

Telencephalon of the Teleost *Macropodus*: Experimental Localization of Secondary Olfactory Areas and of Components of the Lateral Forebrain Bundle

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Connections of the telencephalic hemisphere were experimentally examined to facilitate investigations of the functions of this major brain area in teleosts. The distribution of afferent olfactory tract fibers was traced using a degeneration method and autoradiographic localization of axonally transported protein. Afferents terminate predominantly ipsilaterally in the medial, lateral, and posterior zones of the hemisphere and in the nucleus posterior tuberis of the diencephalon. Afferents also project to the contralateral olfactory bulb through the commissure of Goldstein. The results of similar experiments in five other teleosts are briefly reviewed. Evidence of retrograde transport of tritiated proline or metabolite is also discussed. Components of the lateral forebrain bundle originating in the extreme rostral end of the dorsal zone of the hemisphere were traced to: (1) the corresponding zone of the contralateral hemisphere; (2) nucleus entopeduncularis; and (3) the extreme ventral part of the posterior zone of the diencephalon. The anatomical findings are discussed in relation to previous experiments in *Macropodus* dealing with the location of brain areas which concentrate sex steroids and with the effects of brain lesions on reproductive behavior in the male.

Telencephalon ablation experiments in the paradise fish, *Macropodus opercularis*, indicate that the hemisphere mediates processes which potentiate male reproductive behaviors (Davis, Kassel, & Schwagmeyer, 1976; Kassel, Davis & Schwagmeyer, 1976; Kassel & Davis, 1977; Schwagmeyer, Davis, & Kassel, 1977). Bilateral ablation of the hemispheres and olfactory bulbs results in a decreased incidence of mating with intact females and increased egg cannibalism. Removal of only the bulbs results in increased egg eating, but sexual behavior is unimpaired. The results indicate olfactory areas in the brain are necessary for normal egg care and that nonolfactory structures are sufficient for the potentiation of sexual behavior (Davis, Kassel, & Martinez, 1981).

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The olfactory functions of the hemisphere in teleosts have received little direct attention in behavioral studies. Brain lesion and electrical stimulation experiments have concentrated on nonolfactory processes such as reinforcement (Flood, Overmier, & Savage, 1976), arousal (Laming, 1980, 1981), potentiation of sexual behavior (Davis et al., 1981; Demski & Knigge, 1971; Demski, Bauer, & Gerald, 1975), and startle reactivity (Davis, Reynolds, & Ricks, 1978). The nature of these processes remains ill-defined, and attempts to localize the structures responsible have generally produced ambiguous results. We believe that the olfactory functions of the hemisphere may be more amenable to investigation. As a foundation for such research, the cytoarchitecture of the telencephalon of *Macropodus* was described, and the afferent projections of the olfactory tracts were investigated using a degeneration method and autoradiographic localization of axonally transported protein.

METHOD

Cytoarchitecture and Terminology

The major brain-cell groups, or nuclei, were identified in transverse and horizontal sections of reference brains, which were stained either by the Bodian method, by the Kluver-Barrera method, or with cresyl-violet acetate. The identifications were based on descriptions of the brain of *Gasterosteus* (Nieuwenhuys, 1962), *Carassius* (Peter & Gill, 1975), and *Lepomis* (Northcutt & Braford, 1980). The terminology of Nieuwenhuys (1962, 1963), as modified by Northcutt and Braford (1980), was adopted (see Table 1).

Autoradiographic Method

Adult male *Macropodus opercularis* (L.), 4.7–5.5 cm body length, were obtained from a local supplier. Fish each received 0.2–0.5 μCi of L-[2,3- ^3H]proline which was air-dried on a 60–80- μm Dowex resin bead (Davis and Agranoff, 1977). The bead was implanted unilaterally in the olfactory bulb. In additional fish, the contralateral bulb was immediately removed to prevent transport of radiolabeled protein in the contralateral olfactory tracts. The results are based on four fish for which the autoradiograms revealed that uptake of radiolabeled proline was confined mainly to the olfactory bulb and peduncle and on four other fish that showed extensive uptake of proline in the contralateral bulb and the rostral pole of the ipsilateral hemisphere.

Fish were sacrificed by brief immersion in ice slush 10 days following the proline administration. The brain was fixed in 10% formalin or alcohol-formalin-acetic acid, removed from the cranium, and embedded in paraffin. Serial, transverse, or horizontal sections of the brain, cut 10 μm thick, were mounted on glass slides for autoradiography (Kopriwa

TABLE 1
Abbreviations Used in Figs. 1-10

Abbreviation	Meaning
AC	Anterior commissure
CG	Commissure of Goldstein
D	Area Dorsalis telencephali
Db	Dowex resin bead
Dc	Central part of D
Dc-1,2,3,4	Divisions of Dc
Dd	Dorsal part of D
DI	Lateral part of D
Dld,p,v	Dorsal, posterior, and ventral divisions of DI
Dm	Medial part of D
Dm-1,2,3,4	Divisions of Dm
DMC	Commissure of the medial part of D
Dp	Posterior part of D
Ed,v	Dorsal and ventral divisions of nucleus entopeduncularis
GL	Glomerular layer
ICL	Internal cell layer
It	Lateral olfactory tract
LFB	Lateral forebrain bundle
M	Meningeal tissue
MO	Medulla oblongata
mc	Caudal branch of mt
md	Dorsal branch of mt
mm	Medial branch of mt
mt	Medial olfactory tract
N	Nervus terminalis
NAT	Nucleus anterior tuberis
NDLI	Nucleus diffusus lobi inferioris
NDTL	Nucleus diffusus tori lateralis
NG	Nucleus glomerulosus
NH	Nucleus habenularis
NLTi	Nucleus lateral tuberis parts inferioris
NPG	Nucleus preglomerulosus
NPT	Nucleus posterior tuberis
NSV	Nucleus saccus vasculosus
NT	Nucleus taenia
Ob	Olfactory bulb
T	Telencephalon
ON	Olfactory nerve layer
Pit	Pituitary
POA	Preoptic nuclei
PR	Preoptic recess
SOF	Secondary olfactory fiber layer
Otec	Optic tectum
V	Area Ventralis telencephali
Vc,d,i,l,p,s	Central, dorsal, intermediate lateral, postcommissural, and supracommissural parts of V
X	Nucleus X

& Leblond, 1962; Landreth & Agranoff, 1976). The slides were dipped in Kodak NTB-3 emulsion, dried, and stored at 4°C in darkness for 21 days. The emulsion was developed in Kodak Dektol for 3 min at 16°C, washed, and fixed. The sections were lightly stained with cresyl-violet acetate. The distribution of grains of reduced silver in the emulsion over the sections was examined by bright and dark field microscopy.

Degeneration Method

The olfactory bulb was unilaterally aspirated in 14 fish. Following a survival time of 2 to 10 days, the fish was sacrificed, the roof of the cranium removed, and the head fixed in 10% formalin for a minimum of 1 week. In preparation for the silver impregnation procedure (Wiitanen, 1969), the fixed brain was removed, washed overnight, and infiltrated and embedded in 20% gelatin. The gelatin block was subsequently fixed in 25% formalin overnight and transferred to 10% formalin for storage prior to cutting. The block was frozen on the stage of a sliding microtome following overnight immersion in 1:1 10% ethanol and 10% formalin. Frozen transverse or horizontal 35- μ m sections were cut and stored in 2% formalin at 4°C until the impregnation process was carried out.

Degenerating fibers were identified by the occurrence of localized deposits of grains of reduced silver in the tissue, which could be traced in successive sections from the lesioned olfactory bulb. The results are based on four fish that survived 2, 4, 8 or 10 days. In these fish, the lesion included the bulb and part of the olfactory peduncle and the contralateral bulb was not visibly damaged.

RESULTS

I. Cytoarchitecture

A. Olfactory Bulb

The bulb is sessile, or closely apposed to the hemisphere, as in most other teleosts (Nieuwenhuys, 1967). The short olfactory peduncle, which contains the olfactory tracts, is overhung by the rostral pole of the hemisphere. The olfactory nerve layer and the underlying glomerular layer surround the bulb rostrally (Fig. 1A). In the caudal region, these layers are restricted to the ventral and lateral quadrants. In addition to the primary afferent fibers, the nerve also contains fibers that terminate in the receptor epithelium as described elsewhere (Davis, 1979). The mitral cells and secondary olfactory fibers are intermingled and do not form distinct layers. Scattered, large perikarya, presumed to be mitral cells, also occur in the glomerular layers (Holmgren, 1920). The internal cell layer, which may be homologous with the anterior olfactory nucleus

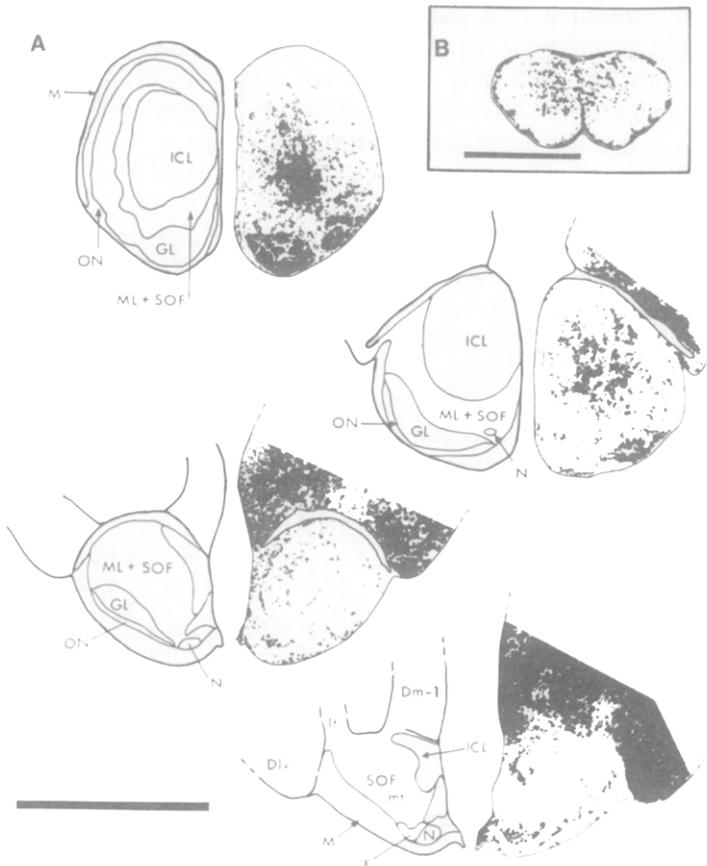


FIG. 1. (A) Transverse sections of the olfactory bulbs. The most rostral section (top) is from the middle of the bulb. Kluver-Barrera stain. (B) Example of fused olfactory bulbs. Cresyl-violet acetate stain. Scale bars = 0.5 mm. Abbreviations are presented in Table 1.

of land vertebrates (Northcutt & Braford, 1980), tapers caudomedially into the peduncle.

In the peduncle, the mass of secondary olfactory fibers separates into the lateral and medial divisions of the olfactory tracts. The lateral tract enters the hemisphere dorsolaterally and the medial tract ventromedially. Large perikarya, designated as nervus terminalis ganglion cells (N), are distributed rostrocaudally in the ventromedial zone of the bulb and peduncle. As shown in Fig. 1A, in the peduncle the N cells occur in close association with a small cluster of very large perikarya, which are identified as nucleus "X" (Ariens-Kappers, Huber, & Crosby, 1936). This nucleus may be a part of the nervus terminalis system (Northcutt & Braford, 1980).

In some individuals, the olfactory bulbs are fused, forming a common internal cell layer (Fig. 1B). Whether the bulbs exchange fibers in this "commissure" has not been determined. The trait occurred in one of eight reference brains. Several experimental fish were discarded during surgery because the bulbs appeared to be fused.

B. Hemisphere

The hemisphere of the teleost telencephalon can be divided into a dorsal and ventral zone, which are thought to be homologous with the pallium and subpallium, respectively, of land vertebrates (Nieuwenhuys, 1962, 1963), although the exact boundary between the two is controversial (Northcutt & Braford, 1980). The dorsal zone is denoted as area Dorsalis telencephali, or area D. The ventral zone is denoted as area Ventralis telencephali, or area V. In this report, area V is deemed to include the caudoventral region between the anterior commissure and the habenular nucleus of the thalamus. We identified 23 nuclei, or brain-cell groups, as illustrated in Fig. 2.

Area V. The rostral part of area V contains a ventral (Vv), dorsal (Vd), and a lateral (V1) nucleus (Fig. 2.3). Vd is a morphologically complex group, and it extends caudodorsally beyond the anterior commissure (Fig. 2.6). Vv is subjacent to Vd and is a more circumscribed, uniform nucleus. Vv tapers caudodorsally over the preoptic recess, and it is replaced at the anterior commissure by the supracommissural nucleus (Vs; Fig. 2.4). V1 consists of an irregular strand of cells in the lateral wall of V (Fig. 2.3). The cells are larger and more dispersed than the cells of Vv or Vd. Widely scattered cells occur in the central part of V. While they may belong to the central nucleus of V, as seen in *Lepomis* (Northcutt & Braford, 1980), a distinct grouping of these cells was not evident in our material.

Posteriorly (Fig. 2.5–2.7), the medial wall of V contains the caudal part of Vd, the postcommissural nucleus (Vp), and the preoptic region (POA). More laterally, a ventral (Ev) and a dorsal (Ed) entopeduncular nucleus can be distinguished. Ev is a highly compact, elongated group. Ed, a smaller, more dispersed group, extends caudally to the intermediate nucleus (Vi).

Area D. Area D consists of a peripheral zone containing nine relatively compact cell masses and a central zone of larger cells, which are dispersed singly and in several small groups. The peripheral groups appear to be subdivisions of three rostrocaudal columns of brain cells, a dorso-medial (Dm), dorsal (Dd), and a dorsolateral (Dl) column (Northcutt & Braford, 1980). The dorsal column consists of a unitary group (Dd; Fig. 2.1), which tapers caudally and disappears. Column Dm has three parts. The most rostral nucleus, Dm-1 (Fig. 2.2), is the smallest and it is replaced caudally by Dm-3 (Fig. 2.4). The ventral extreme of Dm-1

is apposed to the caudal portion of the olfactory bulb (Fig. 2.2). Dm-2 and 3 expand caudally and, in most individuals, Dm-3 coalesces, forming the commissure Dorsalis telencephali (DMC; Fig. 2.7). DMC has been detected in only a few teleosts, but it is typical in *Macropodus* and other members of the suborder Anabantoidei.

Column Dl includes four groups in the lateral wall of D and the posterior nucleus (Dp). The ventral nucleus of Dl (Dlv) and Dd form the extreme rostral end of the hemisphere (Fig. 2.1). Dlv tapers caudoventrally between the dorsal nucleus of Dl (Dld) and nucleus taenia (NT) and is replaced by the posterior nucleus of Dl (Dlp; Fig. 2.4). NT is a distinct, wedge-shaped group which reaches caudally beneath Dlp to Dp and Vi (Fig. 2.4–2.6).

Four cell masses can be distinguished in the central zone of area D. Dc-1 occurs in the rostral region adjacent to Dm-1. Dc-2 is closely associated with Dm-2 (Fig. 2.5). Dc-3 and Dc-4 are distinctive features of the posterior zone of D (Fig. 2.7).

II. Problems of Method

A. Autoradiography

The autoradiographic method produced the clearest labeling of the central projections of the olfactory bulb. We assume that most of the label seen in the autoradiograms resulted from emissions from radioactive protein. Very little of the tritiated proline should remain in the brain 10 days and, in any case, the histological procedure presumably removes most amino acids. In support of this assumption, Fig. 5.1 shows that, in an olfactory bulb containing a high concentration of radioactivity in the central zone, the surrounding olfactory nerve layer was comparatively lightly labeled. Since protein is synthesized in cell bodies, the nerve layer, which consists mainly of the receptor axons, should contain relatively little radioactivity. The boundary between the nerve layer and the glomerular layer is indistinct, and some of the label grains over the area denoted as "olfactory nerve layer" may in fact mark the location of labeled glomerular elements among the receptor axons. The nerve layer also contains scattered small cells, which have been variously described as subglomerular or periglomerular cells (Nieuwenhuys, 1967). Diffusion of tritiated proline into the nerve layer could have resulted in labeled protein in such cells. Some periglomerular cells have been identified as neurons, possibly efferents, which project in the olfactory nerve to the receptor epithelium (Davis, 1979, and in preparation).

The extensive labeling of the contralateral bulb and the rostral pole of the ipsilateral hemisphere that occurred in some brains was interpreted as being the result of local uptake of tritiated proline that diffused from the implantation site. While the following description of the olfactory

bulb projections is based on brains in which the contralateral bulb was ablated or only lightly labeled, the cases of bilateral labeling aided in visualizing the pathways.

The intense labeling of the hemisphere presented the problem of dissociating olfactory fibers from labeled fibers arising from hemispheric nuclei. Mainly, the hemisphere adjacent to the experimental bulb was affected. The zone of diffusion invaded the extreme rostral parts of areas V and D. Whether fibers arising from cells in these nuclei merge with components of the olfactory tracts cannot be determined from this experiment. However, heavily labeled components of the lateral forebrain bundle were detected and were easily distinguished from the afferent olfactory system, as described below.

Relatively dense, tractlike concentrations of silver grains that could be traced from the olfactory bulb through successive brain sections were inferred to indicate the pathways of the olfactory tract fibers. Diffuse patterns of grains that were continuous with the label over a pathway were assumed to mark the location of fiber terminations.

B. Degeneration Method

Degenerating olfactory tract fibers, marked by coarse-grain deposits, could be traced through the anterior zone of the hemisphere, but caudal to the anterior commissure their distribution became increasingly indistinct (Fig. 3). Caudally, fine-grain deposits characteristic of fiber terminations were visible in various nuclei. In addition to confirming certain autoradiographic findings in the caudal half of the hemisphere, the degeneration results aided in identifying terminal fields in rostral area V, which were difficult to distinguish in the autoradiographs of brains in

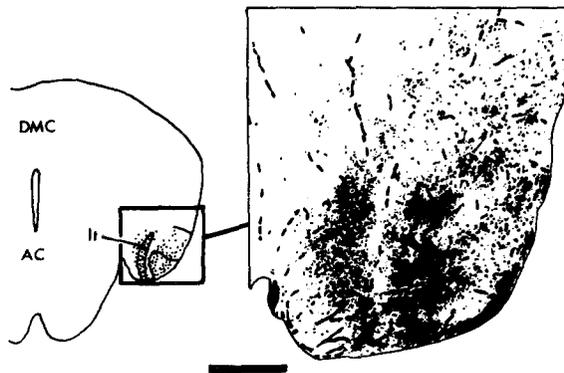


FIG. 3. Transverse section through the rostral end of nucleus taenia showing degenerating olfactory tract fibers. Fine-grain deposits indicative of degenerating terminals, which occurred in the area lateral to the core of the tract, are largely invisible in this photograph. Scale bar = 0.5 mm.

which the zone of diffusion of labeled proline from the experimental bulb was extensive.

III. Distribution of Labeled Olfactory Tract Fibers

The following description is accompanied by high-contrast, low-magnification photomicrographs of autoradiograms of selected horizontal or transverse brain sections that illustrate the most heavily labeled pathways. The distribution of labeled fibers is summarized in Figs. 9 and 10. Table 2 lists the nuclei that are inferred to receive olfactory system afferents and indicates the relative magnitude of the innervation which was inferred from the density of labeling.

A. Ipsilateral Projections

The medial and lateral divisions of the olfactory tract converge and intermingle in the caudal half of the hemisphere, and their separate pathways could not be traced with certainty. Thus, the tract-of-origin of the fiber terminations described below is uncertain. Upon entering the anterior part of V, the medial olfactory tract forms a dorsal (md) and a medial (mm) branch of similar diameter (Figs. 4.3 and 6.5) and a smaller ventral branch corresponding to the interbulbar commissure of Goldstein described below (Figs. 5.4 and 8A). The dorsal and medial branches course caudally through diffuse terminal fields bordering nuclei Vd and Vv. The anterior central part of the area V was also diffusely labeled, particularly in the autoradiograms (Fig. 4.3), suggesting that olfactory afferents terminate there. Degeneration data revealed a minute bundle entering the rostral extreme of area V ventral to the medial olfactory tract (not illustrated) corresponding to the nervus terminalis as described by Sheldon (1912). The degenerating fibers disappeared caudally near the preoptic recess.

The dorsal and medial branches of the medial tract are most readily visualized in horizontal sections (Fig. 7). The medial branch forms a commissural and a caudal (mc) branch (Figs. 4.7, 6.6, and 6.7). The commissural branch appears to merge with fibers of the lateral tract in

TABLE 2
Structures Inferred to Receive Secondary Olfactory Afferents

Brain region	Specific structure and degree of innervation		
	High	Intermediate	Low
Area V	Vi ^a	Vv, Vd	Vs, ^a Vp, ^a POA
Area D	NT, ^a Dlp, ^a Dp ^a	Dc-3	Dlv
Diencephalon		NPT	
Contralateral olfactory bulb	Indeterminant		

^a Innervated bilaterally.

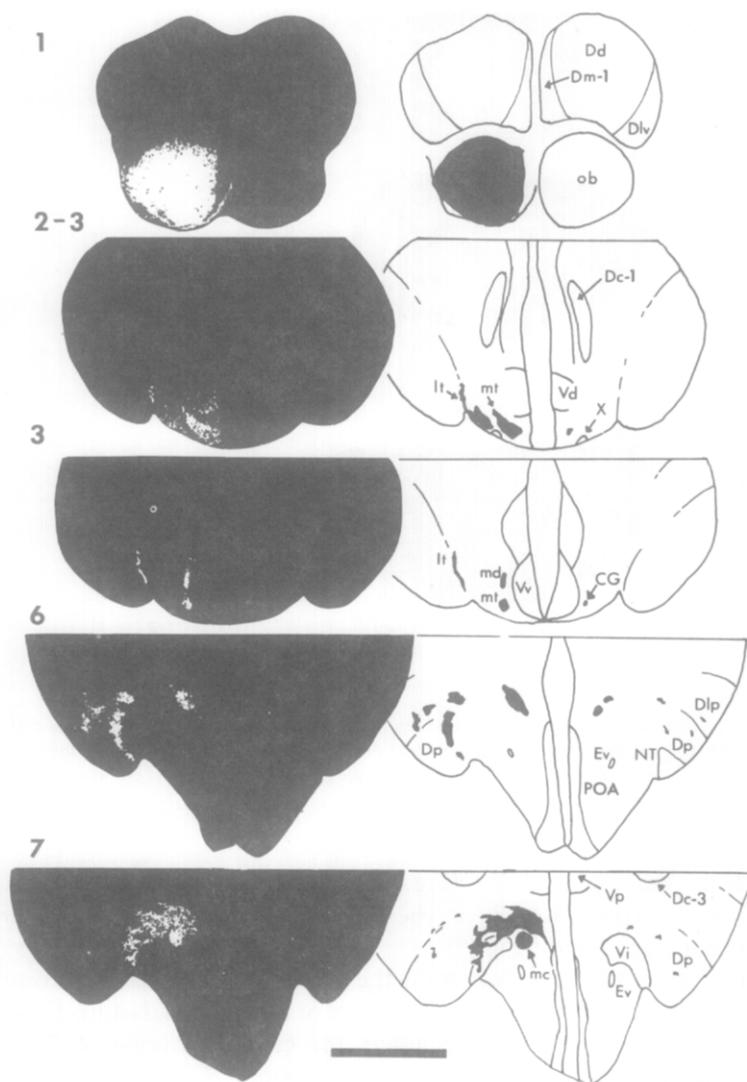


FIG. 4. Autoradiograms of transverse sections of a brain in the zone of diffusion was largely restricted to the experimental bulb. The number to the left of each section refers to the closest reference section shown in Fig. 2. The locations of the main components of the radiolabeled tracts are illustrated on the line drawings. Scale bar = 0.5 mm.

the dorsal region of the anterior commissure (Figs. 6.5, 7A). The caudal branch descends to the diencephalon and terminates in the region of nucleus posterior tuberis (NPT) (Fig. 10). The caudal branch may also be joined by lateral tract fibers as it courses through the postcommissural zone of the hemisphere. In this region, sparse terminal fields invade the

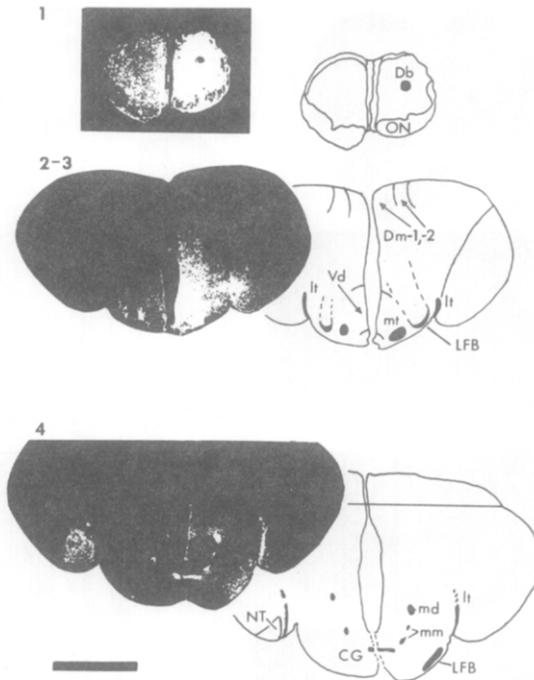


FIG. 5. Autoradiograms of transverse sections of a brain in which the zone of diffusion invaded the contralateral bulb and the rostral pole of the hemisphere. The section numbers indicate the closest reference section shown in Fig. 2. Additional sections of this brain are illustrated in Fig. 6. Scale bar = 0.5 mm.

borders of Vs, Vp, and Vd (Fig. 9.4–9.6). The dorsal branch of the medial tract swings laterally in the post commissural zone and merges with lateral tract fibers (Fig. 7).

Lateral tract afferents penetrate the hemisphere, forming a compact ribbonlike bundle along the medial aspect of Dlv (Fig. 4.2–4.6). Labeling indicative of terminal fields occurs in the caudal extreme of Dlv, the ventral part of Dlp and of Dc-3, and throughout NT, Dp, and Vi (Fig. 10). Some or all of these areas could receive medial tract fibers (md) as well as lateral tract fibers. Based on the density of silver grains over the cells, nuclei Dlp, Dp, and Vi are the predominate targets of the olfactory afferents (Table 2). Some lateral tract fibers appear to swing medially and combine with medial tract fibers in the anterior commissure as described above. A small bundle of fibers descends from the region of Vi to the dorsal margin of POA (Figs. 4.7 and 6.6) and another projects from the caudal extreme of the hemisphere along the medial aspect of nucleus Ev into the habenular commissure (not illustrated).

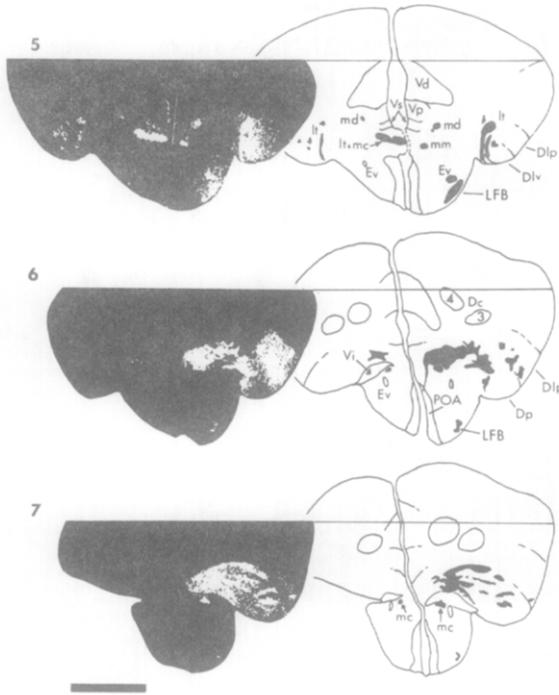


FIG. 6. Additional sections of the brain described in Fig. 5. The dashed line in the midline of section 5 denotes that the tissue was torn.

B. Contralateral Olfactory Projections

Olfactory bulb fibers cross to the contralateral hemisphere in the interbulbar commissure of Goldstein, anterior commissure, and, presumably, the habenular commissure. The commissure of Goldstein loops over the preoptic recess (Figs. 5.4 and 8A) and ascends to the caudal margin of the contralateral bulb, where the trace became illegible. Fibers also cross dorsally in the anterior commissure to the posterior central and lateral zone of the contralateral hemisphere (Figs. 6.5, 7A, and 9.5). Sparse terminal fields occur in the borders of Vs and Vp and in Vi, Dp, Dlp, and Nt (Fig. 9.5–9.7). The labeled habenular commissure fibers disappeared part way through the commissure, and their destination could not be determined.

IV. Distribution of Labeled Fibers of the Lateral Forebrain Bundle

Radiolabeling of brain cells in the rostral zone of the hemisphere, ipsilateral to the experimental bulb, revealed three pathways corresponding to parts of the lateral forebrain bundle (Sheldon, 1912; Nieuwenhuys, 1959). Most of the fibers seem to originate rostromedially, in the region

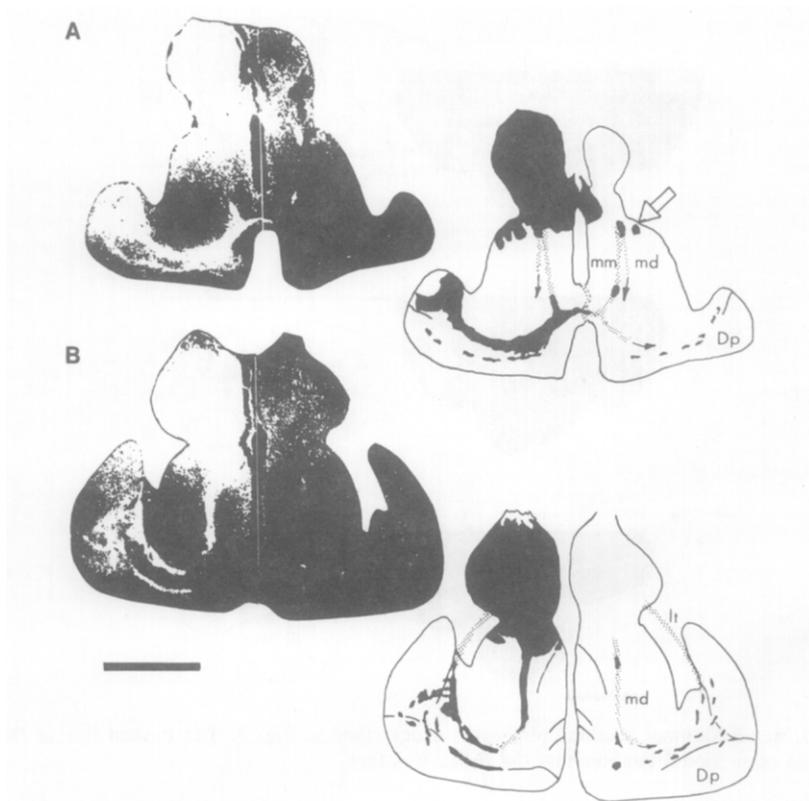


FIG. 7. Autoradiograms of horizontal sections of the telencephalon through (A) and dorsal to (B) the anterior commissure. The zone of diffusion invaded the rostral zone of the hemisphere and the contralateral bulb. Scale bar = 0.5 mm.

of Dm-1 and 2 (Figs. 5.2–3), rather than from Dd or Dlv. The fibers descend to the anterior lateral wall of area V, forming into several ill-defined fascicles (Fig. 8A).

One bundle descends in the lateral wall of V (Figs. 5.4 and 6.5–6.7) to the posterior ventral diencephalon (Fig. 10). The fibers spread out upon entering the diencephalon, but the core of the bundle appears to terminate in the neuropil adjacent to the posterior (NLTp; not illustrated) and inferior (NLTi) parts of nucleus lateral tuberis and the nucleus of the posterior recess (NRP). Another bundle projects caudomedially in V to the medial aspect of Ev (Figs. 6.5 and 8A). The third, and smallest, bundle courses from the rostral extreme of the lateral wall of V across the anterior commissure to the corresponding zone of the contralateral area V (Figs. 5.2–3 and 8A). Its looping path follows that of the commissure of Goldstein. The bundle subsequently turns dorsally then medially to a diffuse terminal field in the region of Dm-1 and 2.

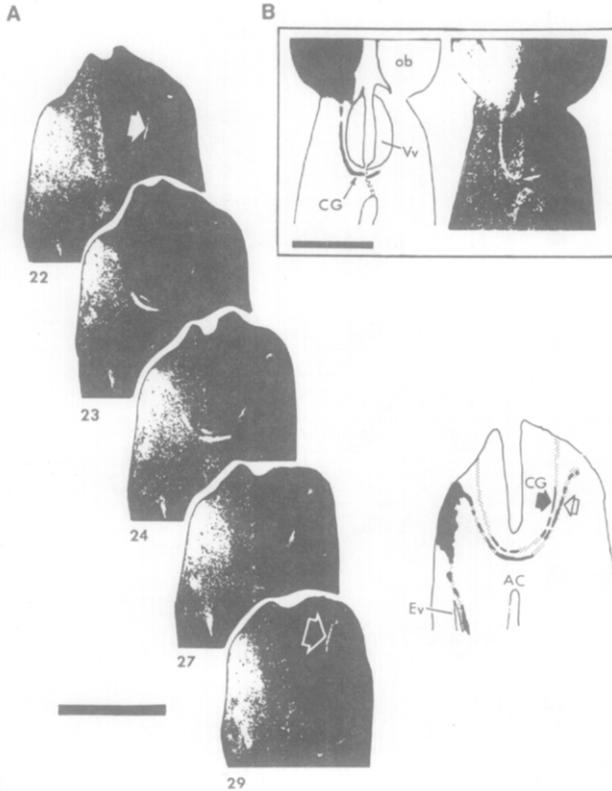


FIG. 8. (A) Autoradiograms of horizontal sections through ventral area V. The experimental bulb and the rostral pole of the ipsilateral hemisphere were intensively labeled (left side of the sections). The contralateral bulb was ablated. Section 22 is the most ventral. The data are summarized in the composite drawing on the right. (B) Horizontal section through area V in a brain in which the zone of diffusion was largely restricted to the experimental bulb. The contralateral bulb was not ablated. The rostral pole of the ipsilateral hemisphere was only lightly labeled and, correspondingly, there was no evidence of labeled lateral forebrain bundle fibers as seen in (A). Scale bars = 0.5 mm.

DISCUSSION

I. Central Projections of the Olfactory Bulb

A. Hemispheric Targets

The location of the major afferent olfactory areas of the hemisphere *Macropodus*, a representative acanthopterygian, resemble those reported for the five ostariophysians that have been examined experimentally. The previous studies employed degeneration methods—*Gymnothorax* (Scalia & Ebbesson, 1971), *Cyprinus* (Ito, 1973), *Carassius* (Ichikawa,

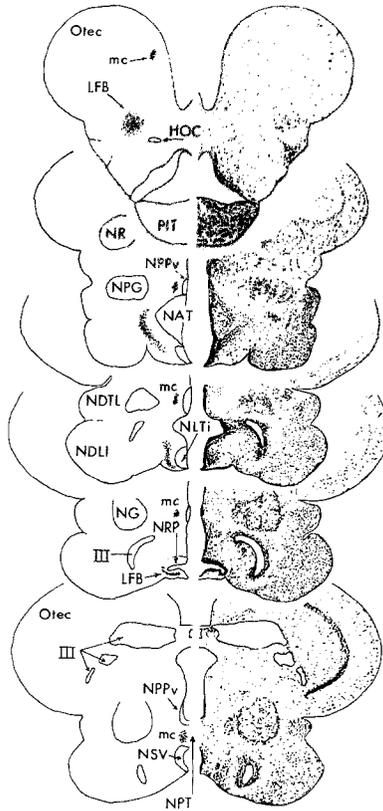


FIG. 10. Summary of the distribution of experimentally localized olfactory tract and lateral forebrain bundle fibers in the diencephalon. The right half of each transverse section is a photomicrograph. These sections and those in Fig. 2 are of the same reference brain.

Area V. The distribution of terminal fields in area V is extensive in some species and relatively restricted in others. The reports for *Gymnothorax*, *I. nebulosus*, and *Carassius* indicate that area V input is confined to the precommissural region, containing Vv and the rostral part of Vd, forming a relatively circumscribed "medial" field. In *Cyprinus*, Ito (1973) localized fiber terminations chiefly in VI but also in Vd and, we surmise from his illustrations, in Vv as well. The prominent input to VI is interesting in that a similar projection apparently does not occur in the other species. In *I. punctatus* and *Macropodus*, nearly all nuclei in V receive olfactory afferents including Vi, which is topographically contiguous with the "lateral" field.

Area D. Olfactory input to D is restricted to the lateral and the posterior central zones. The lateral zone appears to include the rostral part of Dp in *Gymnothorax*; of NT and parts of Dp and Dlp in *I. nebulosus*; and

of the extreme caudal end of Dlv, the ventral part of Dlp, and all of NT and Dp in *I. punctatus*. The targets in *Macropodus* compare closely with those of *I. punctatus*. In *Cyprinus*, terminals were located in the region of Dlv–Dlp.

The posterior central field includes the posterior part of Dc in *Gymnothorax*, *I. nebulosus*, and *Carassius*. In *I. punctatus*, fiber terminals are located in the area ventral and lateral to Dc-3. A similar though less elaborated field occurs in *Macropodus*.

B. Interbulbar Connections

Connections between the two bulbs have been demonstrated in *Gymnothorax* and *Ictalurus* and *Macropodus* but not *Cyprinus* nor *Carassius*. Two projections occur in *Ictalurus*, in the commissure of Goldstein and a component of the olfactory tracts that cross more dorsally in the anterior commissure. Only the former was detected in *Macropodus* and only the latter in *Gymnothorax*. In *I. punctatus*, Bass (1979) traced interbulbar fibers into the internal and mitral cell layers of the contralateral bulb.

C. Diencephalic Connections

The nucleus posterior tuberis receives olfactory input in *Ictalurus* (Finger, 1975; Bass, 1979) and *Macropodus*. A similar diencephalic projection was not reported for *Gymnothorax*, *Cyprinus*, nor *Carassius*.

Nieuwenhuys (1967) noted that olfactory fibers project to the habenula. Fibers from the nearby habenular commissure pathway could innervate the nucleus. Some *Macropodus* autoradiograms suggested the existence of terminals in the core of the nucleus, but the pattern of labeling was unconvincing. Finger (1975) and Bass (1979) traced lateral tract fibers through the habenular commissure to the lateral terminal field of the opposite hemisphere, but they reported no evidence of terminals in the habenula nucleus.

D. Bilateral Connections

Among the 6 species, there is marked variation in the number of zones that are innervated bilaterally. In *Ictalurus* (Finger, 1975; Bass, 1979) and, apparently, *Gymnothorax* (Scalia & Ebbesson, 1971), the projections are completely bilateral. Although the bulb innervates the same zones on both sides of the brain, the ipsilateral targets appear to receive more fibers than the contralateral. In *Macropodus*, bilateral input is restricted to three area V and three area D nuclei (Table 2), and the contralateral input is very sparse. The data for *Cyprinus* indicate that the contralateral input is limited to the ventral zone of V including VI. In *Carassius*, only the posterior–central zone of D is innervated bilaterally (Ichikawa, 1973; Oka, 1980).

II. Lateral Forebrain Bundle

Experimental studies of the efferents of the rostral zone of area D have apparently not been previously reported. The three projections detected in *Macropodus* compose only a small part of the lateral forebrain bundle, which is the largest tract system in the teleost forebrain (Johnston, 1911; Sheldon, 1912; Nieuwenhuys, 1959). The system includes many bundles connecting the diencephalon and virtually all parts of area D. Some bundles decussate in the anterior commissure as described, for example, in *Cyprinus* (Sheldon, 1912) and *Gasterosteus* (Nieuwenhuys, 1959). In *Gasterosteus*, Nieuwenhuys observed that fibers arising from Dm seem to project to Dm in the opposite hemisphere, forming a commissure. The commissural projection detected in *Macropodus* clearly fits this description.

The ventral entopeduncular nucleus, which is embedded in the lateral forebrain bundle (Nieuwenhuys, 1959), is apparently innervated by cells of rostral area D in *Macropodus*. Unfortunately, the identity of the cells is unclear in our limited material. The cells-of-origin of the diencephalic projection also remains to be clarified. Its distribution in the ventral diencephalon partly resembles that of the so-called strio-lobar bundle described in *Eugerres* and *Holocentrus*. Vanegas and Ebbesson (1976) traced degenerating telencephalic fibers following unilateral ablation of the entire hemisphere in these perciform fishes. The strio-lobar, however, ends in the inferior lobe in the region of the lateral recess of the III ventricle, and no projection to the nearby posterior recess was indicated. While the projection in *Macropodus* passes near the lateral recess, it ends medially near the NLTp, NLTi, and NRP. Thus, its relationship to the strio-lobar is unclear.

III. Correlations with Previous Neural and Behavioral Findings in *Macropodus*

Hemispheric and bulbar lesions impair reproductive functions in *Macropodus*. The lesions could act by destroying brain areas that potentiate reproduction or by disrupting essential input to such areas. Neurons that regulate reproductive processes are thought to include those which concentrate sex steroids (Morrell, Kelley, & Pfaff, 1975). In the male *Macropodus*, steroid-concentrating cells occur in Vv, the ventral zone of POA, NLT, the nucleus of the lateral recess of the third ventricle, and the extreme caudal extent of NPPv (Davis, Morrell, & Pfaff, 1977).

A. Afferent Olfactory Connections

The present experiments imply that steroid-concentrating cells in Vv could receive olfactory innervation. Input to the cells in ventral POA seems less likely, since the olfactory afferents were traced to dorsal

POA. The caudal branch of the olfactory tract courses very close to NPPv (Fig. 10). Though the fibers did not visibly penetrate among the nucleus, NPPv fibers might extend laterally into the olfactory pathway.

Bilateral bullectomy results in increased egg cannibalism in the male, but the incidence of various sexual behaviors including spawning is not significantly affected (Davis et al., 1981). Investigations of partial lesions indicated that medial olfactory tract fibers, possibly including those which project to Vv, are necessary for normal egg care. Whether Vv plays a role in egg care or spawning in *Macropodus* remains to be examined. However, Kyle and Peter (1979) showed that, in male *Carassius*, the incidence of spawning is decreased following administration of a localized lesion in Vs-Vv. A similar localized lesion in POA does not impair spawning. Spawning is also decreased following blockade of olfactory input (Partridge, Liley, & Stacey, 1976). Thus, it is possible that the functions of the olfactory input to the Vs-Vv region (Ichikawa, 1975) include potentiation of spawning.

B. Lateral Forebrain Bundle Connections

Telencephalic lesion experiments in *Macropodus* have so far shed little light on the behavioral functions of the complex lateral forebrain bundle system. Though male reproductive behaviors are decreased following complete removal of the hemispheres to the level of POA (Kassel & Davis, 1977), the same behaviors are unaffected by extensive removal of area D and the posterior dorsal parts of V (Davis et al., 1981). If the lateral forebrain connections are located predominantly in area D, as generally believed (Sheldon, 1912; Nieuwenhuys, 1959), they apparently do not play a limiting role in controlling the reproductive behaviors that we observed.

The extensive area D lesions referred to above selectively spared the extreme rostromedial zone, including Dm-1 and in most cases Dm-2 as well. The link between rostral Dm and the steroid-concentrating areas NLTi and NLTp, detected in the present experiments, could have been spared. The possibility that a Dm-NLT projection is necessary for spawning is dashed, however, by the finding that the behavior is not decreased following bilateral ablation of the rostral end of the hemisphere (Davis et al., 1981).

IV. Evidence of Retrograde Transport of Radiolabeled Substance

Kunzle (1977) reported that while tritiated proline is particularly effective in labeling anterograde axonal flow (Elam & Agranoff, 1971), certain neurons in mammal brain show evidence of labeled retrograde flow. Perikarya in specific brain regions contain high concentrations of radioactivity following spinal application of tritiated proline. We detected similarly labeled cells in the hemisphere of *Macropodus* in a pilot study

(Davis & Agranoff, 1977), in which the incorporation period was only 5 hr. Some brains in the present experiment, with a 10-day incorporation period, showed labeled perikarya, but they were less abundant or conspicuous.

Though the radiolabeled cells are few and widely scattered in the hemisphere, a distinct cluster occurs in the ipsilateral Nt-Dlp region, as illustrated by Davis and Agranoff (1977). Efferent cells are reported to occur in this region, among others, in the brain of *Carassius* (Oka, 1980). The coincidence suggests that the radiolabeled cells in *Macropodus* could be efferents whose terminals take up and transport tritiated proline or metabolite. If such transport occurs, the tracts that we traced must be regarded as containing not only secondary olfactory afferents but an unknown number of efferents as well.

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