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Cover illustration. An adult male honey possum, *T. rostratus*. Harman, Coleman and Beazley (p. 31).

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Phylogeny of Putative Cholinergic Visual Pathways through the Pretectum to the Hypothalamus in Teleost Fish

Mario F. Wullimann, Dietrich L. Meyer

Department of Neuroanatomy, School of Medicine, University of Göttingen, Göttingen, FRG

Key Words. Acetylcholinesterase · Evolution · Hypothalamus · Phylogeny · Pretectum · Teleost · Visual system

Abstract. Three patterns of pretectal organization can be discerned morphologically in teleosts. The taxonomic distribution of these pretectal patterns suggests that the intermediately complex pattern (seen in most teleost groups) has given rise to both the elaborate pattern (seen in percomorphs) and the simple pattern (seen in cyprinids). Two pretectal patterns (intermediately complex and elaborate) form part of similar, homologous visual pathways to the hypothalamus; the third pattern is involved in a nonhomologous pathway to the hypothalamus. Acetylcholinesterase (AChE) histochemistry was used in the present study in order to characterize these pretectal patterns further. It is demonstrated that AChE is a highly selective and reliable interspecific marker for all divisions of the superficial pretectum, the nucleus corticalis, the posterior pretectal nucleus (or nucleus glomerulosus) and portions of the inferior lobe. Therefore, the histochemical data support the hypothesis of a homology between the three patterns of pretectal organization in teleosts. Furthermore, the present data provide a basis for more specific investigations regarding the involvement of acetylcholine as a neurotransmitter within the visual pathways to the hypothalamus in teleosts.

Introduction

At least three major patterns of pretectal organization exist within teleost fish (fig. 1). Apparently, the most elaborate pattern occurs in percomorph teleosts (fig. 1b), where some pretectal nuclei form relay centers within visual pathways to the hypothalamus. Morphological and hodological work in percomorphs has shown that a complex array of nuclei and fiber tracts constitutes visual pathways leading from retinal ganglion cells through several pretectal nuclei to the inferior lobe of the hypothalamus [Sakamoto and Ito, 1982; Murakami et al., 1986; Striedter and Northcutt, 1986, 1989; Wullimann and Northcutt, 1986; for a review, see Northcutt and Wullimann, 1988]. The nucleus glomerulosus is the most complex relay center within these pathways. This large and layered nucleus is bilobed, with a smaller rostral lobe extending to-

ward the superficial pretectum and a larger caudal lobe extending far back into the preglomerular cell masses.

There are two possible homologues of this elaborate percomorph pretectal pattern in other teleosts. One is found in some ostariophysines, e.g. cyprinids. It consists of fewer and less conspicuously organized nuclei (fig. 1c). A phyletic analysis has previously shown that many visual nuclei in the diencephalon of cyprinids are not evolutionarily primitive but were reduced secondarily during the ostariophysine evolution; i.e. they represent a derived condition [Northcutt and Wullimann, 1988]. This pattern of pretectal organization in cyprinids will be referred to here as the simple pattern.

A second possible homologue of the elaborate percomorph pretectal pattern is seen in most other teleostean groups, such as osteoglossomorphs, esocids and

salmonids (fig. 1a, d); it is of intermediate complexity compared to the other two patterns (fig. 1a). This intermediately complex pattern is characterized by a large posterior prepectal nucleus which occupies a position similar to that of the nucleus glomerulosus in the percomorph pattern. However, its histology is distinctly different since its neural components are not organized in layers and since it does not extend caudally into the preglomerular cell masses.

Although there is considerable morphological variation among the three patterns, the general topology suggests the hypothesis that they represent different morphotypes of a homologous set of nuclei. A related, but different, topic is the phylogenetic relationship between the patterns. Once a hypothesis of homology between the patterns is corroborated by structural similarities, the systematic distribution of the patterns should be considered. The posterior prepectal nucleus is not as large in elopomorphs and some clupeomorphs as it is in most osteoglossomorphs, esocids or salmonids [M.F. Wullimann, unpubl. observ.]. However, the former two teleostean groups also show a variation of the intermediately

complex pattern of prepectal organization. A survey of the prepectal morphology within the major teleostean groups [Northcutt and Wullimann, 1988; Butler et al., 1989; A.B. Butler, M.F. Wullimann and R.G. Northcutt, unpubl. observ.] strongly indicates that both the simple and elaborate patterns are derived from the intermediately complex one, since the latter is found in all outgroups of the percomorphs and ostariophysines (fig. 1d).

Two approaches to test the hypothesis of homology between the prepectal patterns seem obvious: to look for similarities in connections and for similarities in histochemistry. Recently, we have investigated the visual circuitry to the hypothalamus in an osteoglossomorph, *Osteoglossum bicirrhosum*, which shows the intermediately complex pattern (fig. 1a). We have demonstrated that the connections of the posterior prepectal nucleus are identical to those of the nucleus glomerulosus and intermediate superficial prepectal nucleus [Wullimann and Northcutt, 1989]. This detailed similarity of connections supports the hypothesis that the posterior prepectal nucleus is homologous to the nucleus glomerulosus and intermedi-

Abbreviations used in figures 1-12

A	Nucleus anterior thalami	MO	Medulla oblongata
AOD	Nucleus accessorius opticus dorsalis	NF	Nucleus of the medial longitudinal fasciculus
AOV	Nucleus accessorius opticus ventralis	OT	Optic tract
AP	Nucleus prepectalis accessorius	P	Nucleus prepectalis superficialis, pars parvocellularis
C	Nucleus corticalis	PC	Nucleus paracommissuralis
Ce	Corpus cerebelli	PG	Nucleus preglomerulosus
CM	Corpus mammillare	PLI	Nucleus periventricularis lobi inferiores
CP	Nucleus prepectalis centralis	PM	Nucleus preopticus magnocellularis
CPo	Commissura posterior	PO	Nucleus prepectalis posterior
CS	Commissura supraoptica	PP	Nucleus preopticus parvocellularis
DLI	Nucleus diffusus lobi inferiores	PPd	Nucleus prepectalis periventricularis, pars dorsalis
DOT	Dorsomedial optic tract	PPv	Nucleus prepectalis periventricularis, pars ventralis
E	Eminentia ganularis	SC	Nucleus suprachiasmaticus
FLL	Fasciculus longitudinalis lateralis	T	Tectum opticum
FR	Fasciculus retroflexus	TA	Nucleus tuberis anterior
G	Nucleus glomerulosus	Tel	Telencephalon
GA	Nucleus glomerulosus, pars anterior	TL	Torus longitudinalis
GP	Nucleus glomerulosus, pars posterior	TLa	Nucleus of the torus lateralis
GT	Nucleus gustatorius tertius	TMC	Tractus mesencephalocerebellaris
Ha	Habenula	TP	Nucleus tuberis posterior
Hv	Ventral periventricular hypothalamus	TS	Torus semicircularis
Hy	Hypophysis	V	Vagal lobe
I	Nucleus prepectalis superficialis, pars intermedius	VL	Nucleus ventrolateralis thalami
LH	Nucleus lateralis hypothalami	VM	Nucleus ventromedialis thalami
LI	Lobus inferior	VOT	Ventrolateral optic tract
M	Nucleus prepectalis superficialis, pars magnocellularis		

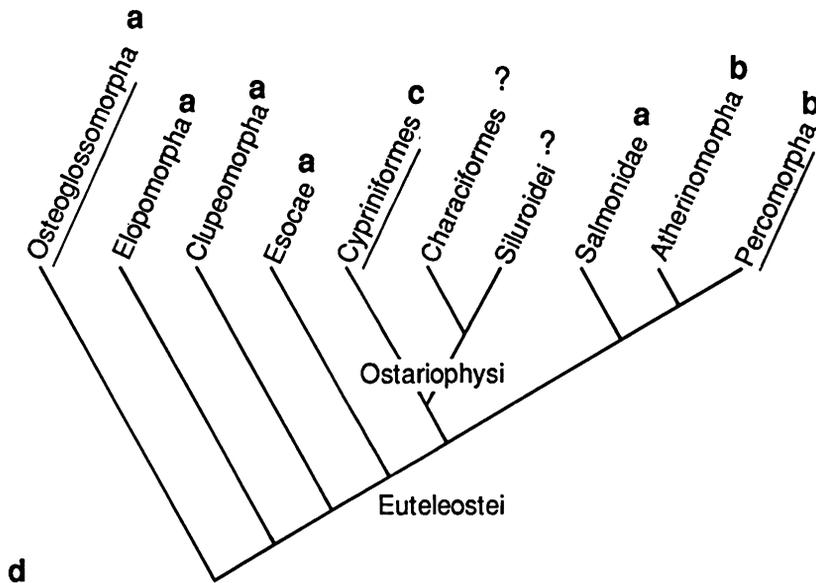
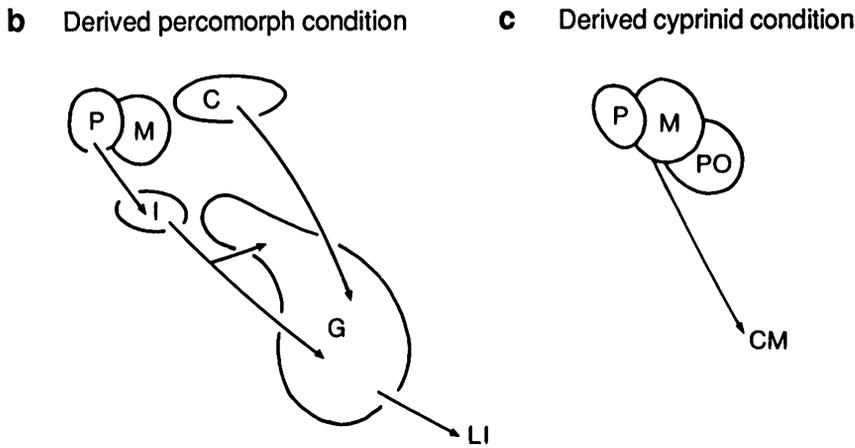
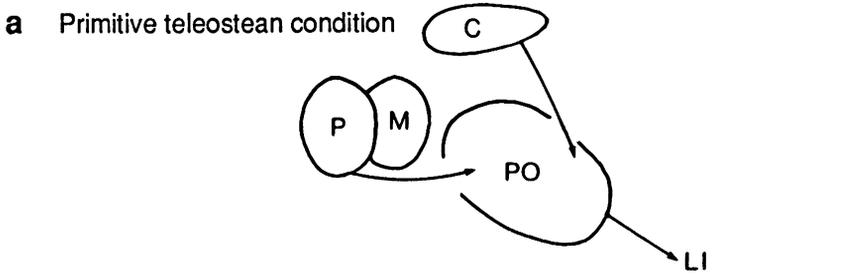


Fig. 1. a-c Schematic illustrations of the three patterns of pretectal organization in teleosts. **a** Intermediately complex pattern with arrows indicating connections according to Wullimann and Northcutt [1989]. **b** Elaborate pattern with arrows indicating connections as reviewed by Northcutt and Wullimann [1988]. **c** Simple pattern with arrow indicating a connection according to Northcutt and Braford [1984]. Based on its distribution (see **d**), pattern **a** is interpreted as the primitive one for teleosts, and patterns **b** and **c** are interpreted as derived ones. **d** Cladogram reflecting the phylogenetic relationships between teleost fish according to Lauder and Liem [1983]. The distribution of the patterns of pretectal organization (**a-c** in this figure) is indicated. The underlined taxa include the three species investigated in the present study.

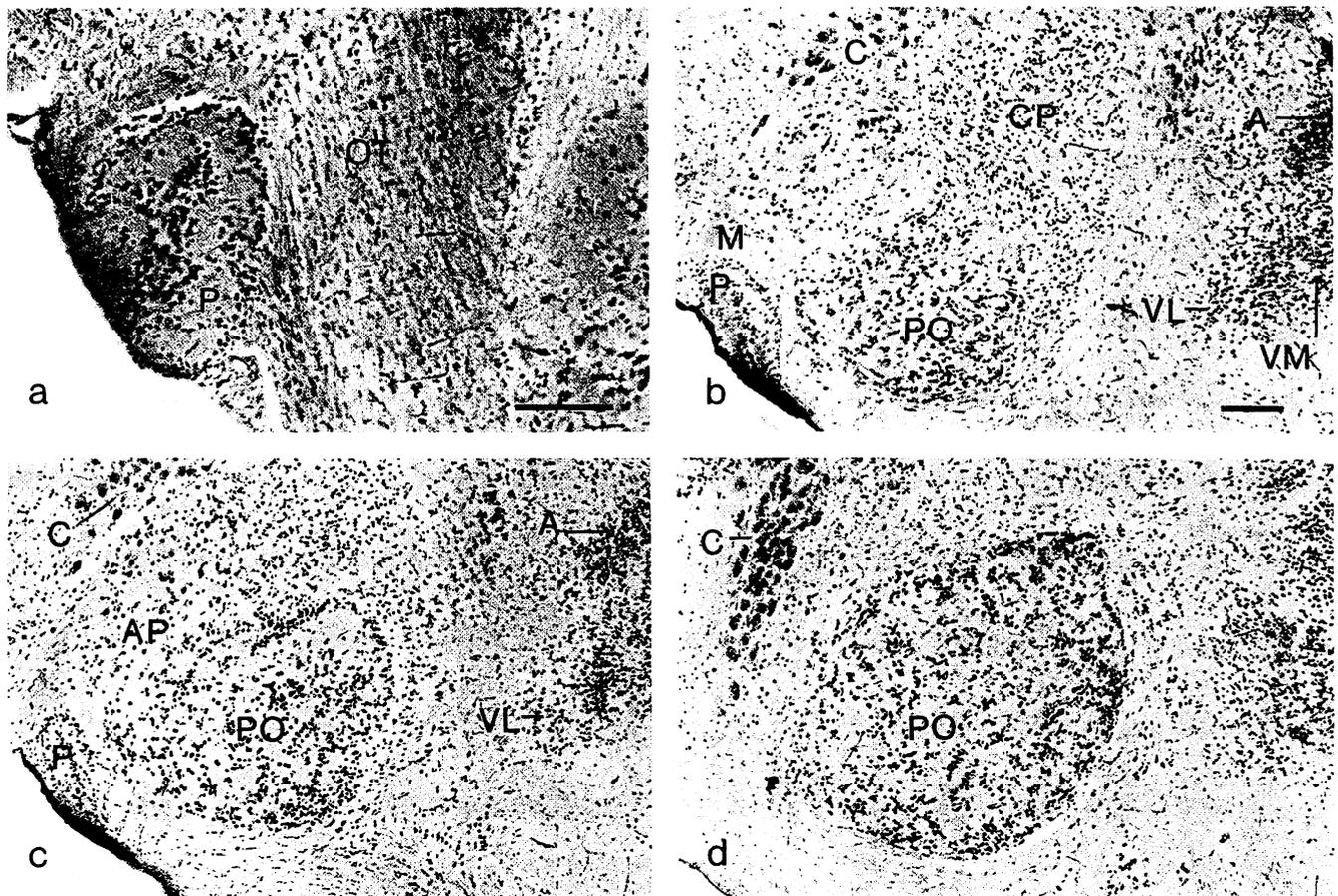


Fig. 2. Photomicrographs of Bodian-stained cross-sections showing the normal anatomy of the pretectum in *Osteoglossum* from rostral (a) to caudal (d). a, b Bars = 0.1 mm. c, d Same magnification as b. Lateral is to the left and dorsal to the top in figures 2-4 and 6-11.

superficial pretectal nucleus (misidentified as central pretectal nucleus in Wullimann [1985]).

The present study provides histochemical information supporting the hypothesis of homology between the three patterns of pretectal organization. Ekström [1987] has shown that the neuropil of the parvocellular superficial pretectal nucleus and the neurons of the magnocellular superficial pretectal nucleus are strongly immunoreactive to choline acetyltransferase (ChAT) in *Phoxinus phoxinus*. This cyprinid species exhibits the simple pattern of pretectal organization. Thus, it is of interest to determine which portions of the pretectum in the intermediately complex and elaborate patterns contain acetylcholinesterase (AChE). Therefore, we chose a cyprinid, *Carassius auratus*, an osteoglossomorph, *O. bicirrhosum*, and a perco-

morph, *Hemichromis lifalili*, as representatives of the simple, intermediately complex and elaborate patterns, respectively, and investigated the diencephalon and mesencephalon in these species with regard to the presence or absence of AChE.

The neuroanatomical nomenclature follows that of Braford and Northcutt [1983] as modified by Northcutt and Wullimann [1988], except where noted. The taxonomic nomenclature is that of Lauder and Liem [1983]. A preliminary report on this topic has been published previously [Wullimann and Meyer, 1989].

Materials and Methods

Brain tissue was prepared either for histochemistry or for normal anatomical analysis. All animal research described in this

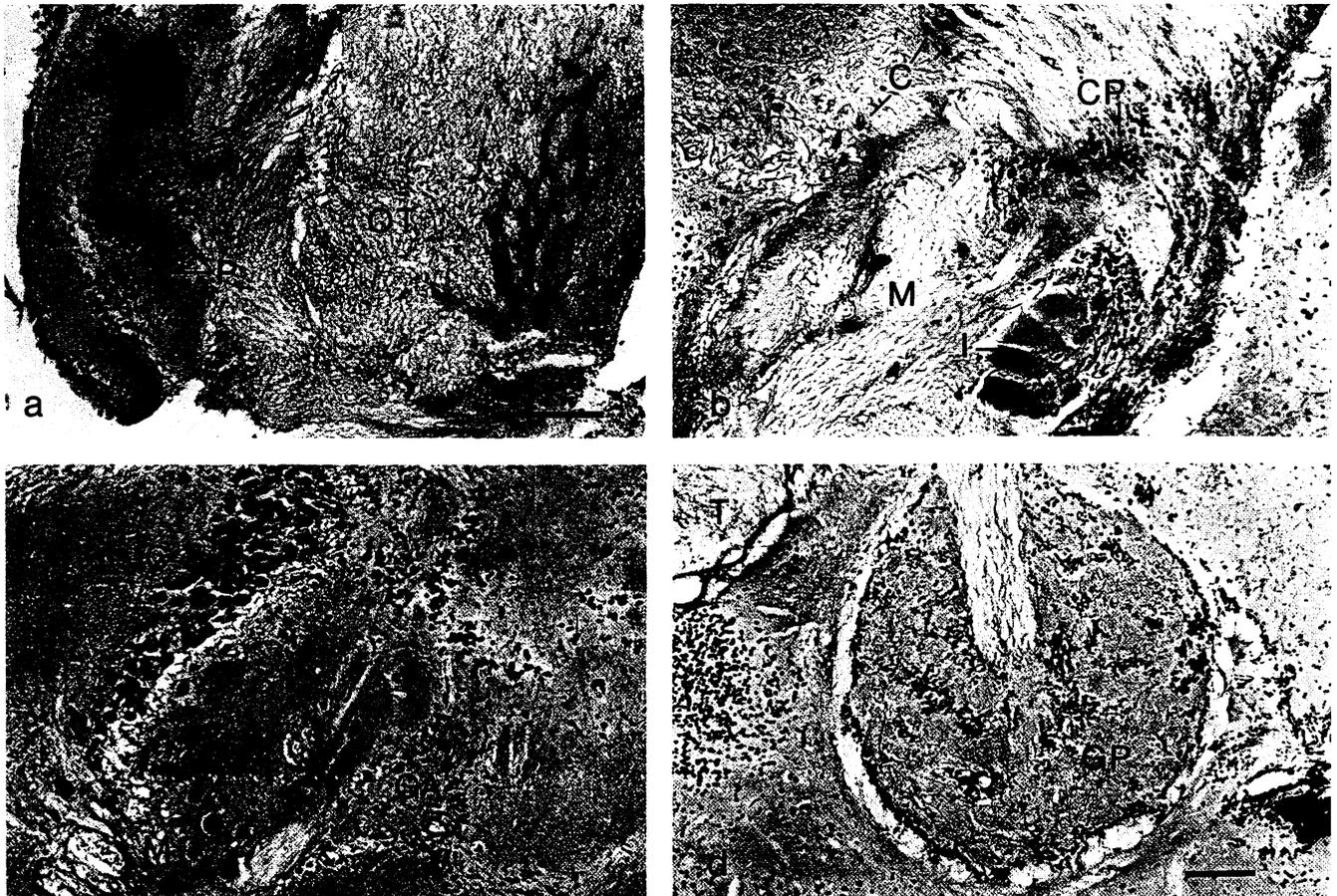


Fig. 3. Photomicrographs of Bodian-stained cross-sections showing the normal anatomy of the prepectum in *Hemichromis* from rostral (a) to caudal (d). a, d Bars = 0.1 mm. b, c Same magnification as a.

study has been approved by the 'Regierungspräsident' of the State of Lower Saxony, FRG.

Histochemistry

Three specimens each of *O. bicirrhosum*, *H. lifalili* and *C. auratus* were anesthetized in a diluted solution of MS222 (Sigma, München, FRG) and perfused transcardially with cold teleostean Ringer's solution containing 1% heparin, followed by cold 10% formalin (Sigma) in 0.05 M phosphate buffer. The brain, liver and, only in *Osteoglossum*, the gut were dissected out, postfixed for 3–8 h in the same fixative supplemented with 30% sucrose, and transferred to phosphate buffer with 30% sucrose overnight. The brains, livers and guts were then sectioned transversely (30 μ m) in a cryostat. The sections were reacted on the slides to detect the presence of AChE after the protocol of Lynch and Killackey [1974].

As controls, promethazine (Sigma), an inhibitor of unspecific cholinesterases (butyrylcholinesterase; BuChE), was added to the incubation solution in parallel experiments. There was no effect on the histochemical staining pattern of the brain tissue. Furthermore,

when acetylthiocholine iodide was replaced by S-butyrylthiocholine iodide (both from Sigma) as a substrate for cholinesterases, no staining was observed in any of the brain tissues at all.

The liver and gut, which have been claimed to contain BuChE [Koelle, 1950, 1951, 1963], were also investigated for the presence of unspecific cholinesterases to provide a further check for the apparent absence of BuChE in the brain tissue. Only the intestinal smooth muscles of *Osteoglossum* showed evidence for the presence of BuChE. The use of S-butyrylthiocholine iodide produced heavy staining of these muscles, whereas the use of acetylthiocholine iodide yielded only weak staining. A similar staining pattern was achieved when promethazine was used in conjunction with S-butyrylthiocholine iodide. By omitting both acetylthiocholine iodide and S-butyrylthiocholine iodide, no smooth muscle staining was observed at all. These control experiments indicate that the smooth musculature of the gut in *Osteoglossum* contains some AChE as well as a high amount of BuChE. Apparently, BuChE can be visualized with this method. However, there was no staining at all in the brain tissue in the three species investigated when S-butyrylthio-

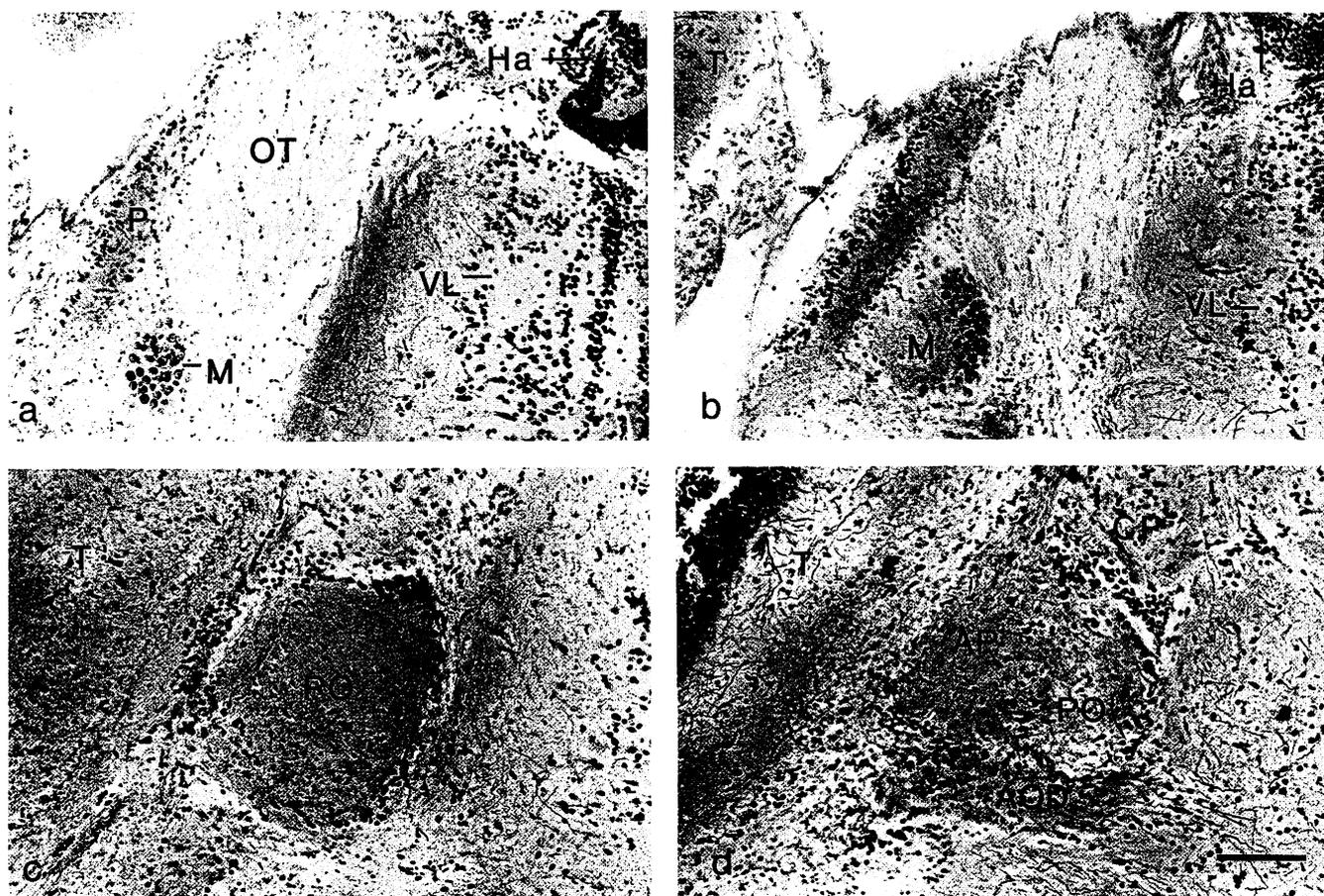


Fig. 4. Photomicrographs of Bodian-stained cross-sections showing the normal anatomy of the pretectum in *Carassius* from rostral (a) to caudal (d). Bar in d equals 0.1 mm and applies to a-d.

choline iodide was used, in contrast to the heavy staining when acetylthiocholine iodide was used. Therefore, the controls also show that all histochemical staining observed in the brain was due to the presence of AChE.

Normal Anatomy

In addition to the specimens examined for AChE histochemistry, one specimen each of the three species was perfused in the same way as described above, except that AFA was used as a fixative (90 ml 80% ethanol, 5 ml formalin, 5 ml glacial acetic acid). The brains were postfixed in AFA for at least 1 month before they were embedded in paraffin, cut transversely at 15 μ m and silver-stained with Protargol (Roques chimie, St. Ouen, France) according to the Bodian method [Romeis, 1989].

Results

The normal anatomy of the pretectum is described in detail in the three species investigated, followed by

a description of the AChE distribution in the dien-cephalon and mesencephalon of these teleosts.

Normal Anatomy

The normal anatomy of the pretectum in the three species is illustrated in figures 2-4.

Osteoglossum. This osteoglossomorph species shows the intermediately complex pattern of pretectal organization (fig. 2). The parvocellular superficial pretectal nucleus emerges furthest rostrally in the dien-cephalon and is embedded in the optic tract. It consists of small cells that rim a pleated band of central neuropil (fig. 2a). Caudally and dorsomedially, a small magnocellular superficial pretectal nucleus emerges. The posterior pretectal nucleus lies ventral to both nuclei; it is histologically distinct with smaller, regularly clustered neurons. This nucleus is the largest and most caudally extending pretectal nuclear group

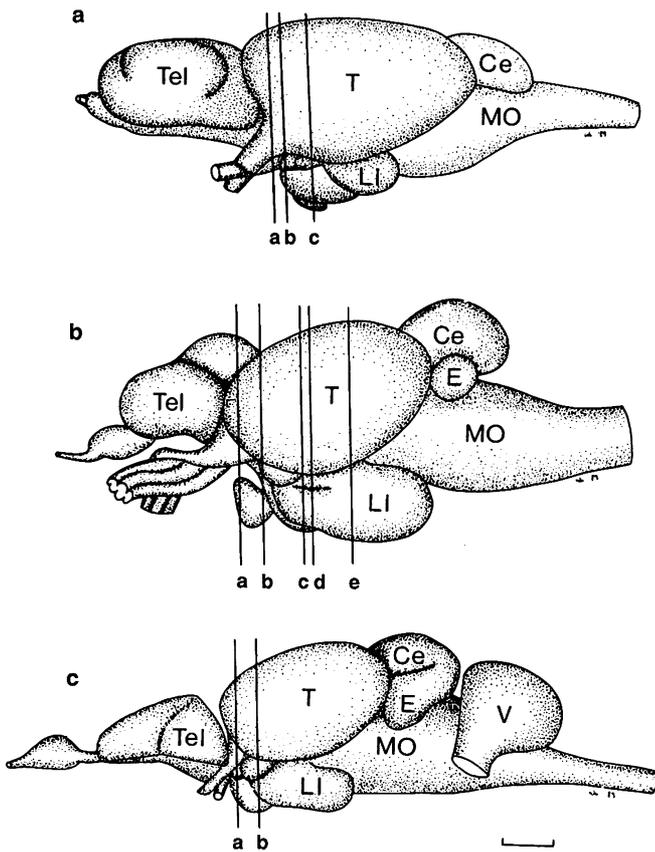


Fig. 5. Lateral views of the brains of *Osteoglossum* (a), *Hemichromis* (b) and *Carassius* (c) indicating the positions of the cross-sections shown in figures 6–8, respectively. Most cranial nerves have been omitted. Bar = 1 mm.

in *Osteoglossum*. The nucleus corticalis is located dorsal to the magnocellular superficial pretectal nucleus. The huge neurons of this nucleus lie at the border to the optic tectum (fig. 2b–d; 6b, c). At more caudal levels, the magnocellular superficial pretectal nucleus is replaced by a smaller-celled nucleus, the accessory pretectal nucleus, which lies sandwiched between the nucleus corticalis and the posterior pretectal nucleus (fig. 2b–d). The more medially located central pretectal nucleus is also situated between these two nuclei (fig. 2b). The dorsal and ventral parts of the periventricular pretectum are found even more medially and caudally, and are characteristically located around the fasciculus retroflexus.

Hemichromis. This percomorph species displays the elaborate pattern of pretectal organization (fig. 3). Most rostrally, a complexly pleated parvocellular su-

perficial pretectal nucleus emerges within the optic tract. Its neurons are located at the rim of a neuropil band in a characteristic fashion (fig. 3a). More caudally and dorsomedially, it is replaced by the large-celled magnocellular superficial pretectal nucleus and ventromedially by the intermediate superficial pretectal nucleus (fig. 3b). The latter is replaced caudally by the anterior part of the glomerular nucleus (fig. 3c). This nucleus is easily identifiable by its specialized neuropil which is organized in spherical glomeruli (fig. 10c). These glomeruli continue to be present more caudally within the posterior part of the nucleus glomerulosus, which extends caudally into the preglomerular cell masses and approaches the inferior lobe of the hypothalamus. The nucleus glomerulosus displays small and large cells. The small cells are found throughout the nucleus, whereas the large cells are mostly found in the periphery (fig. 3d). This histological organization is a variation of the incompletely laminated type of the nucleus glomerulosus (= nucleus rotundus of Ito and Kishida [1975]). Dorsomedial to the magnocellular superficial pretectal nucleus, a smaller-celled nucleus, the accessory pretectal nucleus, can be observed; it is located between the anterior part of the nucleus glomerulosus and the large-celled nucleus corticalis (fig. 3c). Even more medially the central pretectum (fig. 3b) and the periventricular pretectum are seen. The latter surrounds the fasciculus retroflexus.

Carassius. This cyprinid species shows the simple pattern of pretectal organization (fig. 4). Again, the parvocellular superficial pretectal nucleus is located most rostrally. Although not pleated, it consists of the characteristic neuropil band which is rimmed by small cells. Medial to this nucleus lies the large-celled magnocellular superficial pretectal nucleus (fig. 4a, b). More caudally it is replaced by the dorsomedially extending accessory pretectal nucleus and the ventrally located small posterior pretectal nucleus (fig. 4c, d). The latter nucleus disappears at a much more rostral level in *Carassius* than in *Osteoglossum*. The central pretectum lies medial to the accessory superficial and posterior pretectal nuclei (fig. 4d). Again, the periventricular pretectum is located around the fasciculus retroflexus. The nucleus corticalis is absent.

AChE Activity

The levels of the maps of AChE activity (fig. 6–8) described in the following section are depicted in a di-

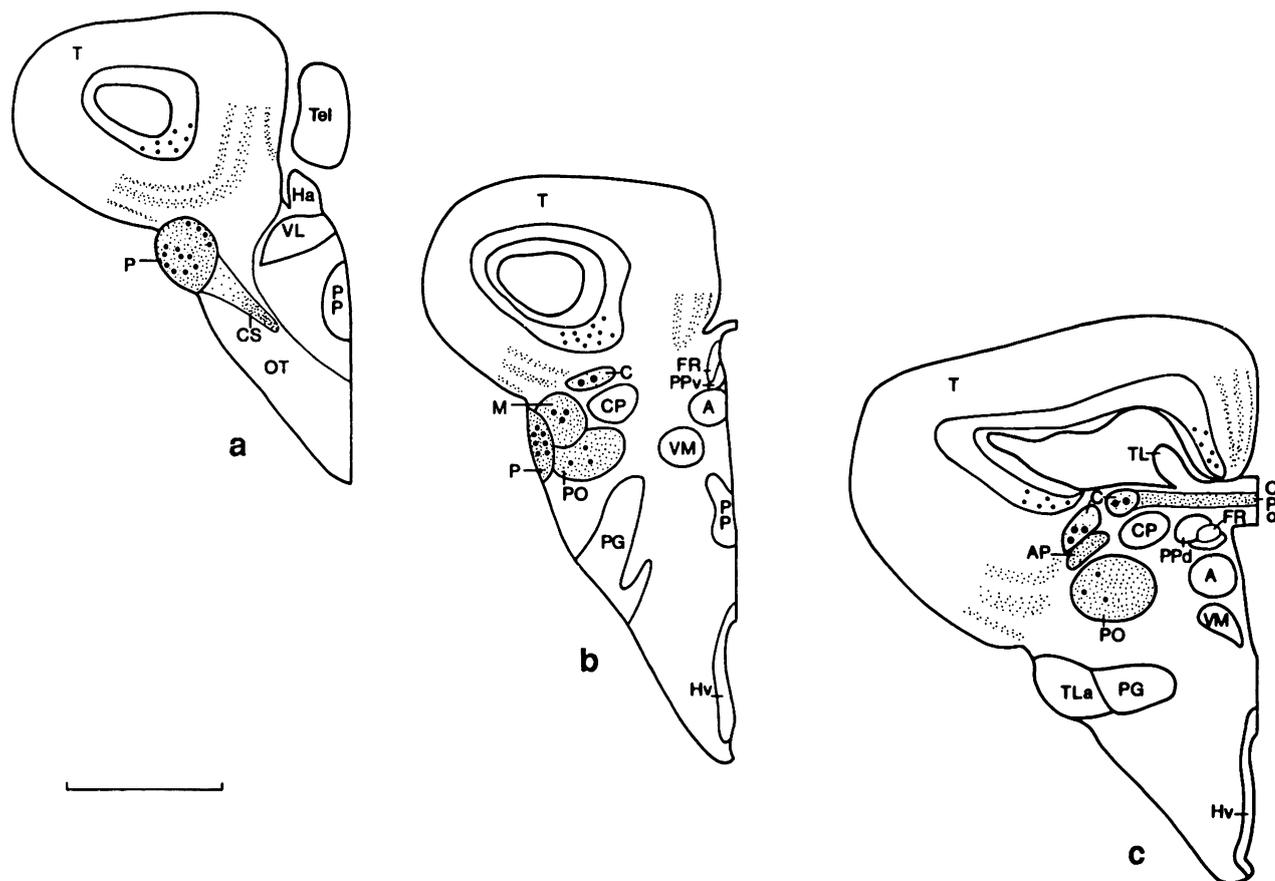


Fig. 6. Maps of AChE activity in the diencephalon and mesencephalon of *Osteoglossum*. Bar = 1 mm.

agram of lateral views of the brains of *Osteoglossum*, *Hemichromis* and *Carassius* shown in figure 5.

Osteoglossum. In the pretectum, we found AChE reactivity in the neuropil and cell bodies of the parvocellular (fig. 6a; 9a) and magnocellular superficial pretectal nuclei, in the nucleus corticalis and in the posterior pretectal nucleus (fig. 6b; 9b-d), whereas only the neuropil of the accessory pretectal nucleus was labeled (fig. 6c; 9c, d). The AChE-containing neurons in the posterior pretectal nucleus were located in the lateral and rostral portions of the nucleus. Additional cell bodies were labeled in the periventricular gray zone of the optic tectum. Other AChE-containing fibers were seen in the posterior (fig. 6c) and supraoptic commissures (fig. 6a; 9b) and, further, in three distinct bands of the optic tectum, two within the superficial white and gray zones and one within the central zone. These AChE bands are continuous throughout the optic tectum in all three species inves-

tigated. However, they are depicted only at the lateroventral and mediodorsal edges of the tectum in figures 6-8.

Hemichromis. In the pretectum, AChE reactivity was observed in the neuropil and cell bodies of the parvocellular (fig. 7a; 10a), magnocellular and intermediate superficial pretectum and in the nucleus corticalis (fig. 7b; 10b). In the accessory pretectal nucleus, such reactivity was restricted to the neuropil (fig. 7c; 10c). The anterior part of the nucleus glomerulosus, which extends toward the superficial pretectum (fig. 7c; 10c), continued to display strong label within specialized neuropil structures, the glomeruli, far caudally into the posterior part of the nucleus (fig. 7c-e; 10d). Additional AChE-containing fibers were observed in the supraoptic commissure (fig. 7a), the habenula (fig. 7c), the torus longitudinalis (fig. 7e), a restricted area within the ventrolateral optic tract (fig. 7b-d), the nucleus of the torus lateralis

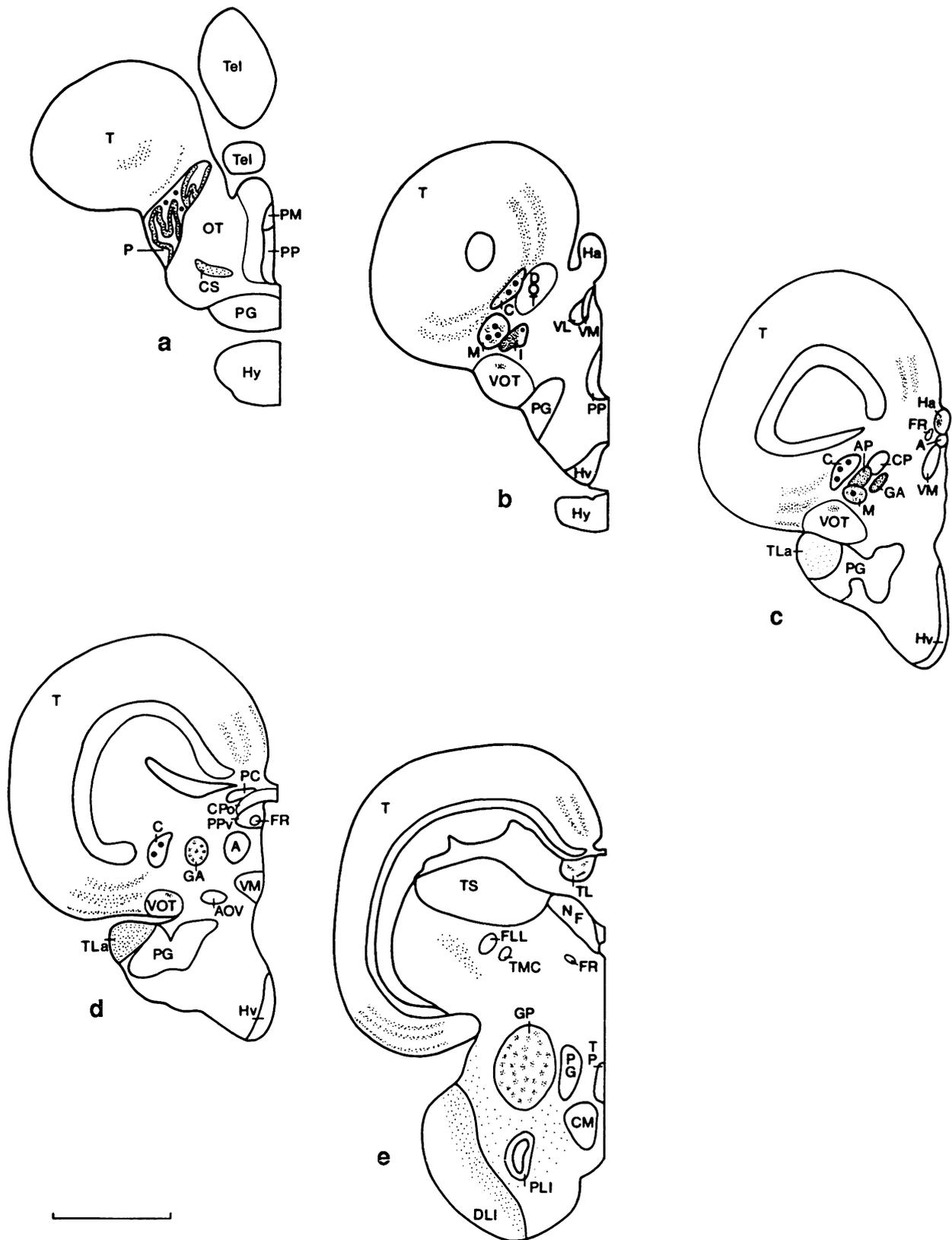


Fig. 7. Maps of AChE activity in the diencephalon and mesencephalon of *Hemichromis*. Bar = 1 mm.

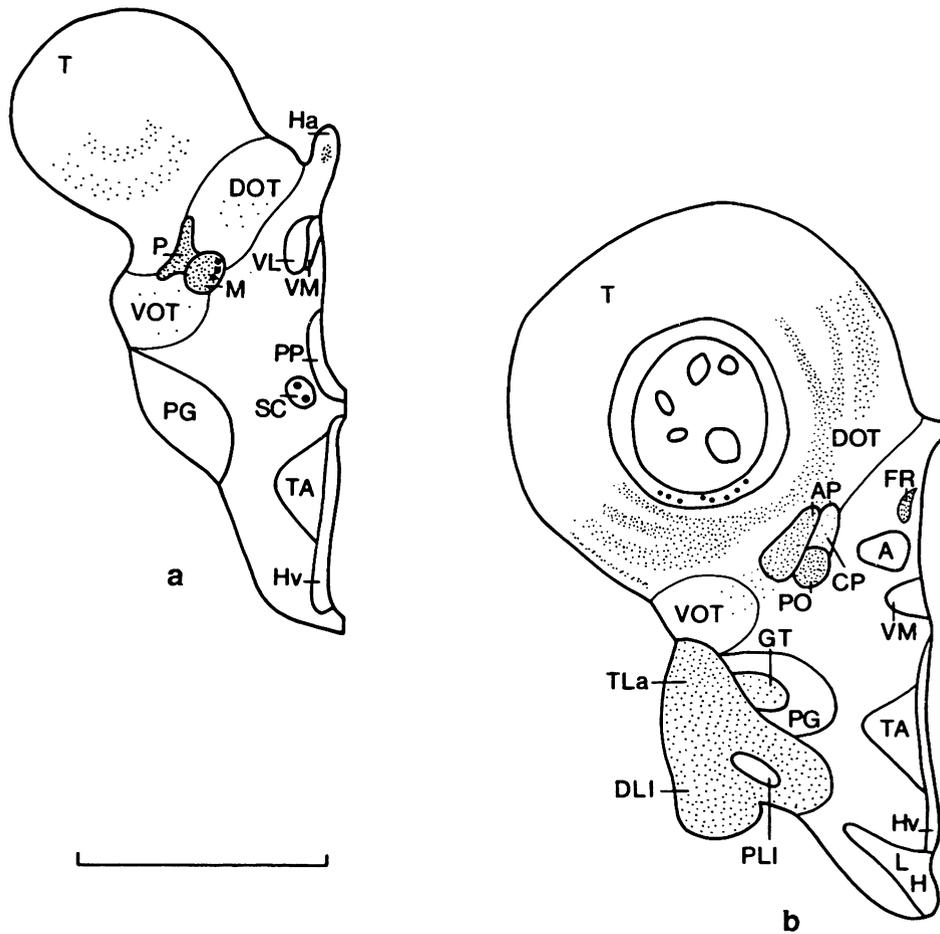


Fig. 8. Maps of AChE activity in the diencephalon and mesencephalon of *Carassius*. Bar = 1 mm.

(fig. 7c, d), and, lightly, in the inferior lobe, particularly in the diffuse nucleus (fig. 7e). AChE-reactive fibers were also found in three distinct bands within the optic tectum, two within the superficial white and gray zones and one within the central zone.

Carassius. In the pretectum, AChE reactivity was seen in the neuropil of the parvocellular and magnocellular superficial pretectal nuclei (fig. 8a; 11a, b), the accessory and posterior pretectal nuclei and, more lightly, in the central pretectum (fig. 8b, 11c, d). The cell bodies of the magnocellular pretectum were also labeled (fig. 8a; 11a, b). In *Carassius*, however, no continuation of AChE activity was observed as far caudally and ventrally as in *Hemichromis* or *Osteoglossum*. Additional cell bodies were labeled in the suprachiasmatic nucleus and in the periventricular

gray zone of the optic tectum. Labeled fibers were observed in the habenula (fig. 8a), fasciculus retroflexus, nucleus of the lateral torus, diffuse nucleus of the inferior lobe and, more lightly, in the tertiary gustatory nucleus [see Wullimann, 1988] (fig. 8b). Furthermore, AChE-reactive fibers were seen in four bands within the optic tectum, two each in the superficial white and gray zones and in the central zone. Some labeled fibers were also seen in the ventrolateral and dorso-medial optic tracts.

In summary, these histochemical data reveal that, in teleosts, AChE is a highly specific and reliable interspecific marker for all divisions of the superficial pretectum, nucleus corticalis, posterior pretectal nucleus (or nucleus glomerulosus) and portions of the inferior lobe.

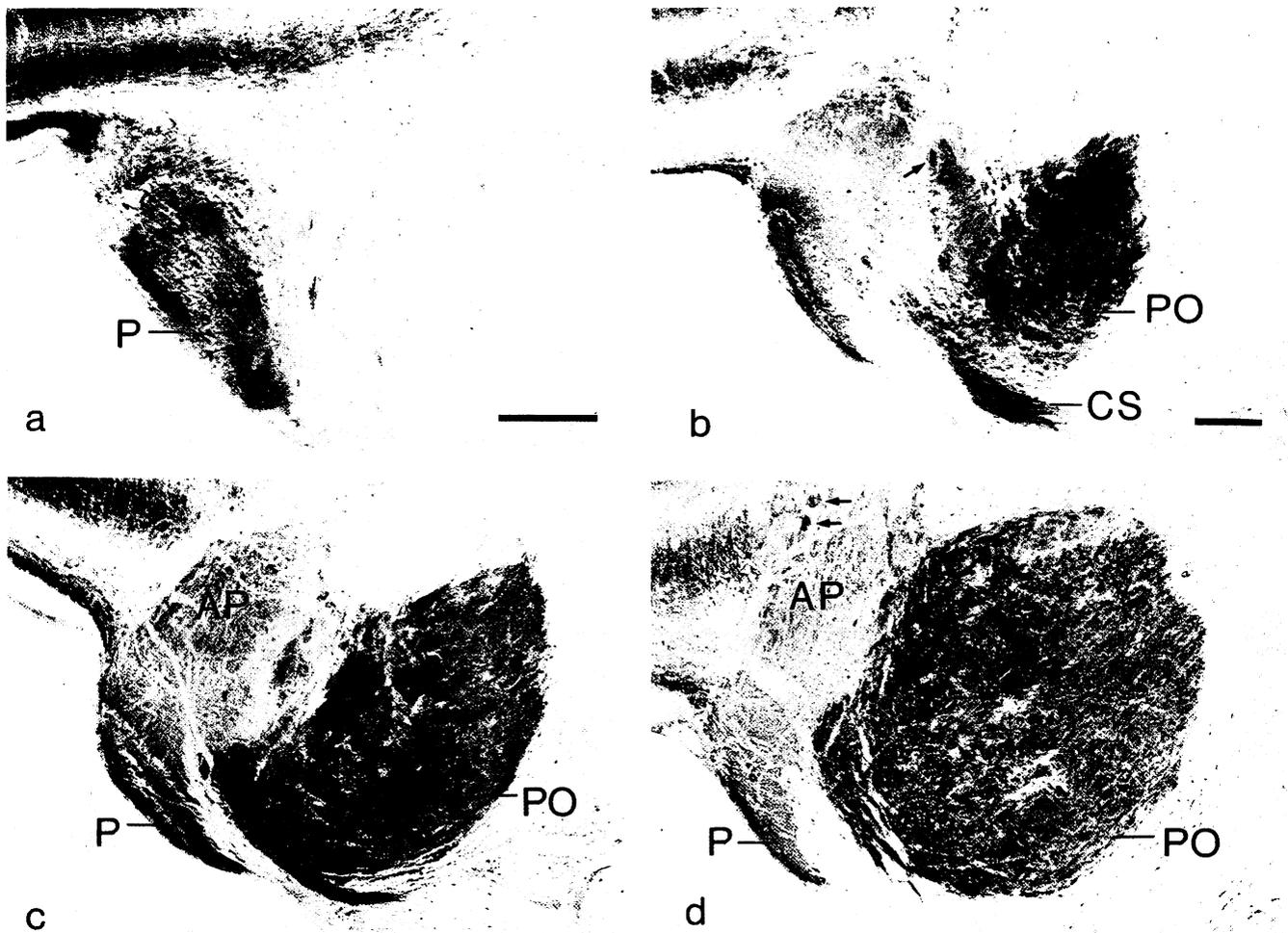


Fig. 9. Photomicrographs of cross-sections through the pretectum of *Osteoglossum* from rostral (**a**) to caudal (**d**) showing the AChE distribution. **b** Arrow points to a neuron in the magnocellular superficial pretectal nucleus. **d** Arrows mark nucleus corticalis neurons. **a, b** Bars = 0.1 mm. **c, d** Same magnification as **b**.

Discussion

The phylogenetic implications of the observed distribution of AChE activity in the diencephalon and mesencephalon of the three species investigated will be discussed first, followed by some functional considerations.

Phylogenetic Considerations

The neuronal circuitry in the three patterns of pretectal organization in teleosts (see fig. 1a-c) will be discussed and reviewed in detail in a companion paper reporting the neuroanatomical connections within the intermediately complex pattern [M.F. Wul-

limann, D.L. Meyer and R.G. Northcutt, in preparation]. For the purpose of the present study, however, it is important to emphasize that the connections of the posterior pretectal nucleus in *Osteoglossum* are identical to those of the nucleus glomerulosus and intermediate superficial pretectal nucleus of percomorphs [Wullimann and Northcutt, 1989]. This observation supports the hypothesis that the anterior and posterior portions of the posterior pretectal nucleus are homologous to the intermediate superficial pretectal nucleus and nucleus glomerulosus of percomorph teleosts, respectively. Visual pathways to the hypothalamus homologous to those observed in the intermediately complex and elaborate patterns of pretectal

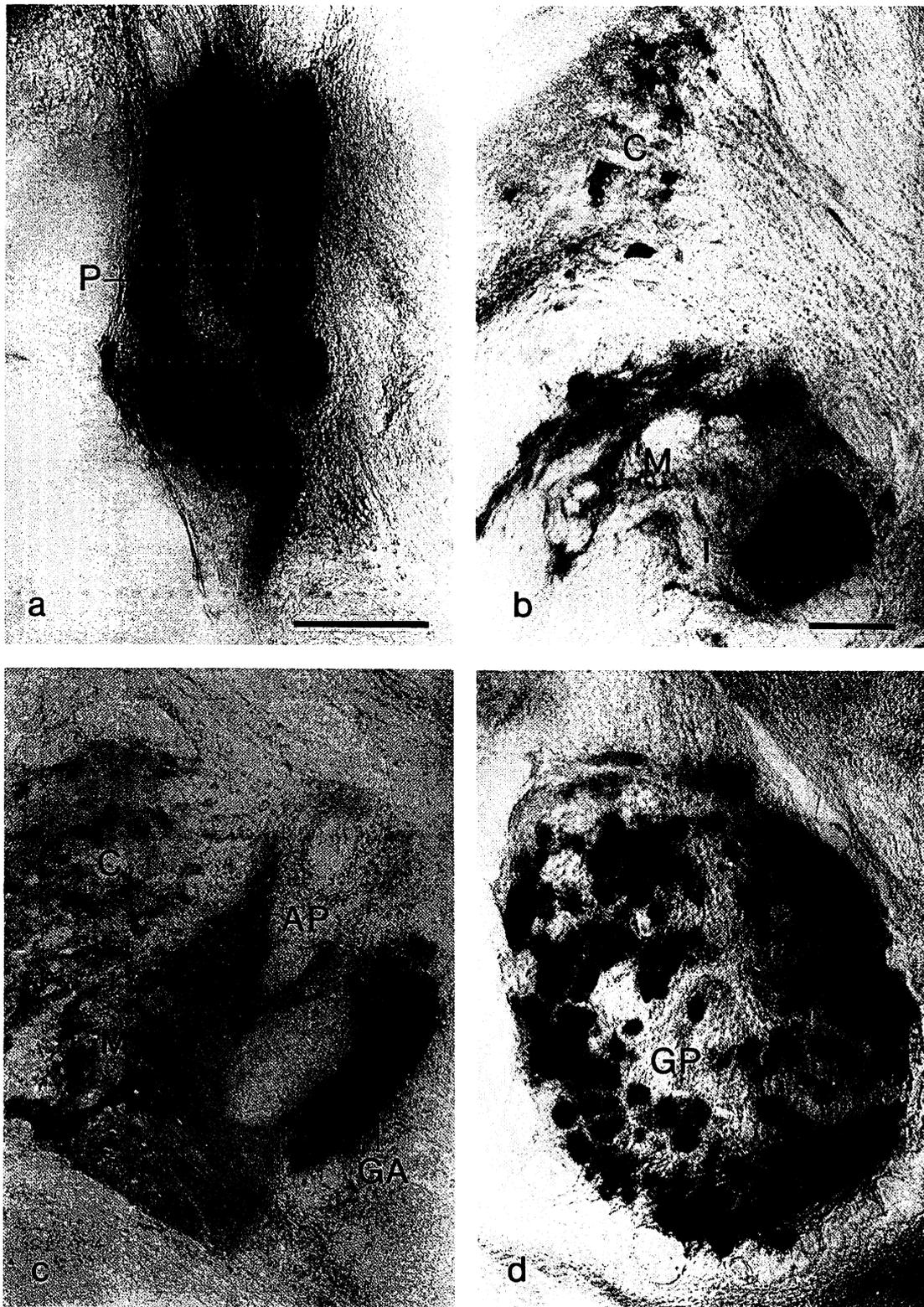


Fig. 10. Photomicrographs of cross-sections through the pretectum of *Hemichromis* from rostral (a) to caudal (d) showing the AChE distribution. a, b Bars = 0.1 mm. c, d Same magnification as b.

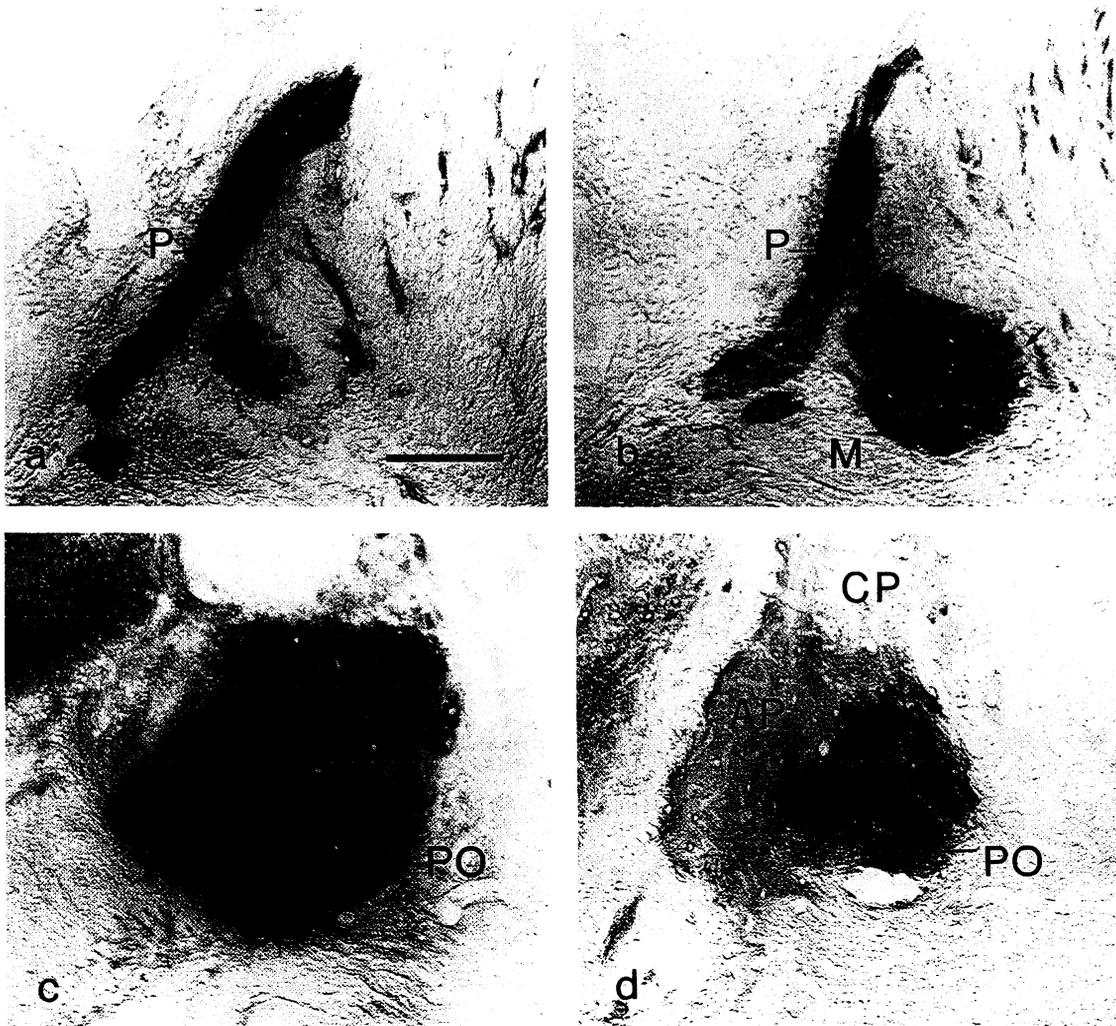


Fig. 11. Photomicrographs of cross-sections through the pretectum of *Carassius* from rostral (**a**) to caudal (**d**) demonstrating the AChE distribution. **a, b** Arrows point to neurons of the magnocellular superficial pretectal nucleus. Bar in **a** equals 0.1 mm and applies to **a-d**.

organization apparently do not exist in the simple pattern; however, a nonhomologous visual pathway (see fig. 1c) leading through the tectorecipient magnocellular superficial pretectal nucleus to the hypothalamus does exist in *Carassius* [Northcutt and Braford, 1984].

The present findings on AChE activity in the diencephalon and mesencephalon of three teleost species support the hypothesis of homology between the three patterns of pretectal organization to an even greater extent. The histochemical data demonstrate that AChE is a highly specific marker for all parts of the teleostean superficial pretectum, nucleus cortica-

lis (when present), and posterior pretectal nucleus or nucleus glomerulosus (fig. 12). In conjunction with the known connective information, the present data suggest several conclusions. (1) The parvocellular and magnocellular superficial pretectal nuclei and the nucleus corticalis (when present) are homologous between the three patterns. (2) The posterior pretectal nucleus in the intermediately complex and simple patterns is homologous to the nucleus glomerulosus and intermediate superficial pretectal nucleus in the elaborate pattern. In *Osteoglossum*, which displays the intermediately complex pattern, AChE-reactive cells were found in the rostralateral portion of the

posterior pretectal nucleus. In *Hemichromis*, which displays the elaborate pattern, such cells were seen in the intermediate superficial pretectum but not in the nucleus glomerulosus. Therefore, AChE histochemistry as well as the neuroanatomical connectivity support the hypothesis that the rostral and caudal portions of the posterior pretectal nucleus in the intermediately complex pattern are homologous to the intermediate superficial pretectum and nucleus glomerulosus, respectively, in the elaborate pattern. (3) There is an additional pretectal nucleus which we did not recognize previously, the accessory pretectal nucleus, which lies dorsomedial to the magnocellular superficial pretectal nucleus. It contains dense AChE activity in its neuropil in all three species and is therefore considered to be homologous between the three patterns.

In summary, AChE histochemistry of the three patterns of pretectal organization and the neuroanatomical connections in the intermediately complex and elaborate patterns reveal a detailed structural similarity. This is a prerequisite for a hypothesis of homology between them. The phylogenetic relationships between the patterns can therefore be considered by examining their systematic distribution (fig. 1d). The latter suggests first that the posterior pretectal nucleus is the ancestral condition for teleosts. It exhibits all connections of the nucleus glomerulosus and intermediate superficial pretectal nucleus of derived percomorph teleosts and, therefore, has probably given rise to both nuclei in percomorphs. The second suggestion is that, in *Carassius*, the posterior pretectal nucleus is reduced to a small nucleus located caudal to the magnocellular superficial pretectal nucleus. Crucial to this interpretation is whether or not the posterior pretectal nucleus of *Carassius* projects to the inferior lobes. In most teleosts it is almost impossible to expose surgically the inferior lobe in order to inject it with a conventional neuronal tracer. With the newly available DiI technique [Godement et al., 1987], however, this experiment can be performed *in vitro*.

Functional Considerations

Although the presence of immunoreactivity to ChAT, the acetylcholine-synthesizing enzyme, is a more reliable indicator for cholinergic pathways, AChE histochemistry remains valuable as a first approach to localize such pathways in the central nervous system [Butcher, 1983; Bradford, 1986]. The

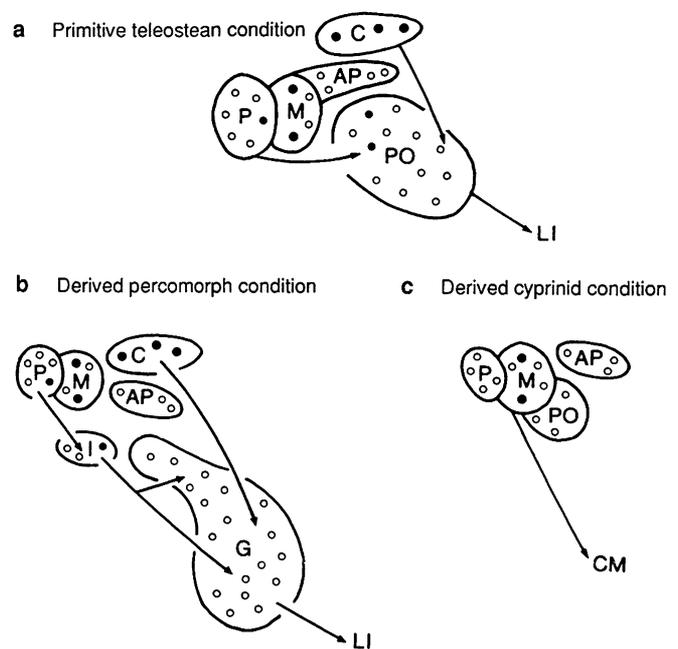


Fig. 12. Diagram summarizing the new information on AChE activity within the three patterns of pretectal organization (a-c). Unlike the maps shown in figures 6 and 7, AChE-reactive fibers in the nucleus corticalis are not indicated here (see Discussion). Filled circles represent cells; open circles represent neuropils.

present data reveal for the first time that some teleosts possess visual pathways to the hypothalamus that can selectively be characterized by AChE histochemistry. These data represent the basis for more specific hypotheses of the involvement of a cholinergic circuitry in the visual pathways to the hypothalamus in teleosts. These hypotheses can be tested further, e.g. by ChAT immunohistochemistry.

The AChE bands and AChE-containing neurons in the optic tectum observed in this study, however, have been reported and corroborated by ChAT immunohistochemistry in teleosts previously [Ekström, 1987; Zottoli et al., 1987]. In these and in the present studies, there is a topological correspondence between three retinal input layers (t1, t2 and t3 of Northcutt [1983]; stratum opticum, stratum fibrosum et griseum superficiale and stratum griseum centrale of von Bartheld and Meyer [1987]) and the discussed AChE bands. It has been argued that retinotectal terminals may be cholinceptive [Zottoli et al., 1987]. Alternatively, it has been proposed that acetylcholine is used

as neurotransmitter in the retinotectal system in teleosts [Contestabile et al., 1980; Oswald and Freeman, 1980] and amphibians [Oswald et al., 1979; Schmidt et al., 1989]. Whatever the case may be, the involvement of acetylcholine as a neurotransmitter of retinal ganglion cells seems to be restricted to teleosts and amphibians, and forms a major difference in the visual systems between these and other vertebrates [Oswald and Freeman, 1980; Wächtler, 1988] such as birds [Sorenson et al., 1989] or mammals [Bigl and Schober, 1977; Fibiger, 1982]. Similarly, the abundant presence of AChE within the visual pathways to the hypothalamus reported in this study may be a unique adaptation in teleosts.

AChE-reactive fibers in the nucleus corticalis lie in the direct continuation of the AChE-reactive band of the central zone of the optic tectum. They may, therefore, not be intrinsic to the nucleus corticalis.

More specific hodological and ChAT immunohistochemical analyses are needed in order to elucidate the cholinergic circuitry within the visual pathways to the hypothalamus in teleosts. However, one subcircuit in the intermediately complex and elaborate patterns should be considered a good candidate for a cholinergic projection: the axons of neurons of the nucleus corticalis in percomorphs project into the nucleus glomerulosus where they form the glomeruli [Sakamoto and Ito, 1982]. In *Osteoglossum*, which displays the intermediately complex pattern, nucleus corticalis neurons project into the posterior pretectal nucleus [Wullimann and Northcutt, 1989]. In this osteoglossomorph species, the nucleus corticalis neurons and the neuropil of the posterior pretectal nucleus contain a high amount of AChE. The same is true for neurons of the nucleus corticalis and the glomeruli of the nucleus glomerulosus in the percomorph *Hemichromis*. Therefore, both neuroanatomy and histochemistry suggest together that at least this portion of the visual pathways leading to the hypothalamus in teleosts may be cholinergic.

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Dr. Mario F. Wullimann
Abteilung Neuroanatomie
Zentrum Anatomie
Georg-August-Universität
Kreuzberggring 36
D-3400 Göttingen (FRG)