesson 1971), *Serrasalmus* (Ebbesson et al. 1981), *Lepomis* and *Salmo* (Northcutt and Davis 1983), and *Carassius* (von Bartheld et al. 1984; Levine and Dethier 1985) enters and travels through the telencephalon as a single tract. In *Ictalurus* (Finger 1975; Bass 1981a), *Sebastiscus* (Murakami et al. 1983) and *Pseudopleuronectes* (Prasada Rao and Finger 1984), separate medial and lateral divisions of the LOT have been reported. In *Pseudopleuronectes,* the medial component of the LOT enters the telencephalon along with the MOT system, whereas in *Ictalurus* and *Sebastiscus,* the two divisions of the LOT enter the telencephalon lateral to the MOT. In *Ictalurus punctatus* (Bass 1981a), the lateral and medial divisions of the LOT separate at extreme rostral levels in the telencephalon, whereas in *Ictalurus nebulosus* (Finger 1975), the LOT enters the telencephalon as two compact bundles that fuse in the rostral telencephalon before separating into several fascicles as the tract courses caudally. In *Ictalurus,* a differentiation of the terminal areas associated with the two divisions of the LOT is also apparent; the lateral components terminate principally in the lateral telencephalic areas, being specialized for bilateral input to the area dorsalis in *Ictalurus punctatus* (Bass 1981a), and the medial component terminates principally in more medial areas of the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telencephalon. In *Gnathonemus,* the LOT appears to show a higher degree of differentiation than that reported in other species. The LOT enters the telencephalon as a dense sheet of fibres that separates into numerous compact fascicles as it courses caudally. We have provisionally grouped the fascicles into dorsal, medial and ventral subgroups on the basis of their position in the rostral telencephalon and projections. Whereas each of the subgroups appears to be principally associated with specific terminal areas (i.e., fascicles from the vLOT with the Dlc nucleus), selective labelling of the individual subtracts is required to determine whether each fascicle projects to a circumscribed terminal area and to determine the distribution of the cells of origin of the fascicles within the olfactory bulb.

(b) Crossing fibre pathways

In *Gnathonemus petersii,* olfactory fibres cross to the contralateral hemisphere via the posterior dorsal part of the anterior commissure and in an area of the ventral midline just rostral to the anterior commissure. The olfactory fibres decussating in the posterior dorsal part of the anterior commissure arise from both the medial and lateral olfactory tracts; a similar projection has been reported in most previous studies (Finger 1975; Oka 1980; Ebbesson et al. 1981; Bass 1981a; Levine and Dethier 1985; von Bartheld et al. 1984; Northcutt and Davis 1983), although in the moray eel (*Gymnothorax funebris*), only the MOT is reported to cross the anterior commissure (Scalia and Ebbesson 1971). The decussating olfactory fibres in the rostral area ventralis arise solely from the ventral part of the medial olfactory tract; some fibres terminate in the contralateral area ventralis, whereas others project to the contralateral bulb. This decussating fiber system appears to correspond to the interbulbar commissure of Goldstein as reported in *Cyprinus* (Ito 1973), *Ictalurus* (Finger 1975; Bass 1981a, b), *Macropodus* (Davis et al. 1981) and *Pseudopleuronectes* (Prasada Rao and Finger 1984). A similar projection also appears to be present in *Carassius* (von Bartheld et al. 1984; Levine and Dethier 1985) where decussating olfactory fibres in the rostroventral and posterior dorsal divisions of the anterior commissure have been reported. Studies on the olfactory projections in *Gymnothorax* (Scalia and Ebbesson 1971), *Serrasalmus* (Ebbesson et al. 1981), *Sebastiscus* (Murakami et al. 1983) and *Lepomis* and *Salmo* (Northcutt and Davis 1983) report solely a projection via the anterior commissure in the telencephalon; whether the absence of the more rostral decussating fibre pathway in these species is a true interspecies difference or merely reflects differences in the sensitivity of the tracing techniques employed remains unclear. Indeed, in *Gnathonemus petersii,* the full extent of the olfactory projections crossing and terminating in the pre-commissural IVP area only becomes evident in the cobalt preparations. In several teleost species, *Ictalurus* (Finger 1975; Bass 1981a), *Macropodus* (Davis et al. 1981), *Carassius* (von Bartheld et al. 1984; Levine and Dethier 1985), *Salmo* and *Lepomis* (Northcutt and Davis 1983) and *Gadus morhua* (unpublished observations), olfactory projections also cross to the contralateral hemisphere via the habenular commissure. Although a few fibres rise towards the habenular nuclei in *Gnathonemus petersii,* we have found no evidence of fibres crossing the habenular commissure in the present study. The absence of decussating olfactory projections in the habenular commissure appears to represent a significant interspecies difference in the olfactory pathways in teleost fish, since it is also absent in *Pseudopleuronectes* (Prasada Rao and Finger 1984), *Gymnothorax* (Scalia and Ebbesson 1981), *Serrasalmus* (Ebbesson et al. 1981) and *Sebastiscus* (Murakami et al. 1983).

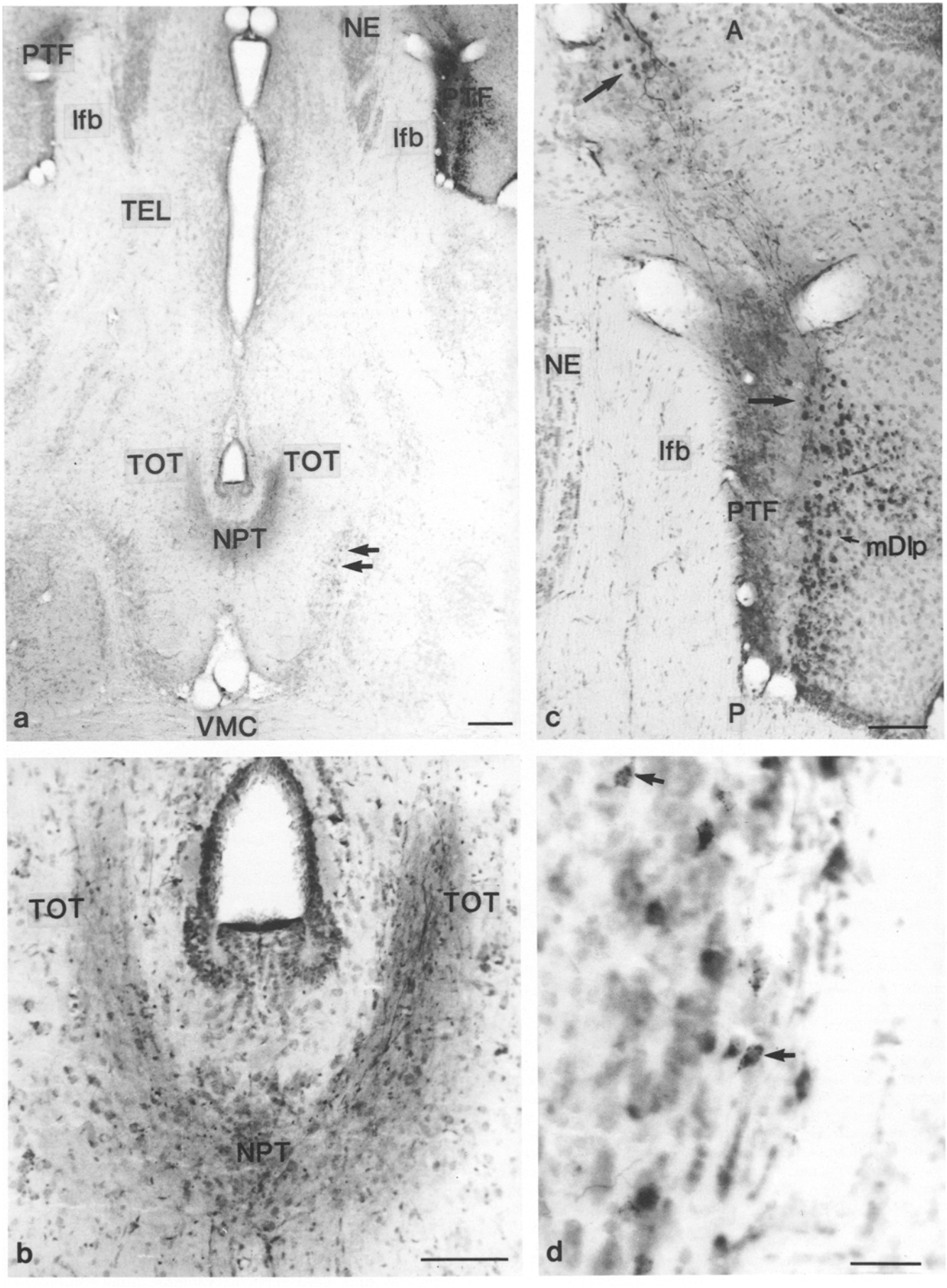
(c) Cell groups projecting to the olfactory bulb

Considerable variation exists in the number of telencephalic cell groups reported to project to the olfactory bulbs in the teleost species studied to date. In *Serrasalamus* (Ebbesson et al. 1981), all ipsilateral cell groups receiving an olfactory tract projection project back to the bulb, whereas in *Sebastiscus* (Murakami et al. 1983) and *Pseudopleuronectes* (Prasada Rao and Finger 1983), only four telencephalic areas contain neurones afferent to the bulb. In *Gnathonemus,* six bulbopetal cell groups are present in the telencephalon, four of which (Vs, Vi, Dcm and cells in the medial Dlp area) are labelled bilaterally. The latter finding of bilateral telencephalic projections to the bulb is in agreement with reports by Prasada Rao and Finger (1984) and von Bartheld et al. (1984) in *Pseudopleuronectes* and *Carassius,* respectively. Earlier studies (Oka 1980; Ebbesson et al. 1981; Bass 1981b; Murakami et al. 1983) have identified bullbopetal cells only in the ipsilateral telencephalon.

Several studies report bulbopetal cells in the nucleus taenia (von Bartheld et al. 1984; Levine and Dethier 1985); no retrogradely labelled cells have been identified within this nucleus in *Gnathonemus.* Some of the reported variation in the number of bulbopetal telencephalic cell groups may be accounted for by the position of some bulbopetal cell groups in the transitional zone between two or more of the designated telencephalic subdivisions; they may thus reflect differences in interpretation of where such divisions lie (von Bartheld and Meyer 1986). However, this possibility cannot account for all the differences reported; indeed, the adoption of a common nomenclature for the major divisions of the telencephalon has generally enhanced comparative studies.

Neurones afferent to the olfactory bulb in the contralateral bulb have been reported in most recent studies (Bass 1981b; Prasada Rao and Finger 1984; von Bartheld et al. 1984). The existence of bulbopetal cell groups in lower brain centres has only been reported in three other studies to

Fig. 5. a Labelling in the telencephalon of *Gnathonemus petersii* after application of HRP to the ipsilateral olfactory bulb. Labelled terminal fibres (*small arrows*) and retrogradely labelled neurons (*large arrows*) within the *Vs* area at mid-anterior commissural (*AC*) level. *Bar:* 25 μm; × 500. **b** Labelled terminals over the dorsal lateral part of the *Vd* area. *Bar:* 100 μm; × 140. *Insert:* High magnification of retrogradely labelled neurons (*arrows*) along the medial edge of the *Vd* terminal area. *Bar:* 25 μm; × 640. **c** Transverse section through the posterior telencephalon showing the extensive posterior terminal field covering *Vp, Vi* and the medial *Dlp* area (*mDlp*) after application of cobalt to the ipsilateral olfactory bulb. Note the *vLOT* fascicles (*arrow*) terminating in the *Dlp* area dorsal to the *NT. Bar:* 100 μm; × 150. **d** Horizontal section through the diencephalon of the brain of *Gnathonemus petersii* showing the distribution of retrogradely labelled neurons in the periventricular area following an injection of HRP into the ipsilateral olfactory bulb (*left-hand side*). Labelled cells are present bilaterally in the periventricular area (*arrows*) medial to the descending tuberal olfactory tract (*TOT*). *Bar:* 100 μm; × 100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date (Prasada Rao and Finger 1984; Levine and Dethier 1985; von Bartheld et al. 1986). The position and size of the bulbopetal cell group, which lies lateral to the nucleus posterior tuberis terminal field in the caudal diencephalon, suggests homologies with the bulbopetal cell group identified in the lateral nucleus preglomerulosus of goldfish (Levine and Dethier 1985). The small number of large retrogradely filled cells in the periventricular diencephalic region in *Gnathonemus* have not been identified in other species. Their position and distribution make it unlikely that these neurones are homologous with the reported small number of mesencephalic cells projecting to the ganglion of the nervus terminalis in goldfish (von Bartheld et al. 1986); future studies should resolve this question. In the present investigation, no bulbopetal cell group comparable to the tegmento-olfactory nucleus of *Pseudopleuronectes* is apparent.

(d) Telencephalic areas receiving olfactory input

As noted in the introductory remarks, previous observations suggest that a reduction in olfactory input to the telencephalon accompanies increased telencephalic differentiation in actinopterygian fish (Nieuwenhuys 1963; Northcutt and Braford 1980). This trend is particularly evident with respect to the extent of olfactory input to the area dorsalis. As the medial region of Dlp is the principal area dorsalis target of descending olfactory projections in *Gnathonemus petersii,* we believe that in future this area should be termed posterior part of the area dorsalis (Dp) to distinguish it (in accordance with Northcutt and Braford (1980)), from the more lateral Dlp cell groups that do not receive olfactory input. The small size of the Dp (mDlp) area in the highly differentiated telencephalon of *Gnathonemus petersii* appears to support the suggestion that a reduction in olfactory input to Dp accompanies increased telencephalic differentiation, and that this trend is not only evident between the major actinopterygian superorders but also within the teleost lineage. However, although Northcutt and Braford (1980) note a reduction in the size of Dp between *Salmo* and the more advanced euteleost *Lepomis,* neither the degree of telencephalic differentiation (Nieuwenhuys 1963; Northcutt and Braford 1980) nor the extent of olfactory input to the telencephalon follow the proposed systematic scale (Lauder and Liem 1983). Indeed, with respect to the extent of telencephalic differentiation and the degree of reduction of olfactory input to area Dp, *Gnathonemus* resembles the advanced teleosts rather than the primitive teleost species.

Olfactory input to the area ventralis appears to show less variation than that to the area dorsalis. However, the presence and extent of a pre-anterior commissural interventral terminal area differs between species. The interventral terminal area has been reported in *Carassius* (Levine and Dethier 1985), *Ictalurus nebulosus* (Finger 1975), *Pseudopleuronectes americanus* (Prasada Rao and Finger 1984) and appears to be present in the anterior central part of area V in the autoradiograms of *Macropodus* (Davis et al. 1981).

Nervus terminalis

Although the position of the ganglion cells (Munz et al. 1982; Springer 1983; Demski and Northcutt 1983; Stell et al. 1984; von Bartheld et al. 1986) and some of the central connections of the nervus terminalis system in goldfish are known (Demski and Northcutt 1983; von Bartheld and Meyer 1986; von Bartheld et al. 1986) the location of the ganglion cells of the nervus terminalis system in *Gnathonemus petersii* has yet to be demonstrated. A study of the retinal projections in *Gnathonemus* (Lazar et al. 1984) has failed to identify projections to or from the olfactory system, although the authors note that a number of labelled fibres in the lfb appear to have a similar position and course to the retinopetal fibres of the nucleus olfactoretinalis as described by Springer (1983) in the goldfish. Preliminary LHRH-immunocytochemical studies and our attempt to label the system by applying HRP to the cut olfactory nerves have also failed to identify the ganglion cells of the nervus terminalis in *Gnathonemus.* However, in the present study, a number of large fibres were identified in the pre-anterior commissural area ventralis, optic chiasma and posterior commissure in two of the fish that received cobalt injected into the olfactory bulb. These projections were interpreted as being part of the nervus terminalis system (von Bartheld and Meyer 1986) and thus not included in our description of the "classical" olfactory projections in *Gnathonemus.* The application of cobalt to the olfactory epithelium or to the ganglion of the nervus terminalis has recently been employed to identify the primary olfactory projections in king salmon fry (Bazer et al. 1987) and the central connections of the nervus terminalis in goldfish (von Bartheld and Meyer 1986; von Bartheld et al. 1986), respectively. A similar approach may facilitate the identification of these systems in *Gnathonemus.*

Finally, although little is known about the role of olfaction in the behavioural repertoire of the weakly electric fish *Gnathonemus petersii,* there is little evidence from the present results to suggest that the olfactory system is less developed than that in other teleost species, as shown for the visual system (Lazar et al. 1984). Indeed, it will be interesting to establish the functional significance of the olfactory system in *Gnathonemus* and its interaction with the electro-sensory system in, for example, feeding and reproductive behaviour.

Fig. 6. **a** Labelling in the telencephalon and diencephalon of the brain of *Gnathonemus petersii* following HRP injection into the ipsilateral *(right-hand side)* olfactory bulb. A horizontal section showing the position of the posterior telencephalic terminal area *(PTF)* arching around the lateral forebrain bundle *(lfb)*. Note the less extensive labelling in the contralateral hemisphere. *Bar:* 200 μm; ×40. **b** Higher magnification of the terminal field over the nucleus posterior tuberis *(NPT)*. *Bar:* 100 μm; ×160. **c** Horizontal section through the brain of *Gnathonemus petersii* showing the location of labelled neurons in the pre- and post commissural areas *(arrows)*. *A–P* antero-posterior orientation. *Bar:* 100 μm; ×100. **d** High magnification of retrogradely labelled neurons *(arrows* in **a**) in the caudal diencephalon lateral to the *NPT* terminal field. *Bar:* 25 μm; ×520

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