

Olfactory Bulb Efferents in the Channel Catfish, *Ictalurus punctatus*

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ABSTRACT Autoradiographic, HRP, and Fink-Heimer techniques define olfactory bulb efferents in the channel catfish. The olfactory bulb projects bilaterally to eight targets in the area ventralis telencephali including the preoptic area, five targets in area dorsalis telencephali, and the posterior tuber of the diencephalon. There is additional input to the peripheral margin of the internal cell layer of the contralateral olfactory bulb. Fibers cross in rostral (nervus terminalis and commissure of Goldstein) and caudal components of the anterior commissure and the habenular commissure. HRP techniques reveal the origin of bulb efferents from the internal and mitral cell layers of the olfactory bulb. The olfactory tract is divided into five major components, each with a unique subset of ipsilateral and commissural pathways.

The actinopterygians, or ray-finned fishes, are traditionally separated into three superorders (Romer, '70), namely the Chondrostei, Holostei, and Teleostei. The teleosts comprise at least 20,000 species and are further divided into three divisions: I, the eels and eel-like fishes; II, the mormyriforms and osteoglossiforms; and III, the bulk of teleost species (Greenwood et al., '66). Teleosts display a wide range in variation of telencephalic organization, which suggests that this is adapted to divergent ecological and behavioral niches.

This study reveals the olfactory bulb efferents in a Division III teleost, the channel catfish, *Ictalurus punctatus*. The catfishes, order Siluriformes, form a major division (about 2,000 species) of the superorder Ostariophysi, which includes the majority of freshwater fishes (5-6,000 species, Greenwood et al., '66). This demonstration of olfactory bulb efferents is critical to understanding telencephalic organization in catfish, and indeed, other teleosts for four reasons: (1) The early anatomists portray the telencephalon of teleosts as an olfactory-dominated structure (Johnston, '11; Herrick, '21). (2) The olfactory system is the only sensory system with direct input to the telencephalon. (3) It is impossible to correlate the topography of telencephalic olfactory bulb targets recognized in previous experimental studies (Scalia and Ebbesson, '71; Ito, '73; Finger, '75) with telencephalic subdivisions identified in the majority of cytoarchitectural studies (Nieuwenhuys, '63; Bass, '81a; Northcutt and

Braford, '80). (4) Comparable experimental data are available for a representative member of each of the other groups of actinopterygians (Braford and Northcutt, '74; Northcutt and Braford, '80).

This study is presented within the context of a cytoarchitectural analysis of the telencephalon of the channel catfish, whose nomenclature is based on a series of normal and experimental studies of several actinopterygians (Bass, '81a). This is the first experimental report that utilizes axonal transport methods to define olfactory bulb efferents in a nonmammalian vertebrate. The results offer new data on: (a) the separate projections of the medial and lateral olfactory tracts, (b) the number of olfactory bulb targets in the subpallium and pallium, (c) olfactory bulb input to the preoptic area and the contralateral olfactory bulb, and (d) the cells of origin of olfactory bulb efferents.

A portion of these results appeared earlier (Bass, '78).

MATERIALS AND METHODS

Experimental animals

Adult and juvenile specimens of channel catfish, *Ictalurus punctatus*, were collected on the grounds of the Spring Valley Trout Farm, Dexter, Michigan. Animals were housed in aquaria

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for at least 1 week prior to surgery, the water temperature being maintained pre- and post-operatively at 24–27°C.

Experimental methods

Olfactory bulb efferents were traced by transections of the olfactory peduncle and olfactory bulb injections of tritiated proline or horseradish peroxidase (HRP). Prior to all surgical procedures, animals were anesthetized by immersion in home tank water containing tricaine methanesulfonate (MS222).

Transections

In four juvenile (18–10.5 cm, snout-tail length) and four adult (27–28 cm) channel catfish, the olfactory peduncle was transected rostral to the anterior pole of the telencephalon. Following survival times of 1–12 days, animals were anesthetized with MS222, and perfused transcardially with 0.7% saline followed by 10% Formalin. All brains were fixed for at least 1 week in 10% Formalin and were embedded in 25% gelatin. Frozen sections were cut at 33 μm in the transverse plane and stored in 2% Formalin. Sections were stained for degenerating axons and terminals according to the Wiitanen modification of the Fink-Heimer technique (Wiitanen, '69).

Proline injections

Eight adult (28- to 42.5-cm) channel catfish received olfactory bulb injections of 0.1–0.5 μl of tritiated proline (New England Nuclear NET-323, concentrated to 20 $\mu\text{Ci}/\mu\text{l}$). All injections were made under pressure via a 1-lambda pipette (drawn and bevelled to a tip of 75 μm) attached to a stereotaxic device. Injections were made over a 5- to 10-min period. Following postoperative survival times of 1–12 days, animals were reanesthetized with MS222 and perfused transcardially, with 0.7% saline followed by AFA (see Bass, '81a). The brains were removed from the skull and placed in fixative for at least 1 week, with subsequent embedding in paraffin. Material was serially sectioned at 15 μm , in either the transverse or horizontal plane. The mounted sections were deparaffinized, coated with Kodak NTB2 or 3 emulsion, and exposed at 7°C for 28 or 40 days. After development in D-19 or Dektol, sections were counterstained with cresyl violet. Tracings of high-contrast photographs from an earlier cytoarchitectural study (Bass '81a) were used to plot the distribution of silver grains.

In seven adult (31- to 37-cm) channel catfish, a portion of the medial and/or lateral division

of the olfactory peduncle was transected prior to injection of proline into the ipsilateral olfactory bulb (as above). These experiments were designed to elucidate the separate projections of the medial (lateral transection) or lateral (medial transection) division of the olfactory tract.

HRP injections

During a subsequent HRP analysis of olfactory bulb afferents, anterograde transport of HRP (following olfactory bulb injections) confirmed the trajectory of olfactory bulb efferents. Experimental details appear elsewhere (Bass, '81b).

For two juvenile (18-cm) channel catfish, the dorsal surface of the ipsilateral telencephalon was exposed. The injection pipette was guided stereotaxically into the rostromedial and caudolateral segments of the hemisphere with subsequent injections of 1.0–2.0 μl of HRP over a period of 5–10 min. These experiments were designed to reveal the cells of origin of olfactory bulb efferents. Following reanesthetization with MS222, animals were perfused transcardially with cold phosphate buffer (pH 7.4) followed by 2% glutaraldehyde in cold buffer. After removal from the skull, each brain was washed in 30% sucrose-fixative (2–3 hr) and then 30% sucrose-buffer (12 hr). Brains were embedded in gelatin-sucrose and sectioned frozen at 33–40 μm . Staining procedures were modifications (cf. Bass, '79) of the O-dianisidine reactions of Colman et al. ('76) and the tetramethylbenzidine (TMB) reaction of DeOlmos et al. ('78).

A distinct advantage of using catfish for the study of olfactory bulb efferents with protein transport methods is that the olfactory bulb is displaced from the rostral pole of the telencephalon along extended olfactory peduncles (Fig. 1). This condition alleviates the problem of local diffusion of labeled protein into adjacent structures (such as the telencephalon in animals with sessile bulbs), thus hindering the interpretation of pathways originating from the olfactory bulb.

RESULTS

Methodological notes

Silver degeneration

Optimal terminal degeneration occurred in juvenile specimens (13–16 cm), surviving 5–7 days at 26°C. By 9 or 10 days, argyrophilia was predominant in the olfactory tracts. Both ax-

onal and terminal argyrophilic debris appeared as small- to medium-caliber black granules (Fig. 9). In all cases, degeneration within the lateral olfactory tract and the area dorsalis telencephali was coarser and heavier than in the medial olfactory tract and the area ventralis telencephali. Excepting the preoptic area, terminal debris occurred in all regions identified as targets with autoradiographic and HRP material. The trajectory of the medial olfactory tract pars medialis could not be accurately traced with Fink-Heimer methods. One bullhead catfish, *Ictalurus natalis*, sustained a unilateral olfactory tract cut and survived 14 days at 26°C. Sparse degeneration appeared in all regions identified as olfactory targets in *I. punctatus*, while moderate- to large-caliber fragmented varicosities distinguished fiber pathways. An autoradiographic analysis in a second bullhead confirmed the pattern of terminal fields seen in channel catfish. Thus, the bullhead degeneration data were taken as representative of the condition in channel catfish. However, even in bullheads, degeneration material did not reveal a clear portrayal of the pathways and terminals for the medial components of the medial olfactory tract.

Autoradiographic techniques

At 1-day survival time, olfactory bulb autoradiographs reveal dense label over all terminal fields, including the preoptic area, with sparse grain accumulations over the ipsilateral and contralateral olfactory tracts. Increased labeling of tracts and terminal fields occurred at longer survival times, with all tracts clearly labeled by 5 days (Figs. 4, 8, 10). Transneuronal transport of proline should not have obscured the profile of terminal fields, as such transport has not been reported for survival times of less than 11 days among non-mammals (Landreth et al. '75; Lázár, '76, Repérant et al. '78).

An advantage of using the autoradiographic method is that the experimental material can be prepared for paraffin histology. This allows serial sections to be made of each brain, optimizing the relationship between terminal fields and pathways, and recognized cytoarchitectural boundaries (Bass, '81a).

HRP techniques

Treatment of HRP material with the TMB procedure yielded the best visualization of olfactory pathways and terminals, with the heaviest label in the ipsilateral olfactory tract.

Fibers appeared as sinuous lines of clumped reaction product oriented within the plane of section. Terminal zones contained a homogeneous distribution of fine granular precipitate.

Following telencephalic HRP injections, labeled cells within the olfactory bulb contain a dense homogeneous and/or a granular precipitate (Fig. 11).

Secondary olfactory pathways and terminal fields: Overview

The following description of olfactory bulb efferents (secondary olfactory pathways) and terminal fields is based on the results of proline injections into the olfactory bulb. Figures 2, 3, and 5-7 are representative cross sections through the forebrain of *I. punctatus* at the levels indicated in Figure 1. Corresponding Nissl-stained sections at the illustrated telencephalic levels appear in the previous paper (Bass, '81a). Chartings of pathways (dashes, Figs. 2, 3, 5-7) are based on animals that survived 5-12 days, while those of terminal fields (stippling, Figs. 2, 3, 5-7) are based on cases that survived 1 and 3 days. HRP and Fink-Heimer material provided additional confirmation of pathways and terminal fields. Figure 12 provides a summary profile of efferents.

The initial series of experiments indicated overlap in the termination patterns of the medial and lateral olfactory tracts. Additional data on their separate pathways was provided by proline cases that involved partial transections of the olfactory peduncle.

The ipsilateral projections of the medial and lateral olfactory tracts are discussed separately from the contralateral projections and the transection experiments. A final section describes the cells of origin of olfactory bulb efferents.

Ipsilateral projections: Medial olfactory tract

In autoradiographs, dense silver grain accumulations occur throughout the bulb, extending over cells of the nervus terminalis ganglion (Fig. 2A,B). The layer of primary olfactory fibers is unlabeled.

At far rostral levels, the medial olfactory tract (mt, Fig. 2C) lies ventromedial to area dorsomedialis (DM, Fig. 2C), and the lateral olfactory tract (11t, 1mt; Fig. 2C). Caudally, as the mt expands, sparse label appears over the ventral aspect of nucleus "n" of area ventralis (Vn, Fig. 2D). A rostral segment of the central nucleus (Vc, Fig. 2D) lies adjacent to the mt and probably receives an input, as Golgi analysis (not described herein) reveals its proc-

esses extending into the olfactory tract.

As area ventralis expands, the medial olfactory tract divides into ventromedial (mm, Fig. 3A) and dorsolateral (md, Fig. 3A) components, terminating densely along the lateral aspect of Vn and the ventral nucleus of area ventralis (Vv, Fig. 3A), and the medial aspect of Vc. Sparse grain accumulations appear over scattered elements of Vn.

Caudally, the dorsal division of the medial olfactory tract adjoins a dorsal segment of the ventral nucleus (Vv-d; Figs. 3B,C; 8A) and a ventral segment of the dorsal nucleus of area ventralis (Vd-v; Figs. 3B,C; 8A). A dense terminal field appears lateral to, and over, the cells of Vv-d, while Vv-v continues to receive input along its lateral surface. Similarly, a dense terminal field surrounds Vd-v, capped by a dorsal division of the dorsal nucleus (Vd-d, Figs. 3B,C; 8A). Golgi material shows that cells within Vv, Vd-v, and Vd-v extend their processes into adjacent terminal fields.

A small fiber system, resembling the nervus terminalis pathway in carp (Sheldon, '09), branches off the ventromedial component of the olfactory tract (nt, Fig. 3B). As the commissural ridge expands centrally, a medial branch of the nervus terminalis passes ventral to the ventral nucleus. A second component branches off ventrolaterally and continues caudally throughout the lateral preoptic area to the surface of the brain (Figs. 3B,C; 5A). Whether the medial branch terminates beneath the fused aspect of the ventral nucleus or the ventral branch amid the scattered elements of the lateral preoptic region is a moot point. Both pathways are recognizable at 1-day survival, but there are no sudden increases in grain densities along their trajectories, demonstrating a distinct terminal zone.

A second branch of the ventromedial olfactory tract crosses a rostral portion of the commissural ridge as the so-called "interbulbar commissure" of Goldstein ('05). This component (cg, Fig. 3C) passes ventral to a small cluster of cells interposed between the symmetrical components of Vv-v. These cells correspond topographically to Sheldon's ('09) medial septal nucleus, which he recognized as a target of the nervus terminalis. In catfish, these cells appear more closely associated with the commissure of Goldstein. As with the nervus terminalis system, it is difficult to determine whether there are terminals in this central zone.

The most formidable zone of crossed olfactory fibers appears within the caudal segment

of the anterior commissure (ac, Fig. 5A). The dorsal and medial divisions of the medial olfactory tract converge (mdm, Fig. 5A), their fibers distributing ipsilaterally to area dorsalis (see below), a supracommissural nucleus (Vs, Fig. 5A), a rostral segment of a postcommissural nucleus (Vp, Fig. 5A,B), and the contralateral hemisphere (see below).

At postcommissural levels, the medial tract continues as a caudal component (mc, Fig. 5B) coursing ventrolateral to a caudal portion of Vp. Fibers distribute to a migrated intermediate zone of the area ventralis (Vi, Fig. 5C). Unlike the supracommissural and postcommissural nuclei, a dense uniform grain accumulation covers the cells of Vi. At this level, fibers distribute to the most caudal segment of the anterior preoptic nucleus (PPa, Fig. 5C). Rostrally, these fibers (mp, Fig. 5A) continue as two bundles through the medial preoptic area. A medial bundle lies near the ventricular surface and corresponds in position to the tractus medialis preopticus pars posterior and a more lateral bundle to the tractus medialis preopticus pars anterior of carp (Sheldon, '12: Fig. 54). Like the nervus terminalis and the commissure of Goldstein, these pathways are labeled at 1-day survival, but it is difficult to define a circumscribed input zone. These bundles traverse a zone intermediate to the magnocellular and anterior divisions of the preoptic zone, with possible input to either zone.

Finally, the caudal medial olfactory tract courses through the medial diencephalon (Fig. 6A,B) to terminate centrally in the medial nucleus of the posterior tuber (MTP, Figs. 7, 8D). This terminal field extends ventrally to appose the most dorsal aspect of the ventricle. Cells of the more lateral nucleus of the saccus vasculosus (NSV, Fig. 7) may receive input if their processes extend medially to engage the terminal zone.

The caudal component of the olfactory tract is often referred to as a hypothalamic branch (cf. Finger, '75). I abandon this designation because these fibers contribute to telencephalic terminal fields and the posterior tuber, a diencephalic zone separate from the hypothalamus proper (Braford and Northcutt, in press).

Ipsilateral projections: Lateral olfactory tract

The lateral olfactory tract, like the medial tract, comprises lateral (1lt, Figs. 2-6) and medial (1mt, Figs. 2, 4) subdivisions. The lateral division corresponds to both the lateral and intermediate divisions of the olfactory tract of carp (Sheldon, '12). Finger ('75) ap-

pears to indicate the intermediate division as the ventral or medial division of the lateral olfactory tract of bullhead catfish. In channel catfish, the medial and lateral divisions of the lateral tract separate at far rostral levels (Figs. 2D, 4), the medial lying adjacent to the ascending component of the medial olfactory tract and the lateral continuing ventral to the pars ventralis of area dorsolateralis (DLv, Fig. 2C,D). While sparse grain accumulations appear dorsal to the lateral division, there is no recognizable terminal zone at rostral telencephalic levels.

The lateral division of the lateral olfactory tract distributes densely to rostral and caudal divisions of the dorsal posterior zone (DPr, DPc, respectively; Figs. 3A-C; 5A,B; 8B; 9) and sparsely to a caudal remnant of the ventral division of area dorsolateralis (DLv, Figs. 3A-C; 5A). Within DP, silver grain accumulations are higher medially and dorsally (Fig. 8B).

At rostral commissural levels, the lateral olfactory tract distributes to the dorsal central zone that lies above DP (DC-3, Fig. 3C). DC-3 contains a medial division receiving a dense olfactory input (Figs. 3C, 5A,B; 8B) and a lateral division receiving a sparse input (Figs. 5A,B).

The most caudal telencephalic target of the lateral olfactory tract is nucleus taeniae (NT, Figs. 5C, 8C). Just dorsal to nucleus taeniae a sparse terminal field appears over a medial component of the posterior division of area dorsolateralis (DLp, Figs. 5C, 8C).

Caudally, the lateral division of the lateral olfactory tract separates as a distinct bundle just dorsal to the caudal entopeduncular nucleus (Ec, Fig. 5C) and continues through the rostral diencephalon (Fig. 6A) to the habenular commissure (hc, Fig. 6B), where it crosses to enter the contralateral component of the lateral division of the lateral olfactory tract.

The medial division of the lateral tract contributes fibers to a dorsal component of the medial tract (Figs. 3, 4). This pathway is confirmed in cases of partial transections of the olfactory tract (see below).

Contralateral projections

The contralateral olfactory bulb projections are sparser and topographically overlap the ipsilateral projections (Figs. 2, 3, 5-7).

There are four possible sources of input to the contralateral targets: (1) the medial branch of the nervus terminalis (Fig. 3B), (2) the commissure of Goldstein (Fig. 3C), (3) the anterior

commissure (Fig. 5A), and (4) the habenular commissure (Fig. 6B).

Both the habenular and anterior commissural components distribute to the area dorsalis as described above for the ipsilateral tract(s). Rostrally, the crossed component of the anterior commissure continues as two bundles along the lateral aspect of the medial and dorsal components of the medial tract (Fig. 3A,B,C). The ventromedial component merges with the crossed components of the commissure of Goldstein (Fig. 3C) and the nervus terminalis (Fig. 3B). The medial and dorsal bundles continue as ascending components (ma, Fig. 2C,D) to the contralateral olfactory bulb (Fig. 2A,B). Within the olfactory peduncle, the medial bundle occupies a lateral position along the medial olfactory tract, while the dorsal bundle lies ventral within the ascending portion of the medial tract. The former corresponds in position to the pars medialis and the latter to the pars lateralis of the ascending component of the olfactory peduncle of carp [Fig. 22 (Sheldon '12)]. At the level of the bulb, the ventral (pars lateralis) component distributes caudal and ventrolateral and the medial component rostral and ventromedial; both components terminate along the outer margin of the internal cell layer (ICL, Fig. 2A,B). At caudal levels, a few fibers split off the ventral component and traverse the medial aspect of the lateral olfactory tract (1t, Fig. 2B). It is not possible to state if elements within both the internal and mitral cell layer (MCL, Fig. 2A,B) receive an input, as the boundary between these zones is diffuse (cf. Bass, '81).

Transections

The separate projections of the medial and lateral olfactory tracts were revealed in specimens that sustained partial or whole transections of, respectively, the lateral or medial tracts.

For autoradiographs of these experimental cases, silver grain accumulations extend over the entire intact portion of the tract, while they end at the level of the transection for the lesioned portion (Fig. 10).

Following total transection of the medial tract (two cases), all pathways and terminal fields are labeled bilaterally, as normal experimental cases, except: medial and lateral components of the medial tract, nervus terminalis bundles, commissure of Goldstein, and the lateral division of DC-3. Sparse label occurs in the medial preoptic area and lateral to Vv. For one additional case involving transection of

only the medial component of the medial tract, only the nervus terminalis pathway is unlabeled, suggesting its specific association with this component.

For cases with medial tract lesions, area ventralis receives input from a medial component of the lateral olfactory tract (Figs. 2C,D; 3; 4). Dense silver grain accumulations appear over the lateral aspect of the dorsolateral component of the medial tract, the anterior and habenular commissures, and the caudal component of the medial tract; sparse label appears over the ventromedial tract and the medial component of the ascending olfactory tract.

After total transection of the lateral olfactory tract (two cases), all pathways and terminal fields are labeled bilaterally except: the medial and lateral components of the lateral tract, the habenular commissure, and the dorsal component of the ascending olfactory tract. The increased silver grain density along the dorsomedial border of DP is absent. For such cases, the medial tract contributes fibers bilaterally at the level of the anterior commissure (Fig. 5A), and ipsilaterally at caudal telencephalic levels (Fig. 5C) to area dorsalis.

Additional data on the projections of the lateral component of the lateral tract was revealed in two cases. The first involved transection of only this component (Fig. 10). The lateral component and the habenular commissure are unlabeled, while the dorsal grain density in DP is absent. A stria medullaris component of the olfactory tract is labeled bilaterally (Fig. 10F), but label is not continuous rostrally or caudally with any portion of the olfactory tract. The latter remains unexplained.

A second case involved total transection of the medial tract and partial transection of the medial component of the lateral tract (this data is not included with the above mentioned medial tract lesions). The results match those for medial tract lesions with the following additions: The anterior commissure and all area ventralis targets are sparsely labeled; the preoptic area and ascending olfactory tract are unlabeled. All dorsolateral terminal fields are densely labeled (excepting lateral DC-3, as described earlier) as normal cases. The data emphasize: (a) the contribution of the medial component of the lateral tract to area ventralis, the preoptic area, and the anterior commissure and (b) the specialization of the lateral component for bilateral input to area dorsalis.

The transection experiments underline a subdivision of the olfactory tract into multiple

components. On the basis of topography, I divide the tract into five components, each with a unique subset of ipsilateral and commissural fiber bundles (Table 1), each with a general subset of olfactory targets. The lateral division of the lateral tract, the medial division of the medial tract, and the ascending division of the medial tract appear specialized for input, respectively, to area dorsalis, area ventralis (especially Vv and the lateral preoptic area), and the contralateral olfactory bulb. The dorsal division of the medial tract projects to areas ventralis and dorsalis; the caudal division to the caudal telencephalon, the medial preoptic area, and the posterior tuber of the diencephalon. All components, excepting the lateral component of the lateral tract, carry fibers of both the medial and lateral tracts.

Origin of olfactory bulb efferents

Following telencephalic HRP injections, dense label occurs throughout olfactory bulb targets of areas dorsalis and ventralis; the medial and lateral olfactory tracts are densely labeled. HRP-filled cells appear bilaterally within the internal and mitral cell layers of the olfactory bulb. Golgi-like filling of cells with brown reaction product (buffered DIA procedure) distinguishes a variety of morphological types within the mitral cell layer (Fig. 11A). Elements within the internal cell layer contain a small number of fine-caliber black reaction granules (TMB reaction, Fig. 11B). More detailed accounts of telencephalic HRP experiments will appear in future reports.

DISCUSSION

Autoradiographic analysis distinguishes olfactory bulb input to 15 targets in the forebrain of *Ictalurus punctatus*. While the data reveal a circumscribed olfactory input to the telencephalon, as earlier degeneration analyses of teleosts (Scalia and Ebbesson, '71; Ito, '73; Finger, '75), they emphasize the differentiation of the olfactory tract and its recipient zones into multiple subdivisions.

Comparisons with earlier teleost studies

Ito's ('73) study of the secondary olfactory pathways in carp, *Cyprinus carpio*, provides little insight into the position and extent of secondary olfactory targets, as terminal fields are indicated by plus symbols on unlabeled line drawings. Employing Neiuwenhuys' ('63) terminology, Ito ('73) describes ipsilateral input to area ventralis and posterior dorsolateralis

and contralateral input to area ventralis. Its study essentially described ultrastructural profiles of degenerating terminals and axons following olfactory tract transections.

The more detailed light microscopic analyses of Scalia and Ebbesson ('71) in the moray eel, *Gymnothorax funebris*, and Finger ('75) in bullhead catfish, *Ictalurus nebulosus*, form a basis for comparison with the present results in the channel catfish, *I. punctatus*. For these species, secondary olfactory input occurs bilaterally to areas ventralis and dorsalis telencephali, the majority of terminals occurring ipsilateral. The ipsilateral olfactory tract crosses to the contralateral hemisphere via the anterior commissure in the moray eel and the anterior and habenular commissures in catfish. The large number of olfactory targets recognized in channel catfish makes it desirable to frame an extended discussion within the context of the three telencephalic input zones—medial, lateral, and central-posterior—recognized by Scalia and Ebbesson ('71) and Finger ('75) and the diencephalic terminal field described by Finger ('75).

Medial terminal field

In channel catfish, the medial terminal field (area ventralis telencephali) comprises seven divisions. Three precommissural zones—dorsal, ventral, and central nuclei—compare topographically to the medial terminal field reported for the moray eel and bullhead catfish. In channel catfish, additional input is recognized to a supracommissural nucleus and three postcommissural divisions—postcommissural, intermediate, and preoptic nuclei. While the difference in the number of ventral targets identified in the channel catfish and the moray eel may represent a real species difference,

that between channel and bullhead catfish can be accountable to differences in cytoarchitectural criteria and experimental techniques. Excepting some variance in the differentiation of ventral telencephalic nuclei, the same number of olfactory bulb targets are revealed in autoradiographic material for bullhead catfish (unpublished observations, see Materials and Methods). A reexamination of olfactory pathways in the moray eel with the more sensitive autoradiographic methods might reveal additional input to area ventralis.

In both the moray eel and channel catfish, the medial terminal zone gets input from both the medial and lateral olfactory tracts, while in bullhead catfish input from only the medial olfactory tract is reported.

Olfactory input to the preoptic area is described only for channel catfish. The precise terminal zone(s) of this tract remains unclear. Additional electron microscopical analysis might resolve this issue. Such an input could affect a variety of physiological processes via a preoptic-neurohypophysial tract (review: Peter, '77).

Lateral terminal field

The lateral terminal field in channel catfish comprises three major divisions: rostral and caudal divisions of the dorsal posterior zone and nucleus taeniae. Input to a caudoventral component of area dorsolateralis comprises a minor fourth component.

In the moray eel, the lateral terminal zone ends just caudal to the anterior commissure, while in catfish it extends to the caudal pole of the telencephalon. In the moray eel this area gets input from the lateral olfactory tract, while in catfish it gets input from both the medial and lateral olfactory tracts.

TABLE 1. Divisions of the olfactory tract¹

Division	Fiber bundles	Commissural pathway
llt	llt	hc
mm	mm	cg
	nt	nt
	lmt	ac
md	ml	ac
	lmt	
mc-mp	ml	—
	mm	
	lmt	
ma	mm	—
	lmt	

¹ Abbreviations: ac, anterior commissure; cg, commissure of Goldstein; hc, habenular commissure; lmt, medial component of the lateral tract; llt, lateral component of the lateral tract; ma, ascending component of the medial tract; mc-mp, caudal-preoptic component of the medial tract; ml, lateral component of the medial tract; mm, medial component of the medial tract; nt, nervus terminalis component of the medial tract. See Figure 12.

The divisions of the dorsal posterior zone in channel catfish are roughly coincident with those charted for the lateral terminal field of bullhead catfish. It remains difficult to reconcile descriptive differences in the absence of cytoarchitectural analysis in the bullhead study. Nucleus taeniae, as recognized in bullhead catfish, corresponds to the caudal segment of the dorsal posterior zone of channel catfish. I recognize nucleus taeniae as a separate caudomedial region of area dorsalis that corresponds to the caudal contingent of Sheldon's ('12) nucleus taeniae. Berquist ('32) and Källén ('51b) described nucleus taeniae as a pallial zone that arises embryonically from the preoptic area and "pars frontalis thalami" (forms ventral thalamus). Nucleus taeniae occupies a comparable position in channel catfish; it lies adjacent to the intermediate nucleus of area ventralis (Vi) and, along with Vi, is continuous with the medial preoptic area (Bass '80a).

Central posterior terminal field

The central-posterior terminal zone in channel catfish has two major components: a medial central zone (medial DC-3) of dense olfactory input and a lateral central zone (lateral DC-3) of sparser input. A third minor component is a medial segment of the posterior zone (DLP) that lies dorsal to nucleus taeniae.

In moray eels, two components constitute a posterior zone—nucleus limitans and area centralis. These nuclei lie caudal to the lateral terminal field, unlike catfish where central-posterior and lateral zones are contiguous. The medial DC-3 component of channel catfish roughly coincides with the central terminal field of bullheads, while the lateral DC-3 component and possibly medial DLP coincide with the posterior zone.

Both the medial and lateral olfactory tracts project to the central-posterior zone in moray eels and catfish.

Diencephalic input

Olfactory bulb input to the diencephalon is reported only for catfish. Sheldon ('12) referred to this tract in carp as tractus hypothalamo-olfactorius medialis, but rather indicated it as an ascending bundle from the posterior tuber nucleus to the corpus precommissurale (area ventralis). Ariëns-Kappers ('06) referred to a similar bundle as tractus olfacto-lobaris, or tractus olfacto-hypothalamicus, connecting telencephalic olfactory centers with the hypothalamus. His statements are equivocal as to

the presence of secondary olfactory fibers within this tract.

Olfactory bulb input

In channel catfish, there is input to the contralateral olfactory bulb, fibers terminating predominantly at the interface of the internal and mitral cell layers. While degeneration is described within the contralateral peduncle of bullhead catfish, an input to the contralateral bulb is reported only for the moray eel; the location of terminals is undescribed.

In channel catfish, the contralateral olfactory bulb input arises from the mitral cell layer (Bass, '81b). This permits direct modulation of contralateral bulb activity by a primary olfactory target—the mitral cell layer of the ipsilateral olfactory bulb.

Comparisons with other actinopterygians

Northcutt and Braford ('80) employ silver-degeneration techniques to identify the secondary olfactory targets in a polypteriform, *Polypterus* (see also Braford and Northcutt, '74), a chondrosteian, *Schaphirhynchus*, and a holostean, *Lepisosteus*. Intergeneric comparisons are facilitated since a previous cytoarchitectural study of the telencephalon of the channel catfish (Bass, '81a) recognized divisions of the areas ventralis and dorsalis telencephali that are comparable, topographically, to those recognized by Northcutt and Braford ('80).

As in catfish, the areas dorsalis and ventralis of the forementioned species, with the probable exception of *Polypterus* (cf. Braford and Northcutt, '74), show bilateral olfactory bulb input. When contralateral projections occur, they are symmetrical to the denser ipsilateral terminal fields; fibers cross in both the anterior and habenular commissures. For the series *Polypterus-Schaphirhynchus-Lepisosteus-Ictalurus*, the number of targets in area ventralis are, respectively, 4 [this is a minimum with possible additional targets (see Braford and Northcutt, '74)], 6, 7, 7; in area dorsalis 1, 1, 3, 5 (for detailed topographical comparisons see Bass, '79). There is input to the contralateral olfactory bulb and posterior tuber in all species except *Polypterus*. A preoptic input is recognized only for *Ictalurus*. While the number of area ventralis targets is relatively constant, there is a progressive increase in area dorsalis targets for the above series. The increased differentiation of pallial olfactory zones parallels that of other dorsal telencephalic regions (Bass, '79; Northcutt and Braford, '80). For *Ic-*

talurus, parcellation of the pallial olfactory zone into several divisions is associated in part with the appearance of a distinct cell population forming reciprocal connections with the olfactory bulb (Bass, '81b). If other divisions of the olfactory region (or nonolfactory regions) have unique sets of afferents and efferents, it would suggest that subdivision of the pallial olfactory zone (and possibly nonolfactory zones) is coupled with the emergence of unique sets of input/output relationships.

Functional considerations

For channel catfish, subdivision of the telencephalic olfactory zone is associated with multiple components of the olfactory tract. Olfactory autoradiographs emphasize separation of the olfactory tract into five major components. Multiple divisions of the olfactory tract may further relate to a topographical representation of the olfactory bulb, i.e., each component arises from a restricted group of cells. Sheldon ('12) documents for the carp, *Cyprinus*, that the lateral olfactory tract arises from both mitral cells and the anterior olfactory nucleus (internal cell layer of catfish olfactory bulb); the medial component arises from mitral cells. HRP data for channel catfish confirm the origin of bulb efferents from both the mitral and internal cell layers (present study; Bass '81b for interbulbar efferents). Transection experiments coupled with HRP methodology should provide additional topographical data.

Sheldon ('09, '12) further specified a *nervus terminalis* pathway that arises from ganglion cells spread along the ventromedial aspect of the olfactory nerve. While I have identified this ganglion in channel catfish (Bass, '81a), I have not identified an efferent bundle specifically associated with these cells. However, the autoradiographic data presented here for channel catfish describes a pathway reminiscent of the *nervus terminalis*' as described by Sheldon for carp.

If additional experiments demonstrate the origin of olfactory tract components from circumscribed cell groups in the olfactory bulb, such a regional topography might relate to a map of chemical sensitivity that underlies discrete behavioral functions. While physiological evidence for such a map among teleosts is inconclusive (Bodznick, '78; Thommesen, '78), recent experiments in cod, *Gadus*, associate separate divisions of the olfactory tract with different behavior patterns (Döving and Selset, '80).

ACKNOWLEDGMENTS

I thank Drs. R. Glenn Northcutt, Carl Gans, Roger Davis, and Stephen Easter and Ms. Mary Sue Caudle Northcutt for their comments on earlier versions of this manuscript. Additional thanks to Dr. Mark R. Braford, Jr. for his advice during early stages of this work, to Ms. Margaret Ann Marchaterre for assistance with the illustrations, and to Ms. Ricka Robb and Ms. Diane Johnson for typing the final manuscript. This work was supported by a Rackham Dissertation Grant, University of Michigan and NIH Postdoctoral Fellowship 1 F32 NS06309-01 to A. H. Bass and NIH EY02485 to R. Glenn Northcutt.

Portions of this work were submitted in partial fulfillment of the requirements for a Ph.D. degree at the University of Michigan (Bass, '79).

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Abbreviations

ac	anterior commissure	F	facial lobe	mdm	fused portion of the medial and dorsal components of the medial olfactory tract
C	cerebellum	GL	glomerular layer of olfactory bulb	mm	medial component of the medial olfactory tract
cg	commissure of Goldstein	HA	anterior hypothalamic nucleus	mt	medial olfactory tract
DC-1, 2, 3	parts of the central zone of area dorsalis telencephali (D)	Hab	habenular nuclei	mta	ascending component of the medial olfactory tract
Dd	dorsal zone of D	hc	habenular commissure	mtd	descending component of the medial olfactory tract
DL	lateral zone of D	ICL	internal cell layer of olfactory bulb	MTP	median nucleus of the posterior tuber
DLd	dorsal part of lateral zone of D	lt	lateral component of the lateral olfactory tract	N	nervus terminalis ganglion cells
DLp	posterior part of lateral zone of D	lmt	medial component of the lateral olfactory tract	nt	nervus terminalis
DLv	ventral part of lateral zone of D	lr	lateral recess	NT	nucleus taeniae
DM	medial zone of D	lt	lateral olfactory tract	NDIL	nucleus diffusus of the inferior lobe
DPr	rostral portion of posterior zone of D	m	meninges	NSV	nucleus of the saccus vasculosus
DPc	caudal portion of posterior zone of D	ma	ascending component of the medial olfactory tract	OB	olfactory bulb
e	external sulcus	mc	caudal component of the medial olfactory tract		
Ec	caudal entopeduncular nucleus	MCL	mitral cell layer of the olfactory bulb		
Ed	dorsal entopeduncular nucleus	md	dorsal component of the medial olfactory tract		
Ev	ventral entopeduncular nucleus				
Ep	epiphysis				

(continued)

Abbreviations (continued)

OE	olfactory epithelium	rh	rhinocoele	Vd-d	dorsal division of the dorsal nucleus of V
ON	olfactory nerve	S	spinal cord	Vd-v	ventral division of the dorsal nucleus of V
OP	olfactory peduncle	SC	suprachiasmatic nucleus	Vi	intermediate nucleus of V
OT	optic tract	SD	saccus dorsalis	VI	lateral nucleus of V
PG	nucleus preglomerulosus	slt	sulcus limitans telencephali	Vn	another nucleus of V
PLL	posterior lateral line lobe	SOF	secondary olfactory fiber layer of the olfactory bulb	Vp	postcommissural nucleus of V
PM	magnocellular preoptic nucleus	SV	saccus vasculosus	Vs	supracommissural nucleus of V
PP	periventricular preoptic nucleus	sy	sulcus ypsilonformis	Vv	ventral nucleus of V
PPa	anterior segment of the parvocellular part of PP	T	telencephalon	Vv-d	dorsal division of Vv
PPp	posterior segment of the parvocellular part of PP	tc	tela chorioidea	Vv-v	ventral division of Vv
pr	preoptic recess	TeO	optic tectum	z	sulcus z
		ToL	torus longitudinalis		
		V	vagal lobe		
		Vc	central nucleus of area ventralis telencephali (V)		

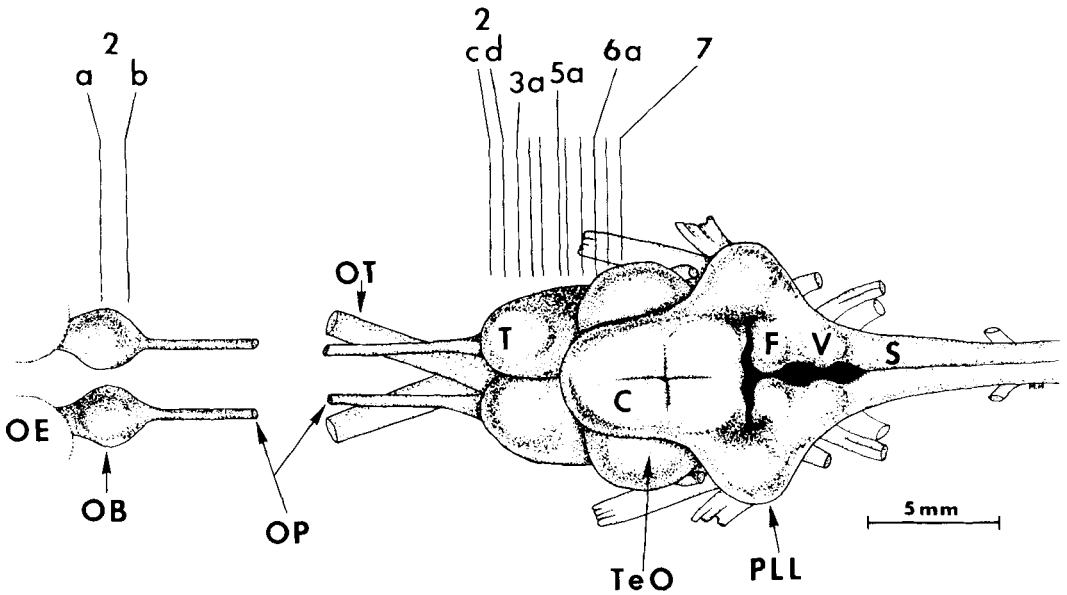


Fig. 1. Dorsal view of the brain of *Ictalurus punctatus*. The numbered lines indicate the levels of the transverse sections in Figures 2, 3, 5-7.

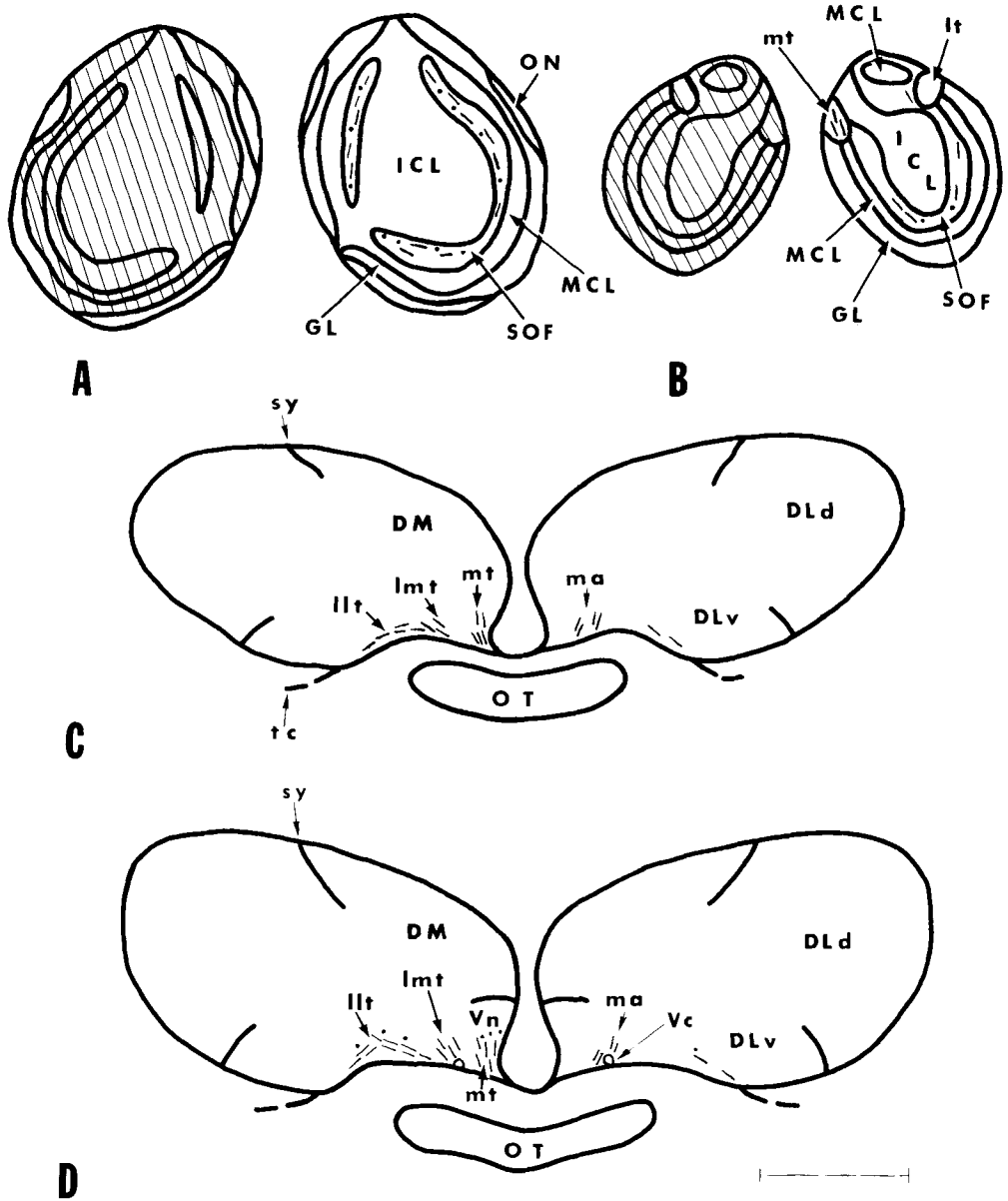
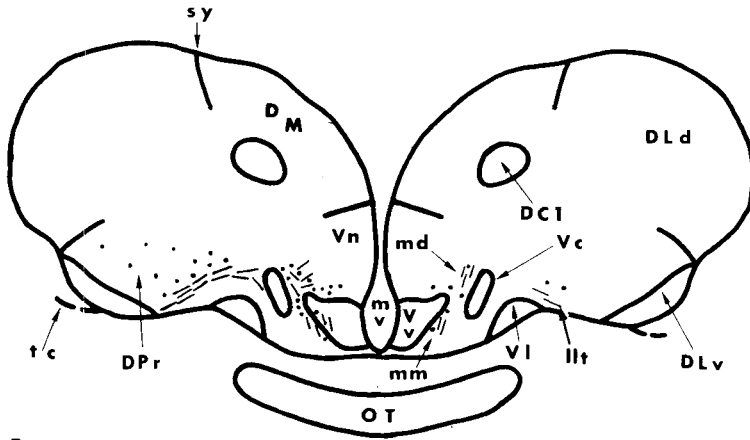
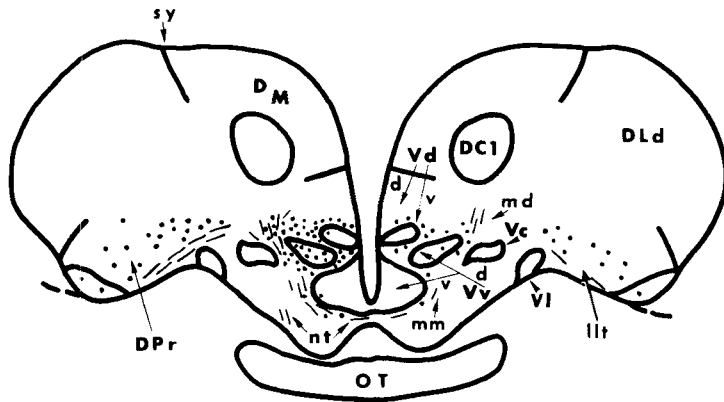


Fig. 2. This figure and Figures 3 and 5-7 are line drawings illustrating the position and extent of the secondary olfactory projections. The chartings are based on animals that received intrabulbar injections of tritiated proline. Drawings to the left lie ipsilateral to the injection site, whereas drawings to the right lie contralateral to the injection site. Olfactory bulb axons are indicated by dashed lines and terminal fields by stippling. The bar scale in Fig-

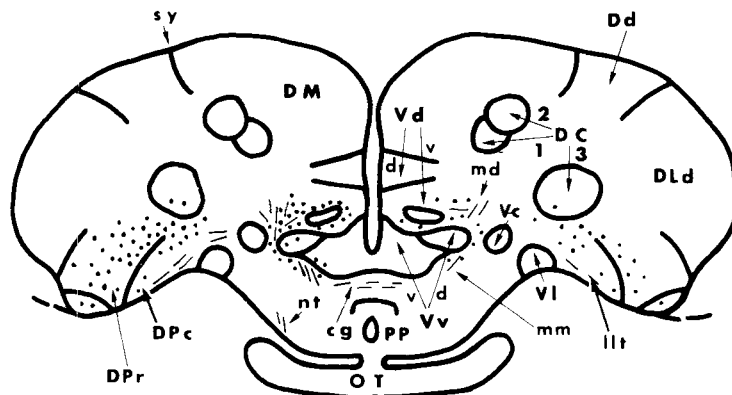
ures 2, 3, and 5-7 is 1 mm. (A) Transverse section through the olfactory bulb. (B) Transverse section through the caudal pole of the olfactory bulb. Cross-hatching in A and B indicates extent of silver grain labeling within olfactory bulb. (C) Transverse section through the rostral telencephalon. (D) Transverse section through the telencephalon at the rostral pole of Vn.



A



B



C

Fig. 3. (A) Transverse section through caudal Vn. Note the medial and dorsal branches of the medial olfactory tract. (B) Transverse section through rostral Vd. Note the medial and ventral branches of the nervus terminalis. (C) Transverse section through the commissure of Goldstein (cg). Bar scale is 1 mm.

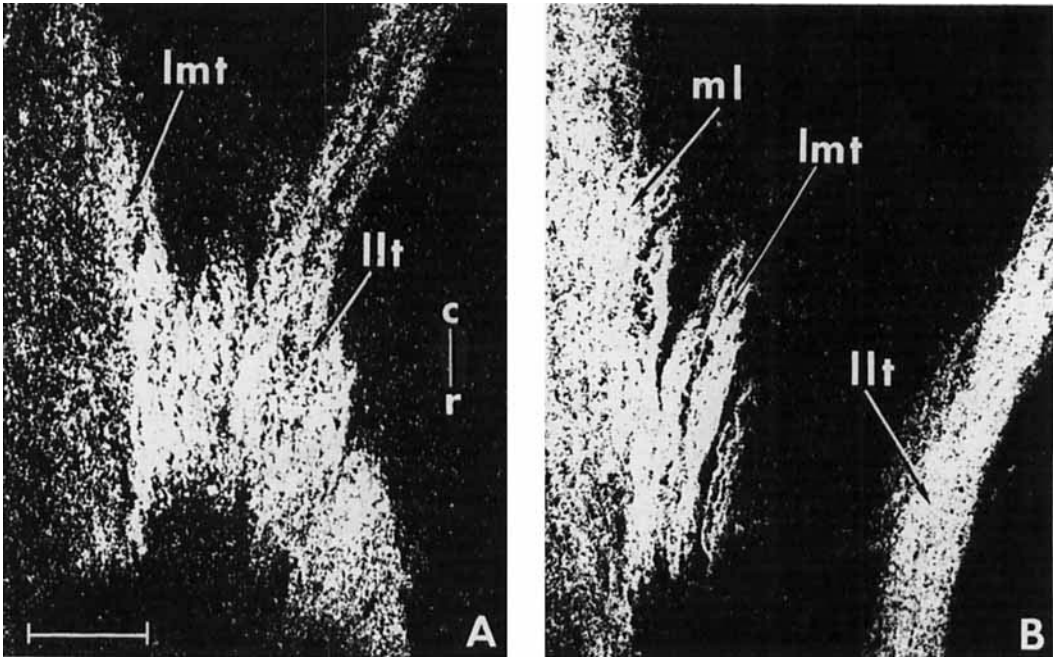


Fig. 4. Photomicrographs of horizontal sections through the olfactory tract as demonstrated by dark-field illumination of autoradiographs following an intrabulbar injection of tritiated proline. The rostral (r)-caudal (c) axis is indicated. The bar scale represents 0.1 mm. for A and B. (A) Ipsilateral olfactory tract illustrating the divergence of the

medial (lmt) and lateral (llt) components of the lateral olfactory tract. (B) A horizontal section caudal and dorsal to that in Figure 4A, illustrating the joining of lmt and the lateral component of the medial tract (ml). The llt continues as a separate component to caudal olfactory targets (see text).

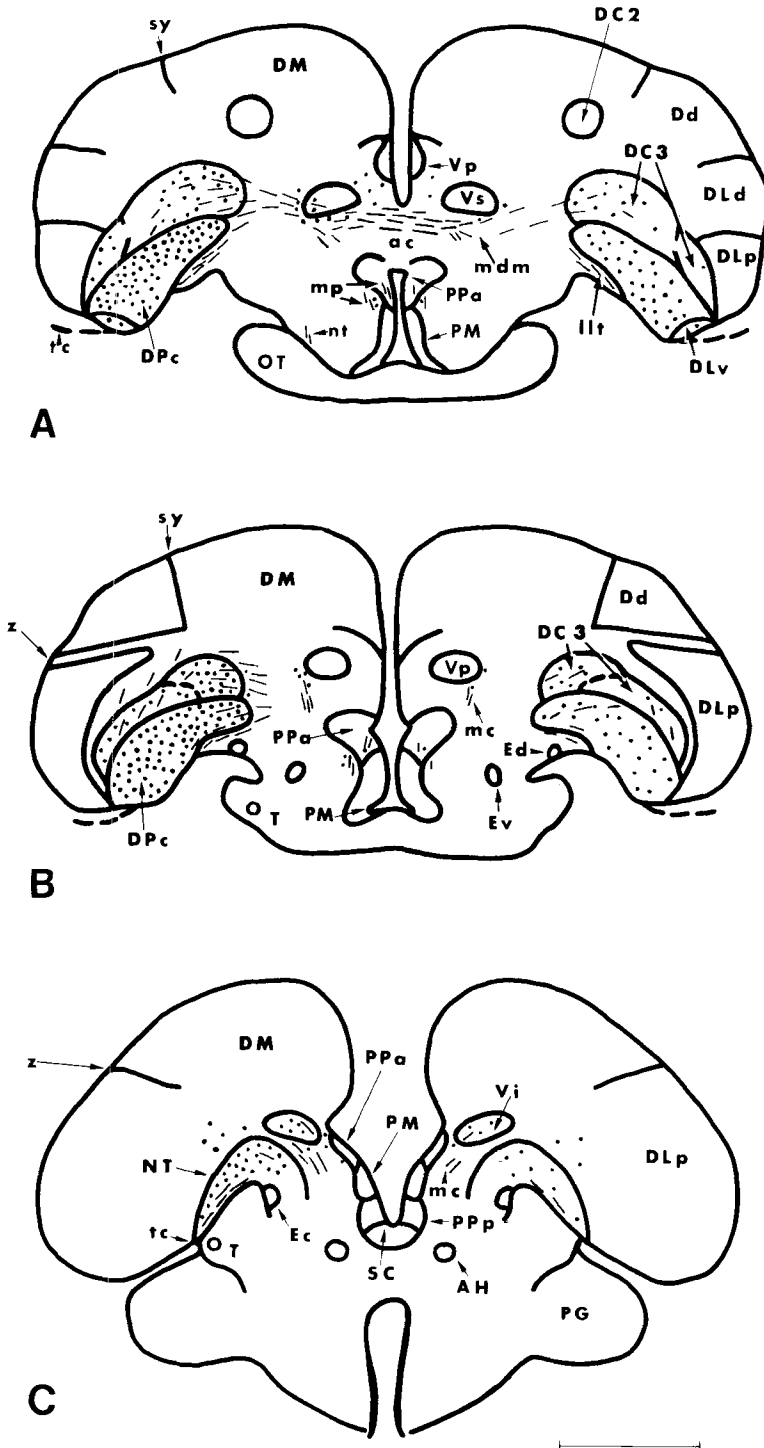


Fig. 5. (A) Transverse section through the caudal pole of the anterior commissure. Note the fusion of the medial and dorsal branches of the medial olfactory tract. (B) Transverse section through the telencephalon. Note the dense input of the caudal DP-DC zone. (C) Transverse section through the caudal pole of the telencephalon. Bar scale is 1 mm.

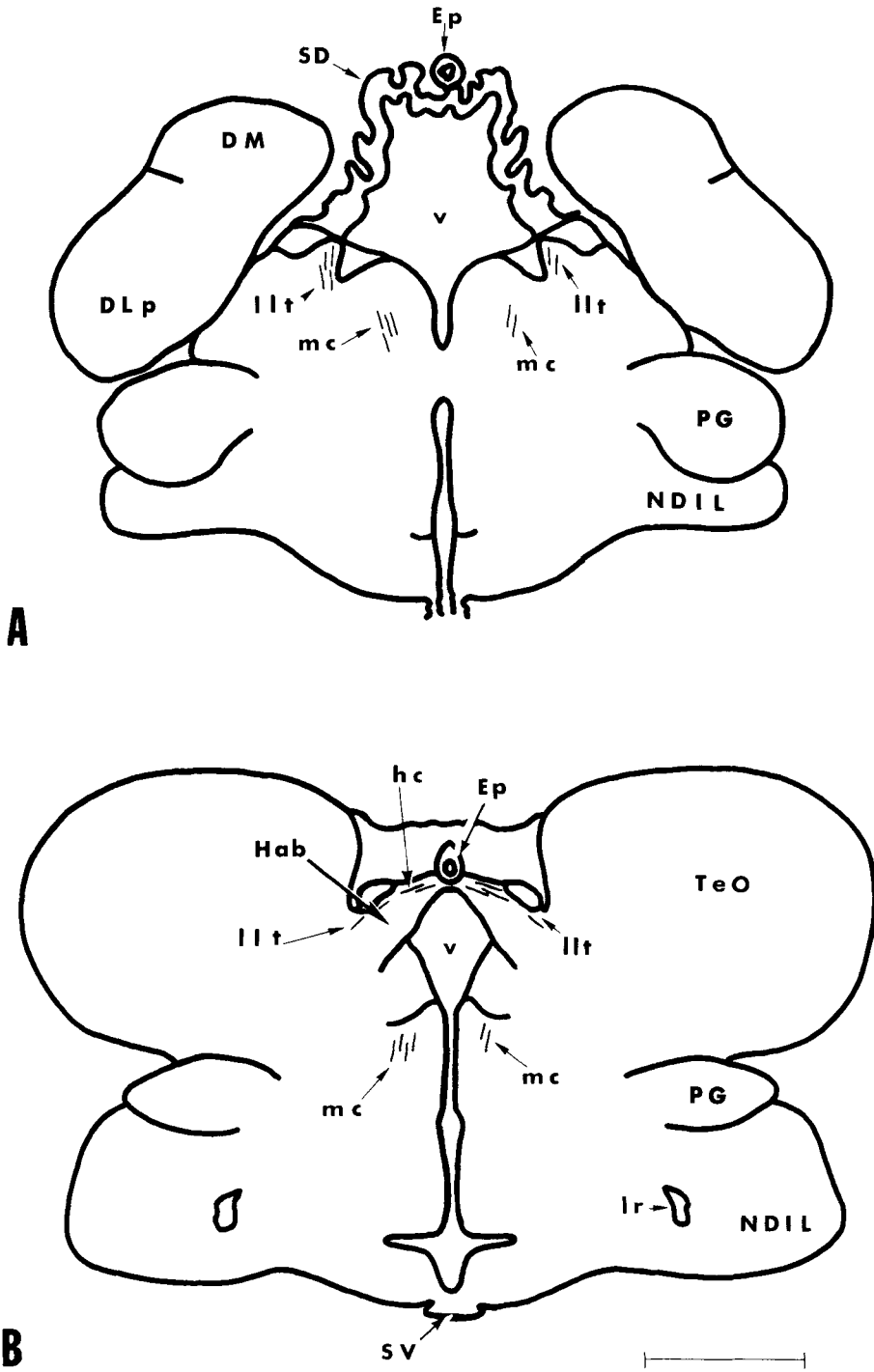


Fig. 6. (A) Transverse section through the rostral thalamus as the lateral components of the lateral olfactory tract (llt) course toward the habenular commissure and the caudal component of the medial olfactory tract (mc) courses toward the posterior tuber. (B) Transverse section at the level of the habenular commissure, indicating the crossed fibers of the lateral component of the lateral olfactory tract (llt). Bar scale is 1 mm.

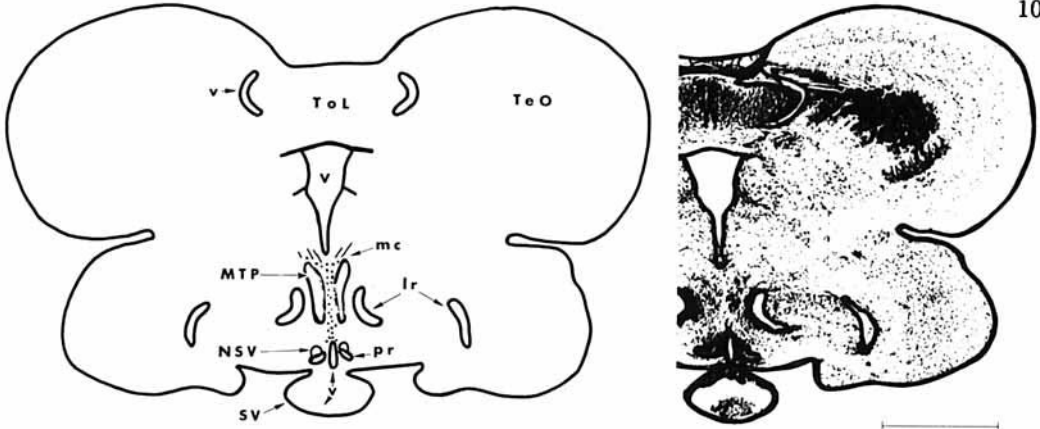


Fig. 7. Transverse section through the posterior tuber. A Nissl preparation appears on the right. To the left is a line drawing indicating the position and extent of the olfactory projection to the median posterior tuber nucleus (MTP). Bar scale is 1 mm.

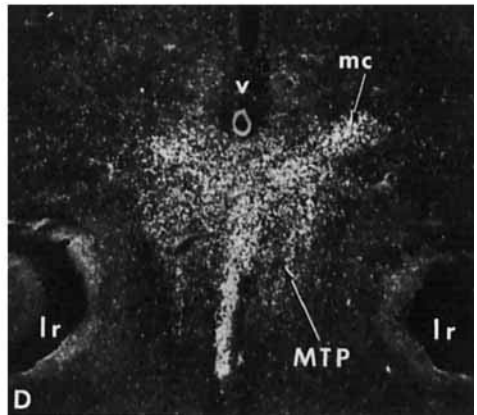
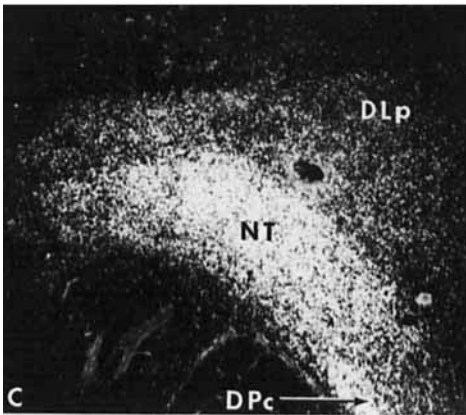
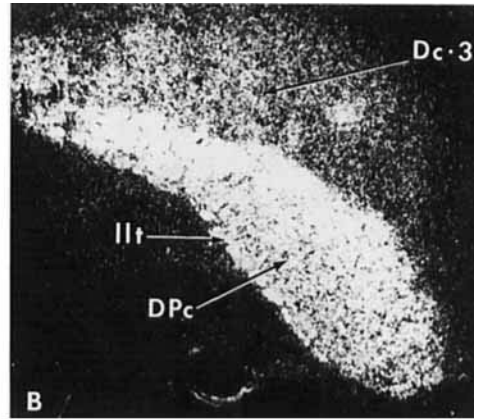
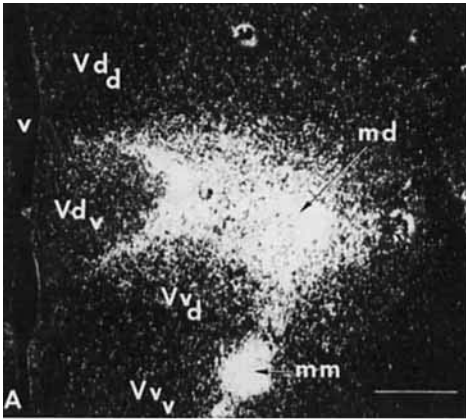


Fig. 8. Photomicrographs of transverse sections of the secondary olfactory projections as demonstrated by darkfield illumination of autoradiographs following intrabulbar injections of tritiated proline. Bar scale represents 0.01 mm for A-D. (A) Ipsilateral terminal field of the ventral (Vv) and dorsal (Vd) nuclei of area ventralis at about the level indicated in

Figure 14B. (B) Ipsilateral terminal field of the caudal DP-DC zone at the level of Figure 5B. (C) Ipsilateral terminal field over nucleus taeniae at the level of Figure 5C. (D) Terminal field over the median posterior tuber nucleus at the level of Figure 7.

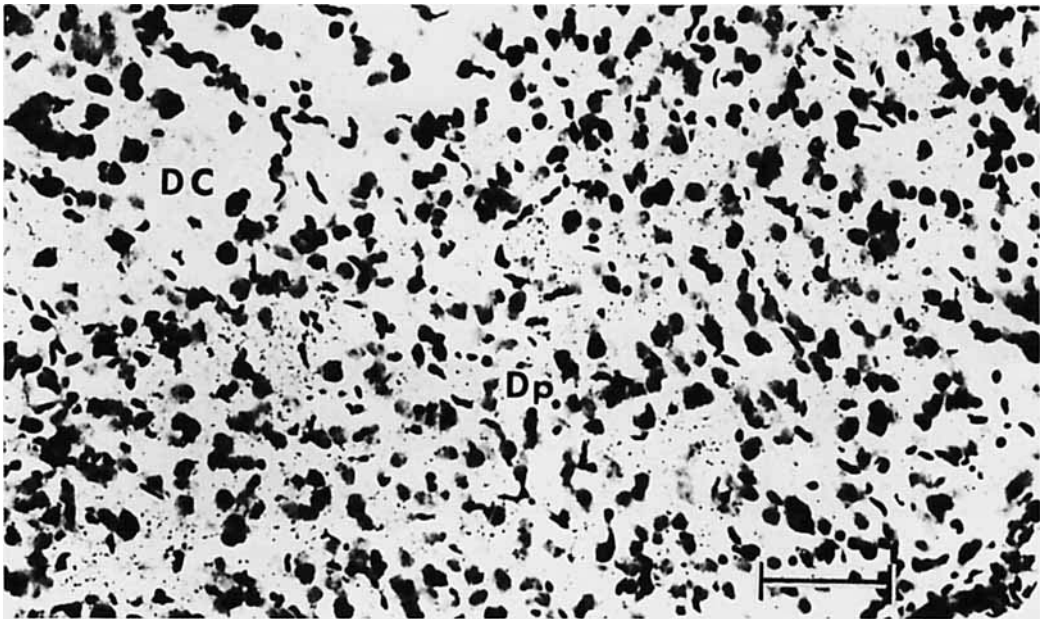


Fig. 9. Photomicrograph of terminal degeneration within the caudal DP-DC zone. Bar scale represents 0.05 mm.

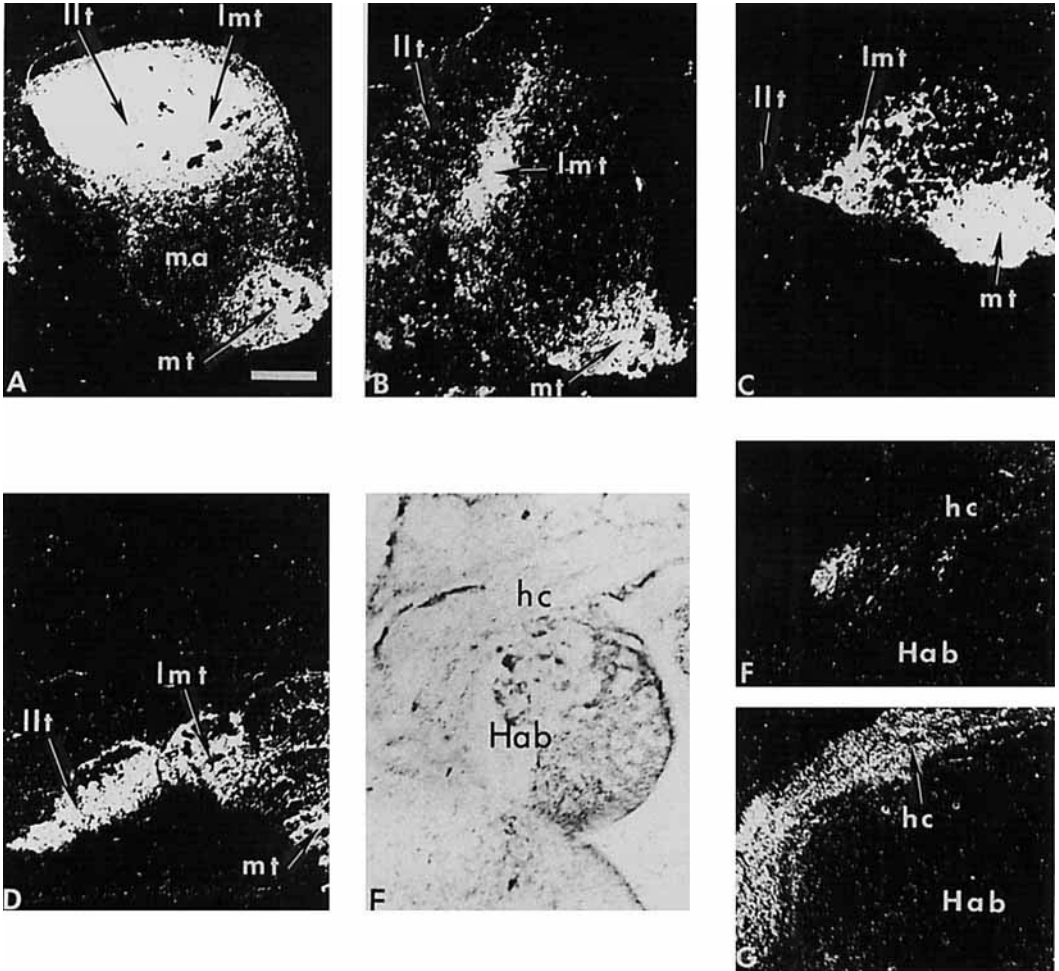


Fig. 10. Photomicrographs A-D, F, and G are transverse sections through the olfactory tract as demonstrated by dark-field illumination of autoradiographs. A-C and F are from a specimen that sustained transection of the lateral component of the lateral olfactory tract (l1t). D and G (as photomicrograph E) are from a specimen that sustained no transections. Bar scale represents 0.2 mm. for A-G. (A) Intact descending medial (mt) and lateral (l1t, lmt) olfactory tracts rostral to transection (ma is ascending component to

medial tract). (B) Caudal aspect of the transection of the l1t. (C) Rostral telencephalon illustrating labeled mt and medial component of the lateral tract (lmt). Note absence of label over l1t. (D) Label over l1t and lmt from specimen with intact peduncle. (E) Transverse section through the habenular nuclei (Hab) at the level of the habenular commissure (hc). (F) Restricted label near hc from specimen with l1t transection. (G) Label over hc from specimen with intact peduncle.

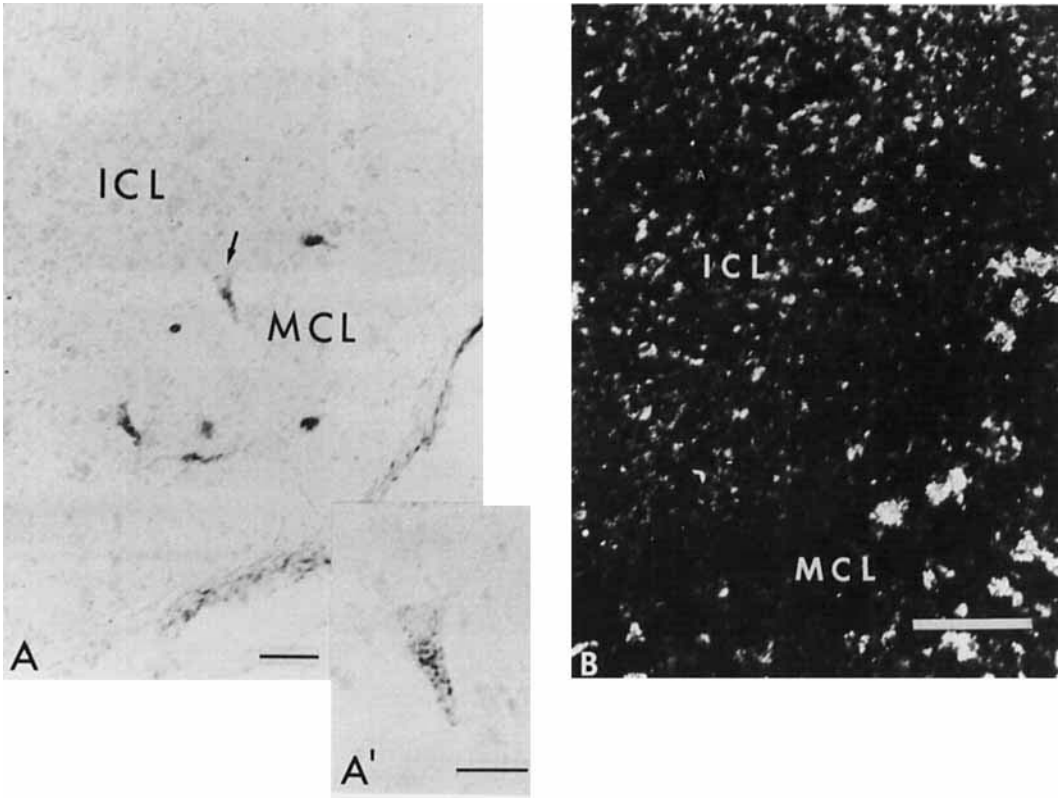


Fig. 11. Transverse sections through the olfactory bulb following HRP injections of the ipsilateral telencephalon. (A) HRP-labeled cells in the mitral cell layer (MCL). Buffered dianisidine (brown) reaction. Bar scale represents 0.1

mm. Arrow points to cell illustrated in A' (bar scale represents 0.05 mm). (B) Dark-field illumination of HRP-labeled cells in internal (ICL) and mitral (MCL) layers. Tetramethylbenzidine reaction. Bar scale represents 0.1 mm.

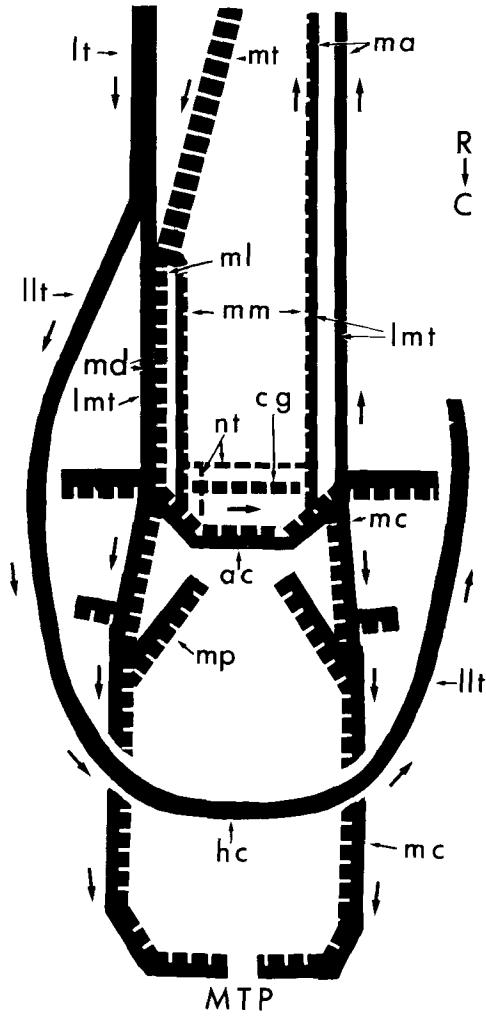


Fig. 12. Summary diagram in the horizontal plane illustrating the trajectory of the lateral (solid-black) and medial (hatched-black) olfactory tracts of the ipsilateral olfac-

tory bulb. The rostral (r)-caudal (c) axis is indicated. See text for details.