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Histochemical, Connectional and Cytoarchitectonic Evidence for a Secondary Reduction of the Pretectum in the European Eel, *Anguilla anguilla*: A Case of Parallel Evolution

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Key Words. Acetylcholinesterase · Horseradish peroxidase tracing · Hypothalamus · Mechanoreception · Phylogeny · Teleost · Visual system

Abstract. There are at least three different patterns of pretectal organization in teleost fishes: a simple pattern observed in cyprinids, an elaborate pattern present in percomorphs, and an intermediately complex pattern seen in many other teleost groups. The taxonomic distribution of the pretectal patterns indicates that the simple and the elaborate patterns are both evolutionarily derived (apomorphic) from the primitive (plesiomorphic) intermediately complex one. In anguillids, the pretectal pattern observed cytoarchitectonically has an anatomical configuration similar to that of the simple pattern in cyprinids. The distribution of acetylcholinesterase positivity in the pretectum (namely acetylcholinesterase positivity in the parvo- and magnocellular superficial and posterior pretectal nuclei, and acetylcholinesterase negativity in the pretectal cell plate and the ovoid preglomerular cell aggregate), as well as the retinal projections (namely retinal terminals in the parvocellular superficial and central pretectal nuclei, and absence of such terminals in the magnocellular superficial and posterior pretectal nuclei and the pretectal cell plate), strongly supports the interpretation suggested by the cytoarchitectonic analysis. As anguillids (elopomorpha) and cyprinids (ostariophysi) are related only distantly, this secondary simplification in the pretectum likely occurred independently, i.e. this simplification represents a case of parallel reduction.

Introduction

Recently, it has been argued that at least three patterns of pretectal organization can be discerned among the different groups of teleost fishes [Wullimann and Meyer, 1990; M.F. Wullimann, D.L. Meyer and R.G. Northcutt, unpubl. observ.]: a simple pattern, seen in some cyprinids, an elaborate pattern, observed in percomorphs, and an intermediately complex pattern, present in most other teleost groups. Both the intermediately complex and the elaborate patterns are characterized by the presence of a large and complexly folded parvocellular superficial pretectal nucleus (SPN) and two pretectal nuclei with the largest cells seen in a teleost pretectum, the magnocellular SPN and nucleus corticalis (fig. 1a, b). In the intermediately complex pattern, a large posterior pretectal nucleus is located caudomedial to the parvocellular SPN and dorsal to the preglomerular complex. In the elaborate pattern, nucleus glomerulosus is in a position comparable to that of the posterior pretectal nucleus in the intermediately complex pattern. In contrast to the posterior pretectal nucleus, however, nucleus glomerulosus displays distinct glomeruli and extends caudally into the preglomerular cell masses. Furthermore, the elaborate pattern has an additional nucleus in the superficial pretectum, the intermediate SPN (fig. 1b).

In contrast to both the intermediately complex pattern and the elaborate pattern, in the simple pretectal pattern seen in cyprinids (fig. 1c), the superficial SPN appears nonfolded in cross-section. The posterior pretectal

List of Abbreviations

A	nucleus anterior thalami
BO	bulbus olfactorius
C	nucleus corticalis
Ce	corpus cerebelli
CP	nucleus pretectalis centralis
DA	nucleus accessorius opticus dorsalis
DLI	nucleus diffusus lobi inferiores
DT	dorsomedial optic tract
E	eminentia granularis
FR	fasciculus retroflexus
G	nucleus glomerulosus
Ha	habenula
HC	caudal periventricular hypothalamus
HD	dorsal periventricular hypothalamus
HH	hypothalamo-hypophysial tract
HV	ventral periventricular hypothalamus
Hy	hypophysis
I	nucleus pretectalis superficialis pars intermedius
LI	lobus inferior
M	nucleus pretectalis superficialis pars magnocellularis
MO	medulla oblongata
NF	nucleus of the medial longitudinal fasciculus
ON	optic nerve
OT	optic tract
P	nucleus pretectalis superficialis pars parvocellularis
PG	preglomerular complex
PLI	nucleus periventricularis lobi inferiores
PO	nucleus pretectalis posterior
PPd	nucleus pretectalis periventricularis pars dorsalis
PPv	nucleus pretectalis periventricularis pars ventralis
PR	pretectal cell plate
PT	nucleus posterior thalami
RT	rostral tegmental nucleus of Grover and Sharma [1981]
SC	nucleus suprachiasmaticus
SO	subcommissural organ
SPN	superficial pretectal nucleus (used in text only)
SV	saccus vasculosus
T	tectum opticum
Tel	telencephalon
TL	torus longitudinalis
TLa	nucleus of the torus lateralis
TPp	periventricular nucleus of the posterior tuberculum
TS	torus semicircularis
V	diencephalic ventricle
VM	nucleus ventromedialis thalami
VT	ventrolateral optic tract

nucleus is small (fig. 1c). Although a magnocellular SPN is present, the intermediate SPN and nucleus corticalis are absent in the simple pattern. Of all the pretectal nuclei mentioned, only the parvocellular SPN and nucleus corticalis are retinofugal targets [Northcutt and Wullimann, 1988; Butler and Saidel, 1991].

Neuropil and/or neurons of all nuclei comprising the three pretectal patterns can be characterized in a highly selective manner by acetylcholinesterase (AChE) histochemistry [Wullimann and Meyer, 1989, 1990; see fig. 1]. Congruence of the histochemical AChE distribution with the cytoarchitectonic delineation of pretectal nuclei, in each of the three patterns supports the hypothesis that the same-named nuclei are homologous among the pretectal patterns. The absence of AChE-positive neurons in a position where nucleus corticalis would be expected reflects the absence of this nucleus in *Carassius*, i.e. in the simple pattern [Wullimann and Meyer, 1990]. The taxonomic distribution of the three patterns indicates that the intermediately complex pattern is ancestral (plesiomorphic) for teleosts, whereas the other two patterns appear to be derived (apomorphic) conditions (fig. 2).

Some nuclei in the intermediately complex pattern and the elaborate pattern have been demonstrated to form homologous visual pathways to the hypothalamus [Sakamoto and Ito, 1982; Murakami et al., 1986; Striedter and Northcutt, 1986, 1989; Wullimann and Northcutt, 1989]. The nuclei involved in these pathways are well developed in these two patterns. However, the homologous nuclei and some of their connections are lost (e.g. nucleus corticalis) or reduced (e.g. posterior pretectal nucleus) in the simple pattern of pretectal organization [Northcutt and Braford, 1984; Striedter and Northcutt, 1989; Wullimann and Northcutt, 1989].

A cytoarchitectonic analysis in the european eel, *Anguilla anguilla*, reveals that the nuclear organization of the pretectal area shares many similarities with that in cyprinids. As *Anguilla* is an elopomorph (fig. 2) and, thus, only distantly related to the ostariophysan cyprinids, these reductions likely occurred independently. However, an alternative interpretation seems possible. A large pretectal cell plate of uncertain identity at the base of the optic tectum may represent the 'nucleus corticalis' that is seen in certain teleost species. Furthermore, the large ovoid portion of the preglomerular cell masses in *Anguilla* may be the 'posterior pretectal nucleus' or 'nucleus glomerulosus' of other species. If this were the case, *Anguilla* would show a mixture of features characteristic of the intermediately complex and elaborate pretectal patterns. In order to test these alternative hypo-

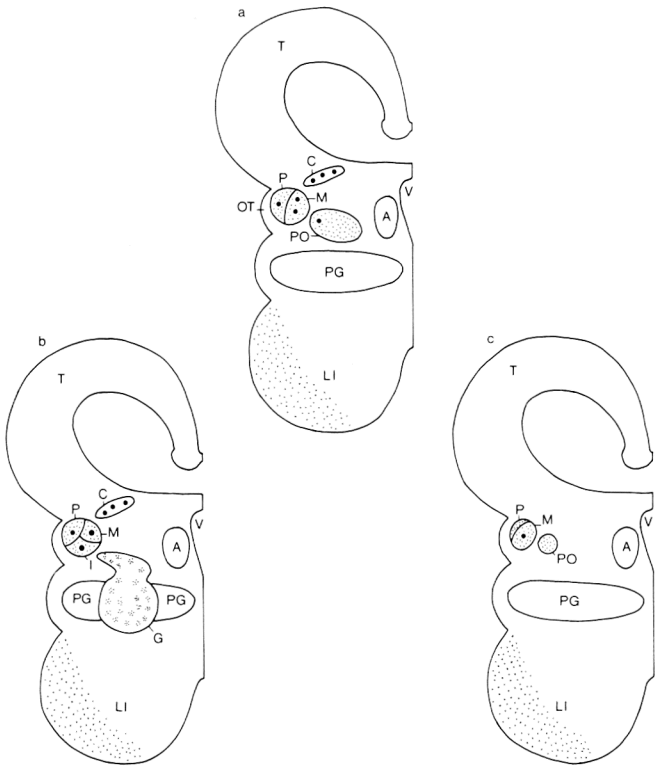


Fig. 1. Schematic drawings of cross-sections through the diencephalon and mesencephalon of a teleost fish showing the general topology of the three recognized pretectal patterns and the acetylcholinesterase distribution in these patterns [after Wullimann and Meyer, 1990]. **a**) intermediately complex pretectal pattern exhibited by most teleost groups; **b**) elaborate pattern observed in percomorphs; **c**) simple pattern present in cyprinids. The taxonomic distribution of the pretectal patterns is shown in figure **2**. Dots indicate AChE-positive cell bodies, stippling AChE-positive neuropil. See list for abbreviations.

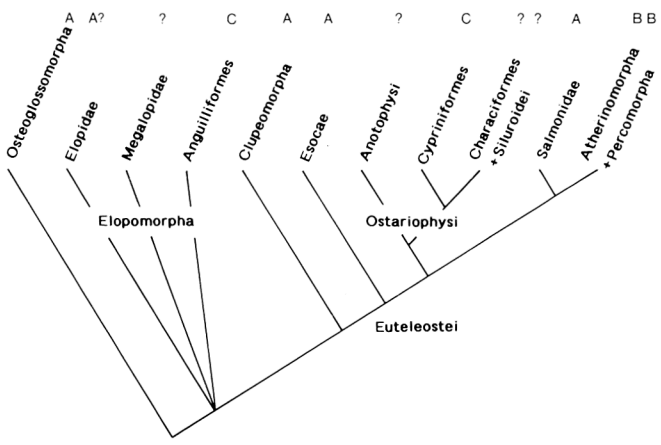


Fig. 2. Cladogram depicting phylogenetic relationships of major teleost groups according to Lauder and Liem [1983]. Distribution of the intermediately complex (A) the elaborate (B), and the simple (C) pretectal patterns is indicated.

theses, we determined (1) the retinal projections, and (2) the acetylcholinesterase (AChE) distribution within the diencephalon and pretectum of *Anguilla*.

The first hypothesis, that of independent reduction, would be supported if retinal projections reach the cell groups designated here as parvocellular SPN, central pretectal, and dorsal accessory optic nuclei, but do not terminate in the nuclei called magnocellular SPN, posterior pretectal nucleus, and the pretectal cell plate (the possible 'nucleus corticalis'). In addition, AChE would be expected in the parvocellular SPN, the magnocellular SPN and the posterior pretectal nucleus but not in the central pretectum, dorsal accessory optic nucleus, the pretectal cell plate ('nucleus corticalis'), or the large ovoid preglomerular nucleus (the possible 'posterior pretectal nucleus'). If the second hypothesis is correct, i.e. that *Anguilla* actually displays a mixture of features characteristic of the intermediately complex and elaborate patterns, then the same retinal projections should exist, with the addition of a retinal projection to the pretectal cell plate ('nucleus corticalis'). Further, the ovoid preglomerular nucleus (the suspected 'posterior pretectal nucleus') and the neurons forming the pretectal cell plate (the suspected 'nucleus corticalis') would be expected to be highly AChE-positive.

The taxonomic nomenclature is according to Lauder and Liem [1983], and the anatomical nomenclature follows Braford and Northcutt [1983] and Northcutt and Wullimann [1988].

Materials and Methods

A total of 12 european eels *(Anguilla anguilla)*, 6.5–49 cm body length were used. All animal research described in this study was approved by the 'Regierungspräsident' of the State of Lower Saxony, FRG.

Horseradish Peroxidase (HRP) Neuronal Tracing

Retinofugal projections were visualized in seven specimens. While the animals were deeply anesthetized with tricaine methanesulfonate (MS 222, Sigma, Deisenhofen, FRG), a 30% HRP solution (Boehringer, Mannheim, FRG), containing 1% dimethylsulfoxide, 1% lysolecithin, and 1% kainic acid, was injected into one eye. After survival times of 3 to 6 days, the eels were again anesthetized in MS 222 and transcardially perfused with cold 0.05 M phosphate buffer (PB; pH: 7.4), containing 0.8% sucrose, 0.4% glucose, and 0.8% NaCl, followed by 2% glutaraldehyde in PB containing 4% sucrose. The brains were removed from the crania and stored in the same fixative overnight. The brains were then cryoprotected in 30% sucrose in PB and cut transversely on a cryostat at 25 µm. The sections were reacted with benzidine dihydrochloride according to the protocol of Ebbesson et al. [1981] and counterstained with neutral red.

Acetylcholinesterase (AChE) Histochemistry. In four specimens the AChE distribution in the diencephalon and mesencephalon was dem-

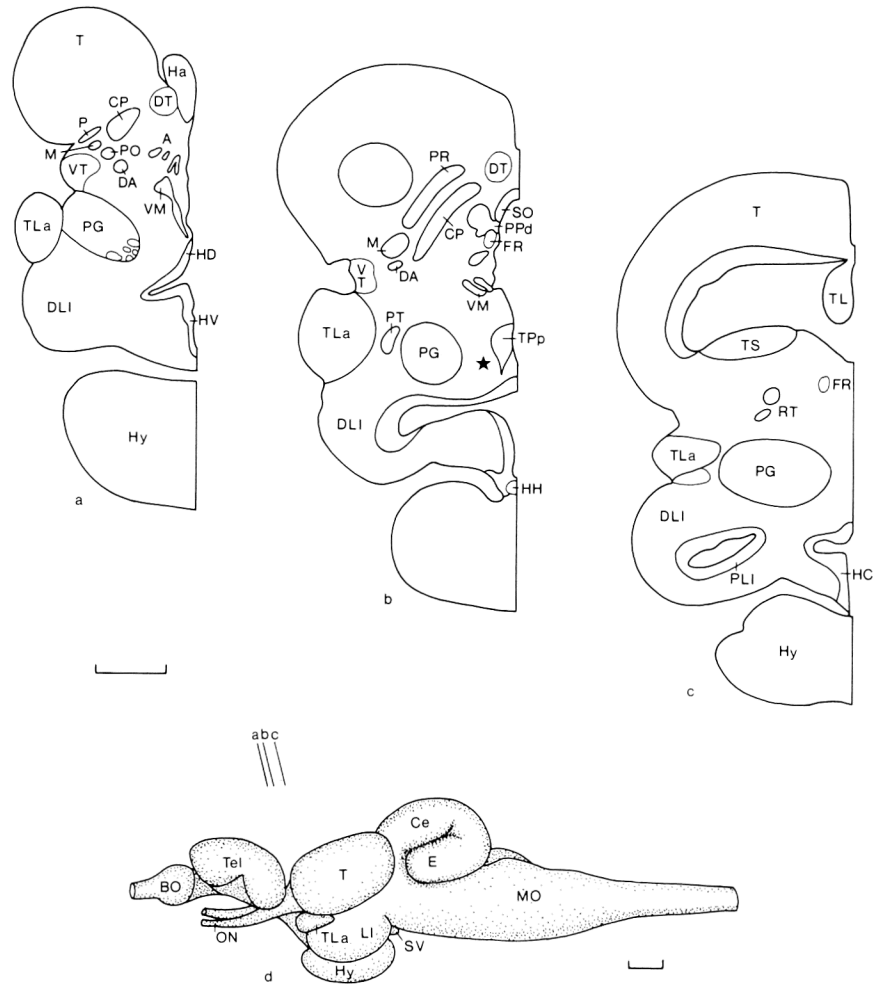


Fig. 3. Drawings of cross-sections through the diencephalon and mesencephalon in *Anguilla* from rostral (**a**) to caudal (**c**) show the nuclear organization. **a**) This level corresponds to that of figure **4a**, which shows the normal anatomy of the superficial pretectal nuclei. **b**) This level corresponds to that of figure **5a**. Asterisk designates position of large AChE-positive neurons, which possibly represent a diencephalic efferent lateral line nucleus (see text). **c**) This level corresponds to that of figure **5c**. **d**) Lateral view of the brain of *Anguilla*. Most cranial nerves have been omitted. Levels of cross-sections shown in (**a**) – (**c**) are indicated. Lateral is to the left. Bar for (**a**) – (**c**) equals 0.5 mm; bar in (**d**) equals 1 mm.

onstrated according to the protocol of Lynch and Killackey [1974]. Promethazine (Sigma) was used to inhibit nonspecific cholinesterases. Replacement of acetylcholine-iodide by S-butyrylthiocholine iodide (both from Sigma) resulted in no staining of the brain tissue at all. A detailed description of histochemical procedures and controls was given in a previous paper [Wullimann and Meyer, 1990].

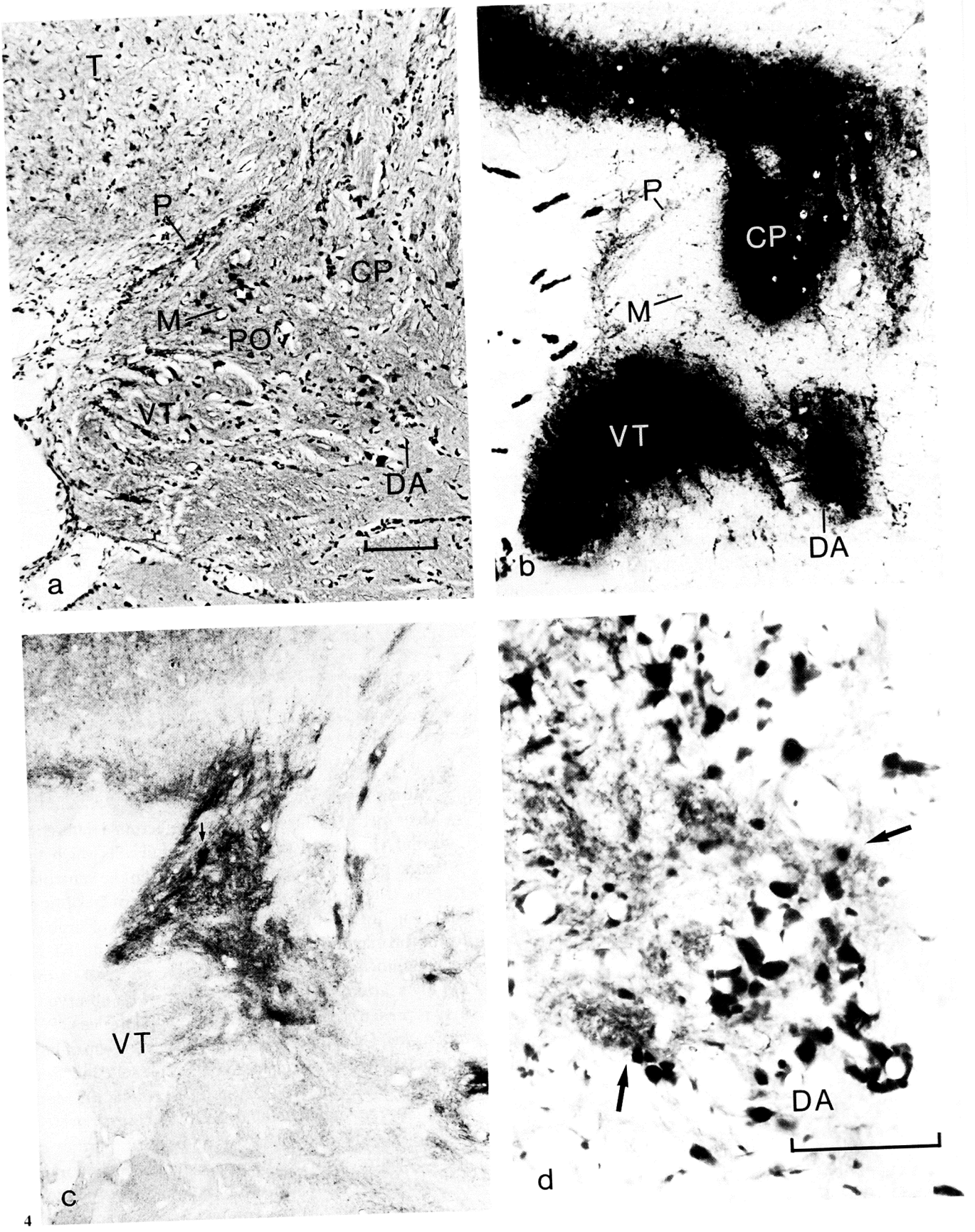
Normal Anatomy. One specimen was perfused in the same way as specimens used for the HRP experiments. However, 4% paraformealdehyde in 0.05 M PB was used as a fixative. The brain was postfixed for six months, then embedded in paraffin and cut transversely at 15 μm. Sections were mounted on albumin-coated slides, silver-stained with protargol (Roques Chimie, Saint-Ouen, France) according to the Bodian method [Romeis, 1989] and counterstained with cresyl violet.

Results

The normal anatomy of the pretectum in *Anguilla* will be described first, followed by descriptions of the retinal projections and the AChE distribution.

Normal Anatomy

Most rostral in the superficial pretectum a thin strip of neuropil, rimmed by two layers of small cells which comprise the parvocellular SPN, lies between the ventrolateral optic tract and the ventrolateral border of the optic tectum (P in fig. 3a, 4a, b). Medial to it, a group of large cells forms the magnocellular SPN (M in fig. 3a, b; 4a, b). Further medioventral, a small nucleus, comprised of more loosely scattered and smaller cells, can be observed. It may represent a small posterior pretectal nucleus (PO in fig. 3a; 4a). Even more medioventral lies a group of larger neurons. They are considered to represent the dorsal accessory optic nucleus (DA in fig. 3a, b; 4a, b). Mediodorsal to the magnocellular SPN, a central pretectal nucleus (CP in fig. 3a, b; 4a, b) can be seen. Further caudal, the central pretectal nucleus expands mediodorsally, and a plate of loosely scattered, large cells emerges dorsal to it (PR in fig. 3b; 5a). This pretectal cell plate may repre-



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sent nucleus corticalis as suggested by Medina et al. [1990].

The preglomerular cell masses extend rostrally to the lateral border of the brain (fig. 3a) and lie underneath the superficial pretectum. Caudally, the preglomerular cell aggregates shift more medially and show an ovoid shape (fig. 3b, c; 5c, d). Even more caudal, the preglomerular complex eventually approaches the ventricle in the posterior tubercular region. Alternatively, the ovoid shaped portion of the preglomerur cell masses may represent a huge posterior pretectal nucleus or a nucleus glomerulosus, as suggested by Ekström [1982].

Retinal Projections

Heavy terminal fields are formed in the parvocellular SPN, central pretectal and dorsal accessory optic nuclei, but not in the magnocellular SPN and the posterior pretectal nucleus (fig. 4b). Additional retinal projections (not depicted) were observed to reach the suprachiasmatic nucleus, the dorsal part of the periventricular pretectum, dorsal and ventral thalamus and four layers of the optic tectum. Within the tectum, there are two layers of retinofugal fibers in the superficial white and gray zones: a thin outer layer and a much thicker inner layer. A third retinorecipient layer is formed by only a few fibers in the central zone of the tectum. Even sparser labeled fibers can be observed immediately peripheral to the periventricular gray zone. A terminal field in a ventral accessory optic nucleus was not observed in retinal preparations. The nucleus was also not seen in normal anatomical preparations.

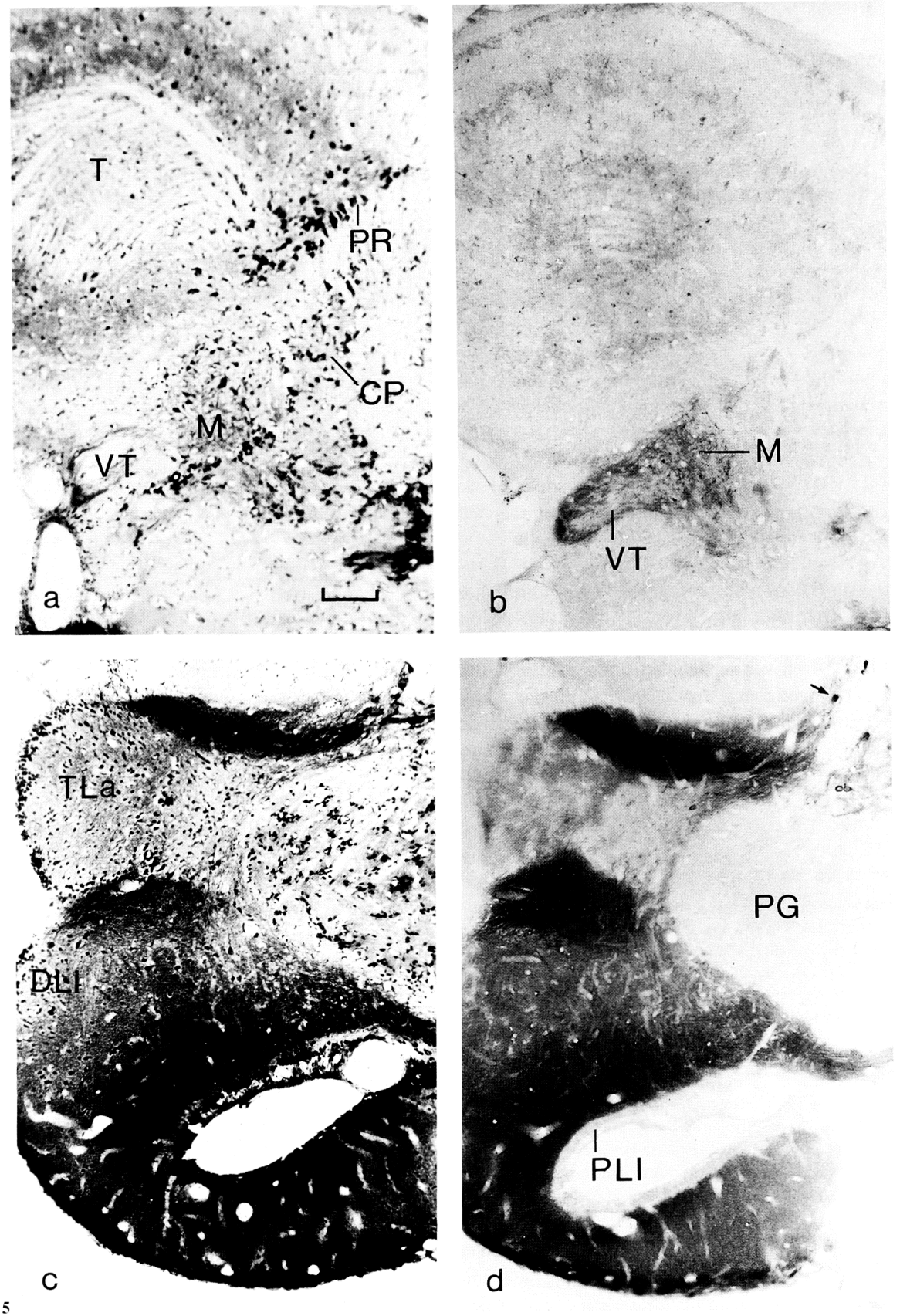
AChE Histochemistry

The AChE positivity was observed in the neuropil of the parvocellular SPN and the magnocellular SPN (in the latter, neurons were also labeled; arrow in fig. 4c), and in the posterior pretectal nucleus (fig. 4c). While some AChE positivity was restricted to the dorsolateral parts of the neuropil of the dorsal accessory optic nucleus (fig. 4d), no such positivity was seen in the central pretectal nucleus and the pretectal cell plate (fig. 5a, b). Even more relevant in the context of this study is that the huge ovoid portion of the preglomerular cell masses was observed to be completely AChE-negative throughout its entire rostrocaudal extent (fig. 5c, d). However, the neuropils of nuclei directly adjacent to it were heavily labeled (fig. 5c, d). These nuclei include the nucleus of the torus lateralis and the diffuse nucleus of the inferior lobe.

Additional AChE positivity in tracts or neuropil was seen in the torus longitudinalis and in the fasciculus retroflexus, as well as in four bands in the optic tectum. Two of these bands are within the superficial white and gray zones, and one is within the central zone. Whereas the bands in the superficial white and gray zones coincide in location and depth with retinorecipient layers seen after HRP injections, the band in the central zone is much thicker than the retinal input layer. The fourth AChE-positive band lies immediately peripheral to the periventricular gray zone. In addition, a tract interconnecting the medial part of the dorsal hypothalamus and the saccus vasculosus was labeled.

Additional AChE-positive diencephalic neurons were present in the preoptic region, including the suprachiasmatic nucleus, in the dorsal periventricular pretectum, in the dorsal and ventral thalamus, and in the periventricular nuclei of the dorsal, ventral, and caudal hypothalamus. However, the lateral and major portion of the periventricular nucleus of the inferior lobe was conspicuously free of label (fig. 5d). A number of giant neurons (asterisk in figure 3b), immediately lateral to the periventricular nuclei of the dorsal hypothalamus and the posterior tuberculum, stain very intensely for AChE. These neurons may represent the diencephalic efferent nucleus of the lateral line system [Puzdrowki, 1989]. Additional AChE-positive neurons in the mesencephalon include some neurons in the periventricular gray zone of the optic tectum, in the medial torus semicircularis, in the nucleus of the medial longitudinal fascicle, in the scattered neurons of the rostral tegmental nucleus (fig. 3c, arrow in fig. 5d), and neurons and neuropil in the mesencephalic reticular formation.

Fig. 4. Four photomicrographs of cross sections through the superficial pretectum at similar levels in *Anguilla*. Compare with figure **3a** for delineation of nuclei. **a**) Normal Bodian stain shows the nuclear organization. **b**) Retinal projections in this area visualized by HRP. Note fine retinofugal terminals in the parvocellular superficial pretectal nucleus and heavier terminal fields in the central pretectum and the dorsal accessory optic nucleus. The magnocellular superficial pretectal nucleus and the posterior pretectal nucleus are devoid of terminals. **c**) AChE histochemistry. Note the positivity in the neuropil of parvocellular and magnocellular superficial pretectal nuclei (in the latter case also in neurons, see arrow) and in the posterior pretectal nucleus. **d**) Higher power photomicrograph of an adjacent AChE-reacted section which is counterstained with cresylviolet in order to demonstrate the histology. Note that the heavy acetylcholinesterase positivity in the posterior pretectal nucleus extends somewhat into the dorsolateral part of the dorsal accessory optic nucleus (arrows indicate boundary between dorsal accessory optic nucleus and posterior pretectal nucleus). Lateral is to the left. Bar equals 0.1 mm in (**a**) and applies to (**a**) – (**c**); bar in (**d**) equals 0.05 mm.



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Discussion

We will first provide some background information on pretectal evolution in teleosts, then discuss the retinal projections and the AChE histochemistry in *Anguilla*, in this context, before offering some conclusions.

Pretectal Evolution

In at least some species of all major teleost groups, the most rostrally located retinorecipient diencephalic target is the parvocellular SPN [see review by Northcutt and Wullimann, 1988]. In the ancestral condition in teleosts, it comprises a complexly pleated band of neuropil, rimmed by two layers of small cells [Northcutt and Wullimann, 1988]. Medial to this nucleus, the tectorecipient magnocellular SPN can be observed in most teleosts [Northcutt and Wullimann, 1988]. Its neurons are large, and it does not receive retinal input. A third obvious pretectal nucleus, the nucleus corticalis, forms a plate of tightly packed, large cells at the base of the tectum in paracanthopterygians, acanthopterygians [Northcutt and Wullimann, 1988], and some osteoglossomorphs, such as the arawana *Osteoglossum bicirrhosum* and the freshwater butterfly fish *Pantodon buchholzi* [Wullimann and Northcutt, 1989; Wullimann and Meyer, 1990; Butler et al., 1991]. Nucleus corticalis has been documented to be retinorecipient in percomorphs and osteoglossomorphs [Northcutt and Wullimann, 1988; Northcutt and Butler, 1991; Butler and Saidel, 1991].

In *Osteoglossum*, both the parvocellular SPN and nucleus corticalis project to the posterior pretectal nucleus, which, in turn, projects to the inferior lobe of the hypothalamus [Wullimann and Northcutt, 1989]. In percomorph teleosts, a similar connectivity pattern can be observed [Sakamoto and Ito, 1982; Murakami et al., 1986; Striedter and Northcutt, 1986; 1989; Wullimann and Northcutt, 1986], except that an intermediate SPN exists as an additional relay center between the parvocellular SPN and the posterior pretectal nucleus. The latter is called nucleus glomerulosus in percomorphs due to its characteristic histological appearance. The pretectal nuclei involved in these pathways in percomorphs have been referred to as the elaborate pretectal pattern, and the pretectal organization in *Osteoglossum* has been referred to as the intermediately complex pattern [Wullimann and Meyer, 1990]. The latter has been argued to be primitive for teleosts.

In contrast, in the cyprinid *Carassius auratus*, the parvocellular SPN is represented merely by a thin strip of tissue which, in cross-section, appears nonfolded [Northcutt and Wullimann, 1988]. Springer and Mednick [1985a] reconstructed the three-dimensional extent of the parvocellular SPN in *Carassius* and demonstrated that this nucleus shows a very simple folding in the goldfish, compared to the complex folding of the parvocellular SPN in percomorphs. Nevertheless, the parvocellular SPN in cyprinids exhibits the histological appeareance and retinal input typical of the ancestral condition. The magnocellular SPN in *Carassius* is well developed and lies medial to the parvocellular SPN.

In some studies of visual projections in *Carassius*, a retinofugal target, innervated via the ventrolateral optic tract, has been identified as 'nucleus corticalis' [Springer and Gaffney, 1981; Fraley and Sharma, 1984; Springer and Mednick, 1985b]. However, it was pointed out in the first such report that 'nucleus corticalis ... appears to be a caudal extension of area opticus pretectalis ventralis' [Springer and Gaffney, 1981: page 409]. These statements, as well as the pictorial descriptions of the suspected 'nucleus corticalis' in *Carassius*, suggest that this nucleus may correspond to the caudal portion of the dorsal accessory optic nucleus [Northcutt and Wullimann, 1988]. The dorsal accessory optic nucleus in *Carassius* and other teleosts not only consistently receives retinal input via the ventrolateral optic tract, but in addition projects to the corpus cerebelli [Wullimann and Northcutt, 1988]. In contrast, nucleus corticalis of percomorphs receives retinal input via the dorsomedial optic tract and, definitely, does not project to the cerebellum [Wullimann and Northcutt, 1988]. This hodological evidence renders it very unlikely that the suspected 'nucleus corticalis' in *Carassius* is homologous to nucleus corticalis as recognized in percomorphs. Our own observations, as well as those in other studies [Braford and Northcutt, 1983; Northcutt and Wullimann, 1988; Wullimann and Meyer,

Fig. 5. Photomicrographs of cross sections through mesencephalon (**a**, **b**) and hypothalamus (**c**, **d**) of *Anguilla*. Compare to figure **3b** and **3c**, respectively, for delineation of nuclei. **a**), **b**) AChE-positivity in the caudal superficial pretectum shown in two adjacent sections. (**a**) is counterstained with cresylviolet. Note that cells forming the pretectal cell plate are not labeled. **c**), **d**) AChE-positivity pattern in the hypothalamus, shown in two adjacent sections. (**c**) is counterstained with cresylviolet to show nuclear organization. Note labeled cells (arrow in **d**) in the rostral tegmental nucleus of Grover and Sharma [1981] dorsal to the preglomerular complex. The latter is entirely free of label. The heavy positivity in two portions of the diffuse nucleus of the inferior lobe and in a strip of neuropil within the dorsal aspect of the nucleus of the lateral torus contrasts with the lighter label in the rest of the latter nucleus. The periventricular nucleus of the inferior lobe is free of label. Lateral is to the left. Bar in (**a**) equals 0.1 mm and applies to (**a**) – (**d**).

1990; Butler et al., 1991] of the cytoarchitecture, retinal projections and AChE distribution in the pretectum of *Carassius* indicate that nucleus corticalis is absent in this species.

The pretectal condition just described for *Carassius* has been argued to be secondarily reduced [Northcutt and Wullimann, 1988] and has been referred to as the simple pretectal pattern [Wullimann and Meyer, 1990].

As outlined in the Introduction, the cytoarchitecture of the pretectum in *Anguilla* is compatible with either of two hypotheses of its organization and evolutionary history. One hypothesis assumes that – as in *Carassius* – the parvocellular SPN and the posterior pretectal nucleus are reduced, while the magnocellular SPN is not. It furthermore assumes that nucleus corticalis has been lost. If this were the case, *Anguilla* would display the simple pattern of pretectal organization.

An alternative hypothesis is that the large, ovoid shaped preglomerular cell aggregate of *Anguilla* corresponds to the posterior pretectal nucleus/nucleus glomerulosus of other species, and that nucleus corticalis is represented by the large plate of loosely scattered cells at the base of the optic tectum. If this were the case, *Anguilla* would exhibit a mixture of features characteristic of the intermediately complex or the elaborate pretectal patterns. Previous studies of different patterns of retinal projections [see Northcutt and Wullimann, 1988], AChE histochemistry [Wullimann and Meyer, 1989, 1990] and inferior lobe connections [Sakamoto and Ito, 1982; Murakami et al., 1986, Striedter and Northcutt, 1989; Wullimann and Northcutt, 1989] in other teleosts provide the basis for tests of these hypotheses. The results of the present study support the first hypothesis and suggest that the posterior pretectal nucleus is minute and that the pretectal cell plate observed in *Anguilla* does not represent nucleus corticalis.

Retinal Projections

Ekström [1982] described the retinal projections in *Anguilla* based on the cobalt technique. While we largely agree with the projection pattern reported, his largely numerical terminology for the pretectum (pretectal nucleus 1–4) reveals that comparative terms could not be applied at that time. As additional criteria for recognizing pretectal nuclei are now available, we interpret the pretectum as follows and give Ekström's [1982] designations in parentheses. In our material, contralateral retinofugal projections are found to reach the suprachiasmatic nucleus (nucleus opticus hypothalamicus), the dorsal periventricular pretectum (area pretectalis), parvocellular SPN (nucleus geniculatus lateralis), central pretectal nucleus (prectectal nucleus 1), dorsal accessory optic nucleus (pretectal nucleus 3), and the dorsal and ventral thalamus (nucleus dorsolateralis thalami). The additional retinal input to the preoptic region that Ekström [1982] reported, which we did not find, was likely due to the higher sensitivity of cobalt in tracing fine projections. A small fascicle of the optic tract was also seen to course within the dorsal marginal layer of the nucleus of the torus lateralis for a short distance in our material. As the fascicle clearly does not form terminals there, and as it eventually rejoins the ventrolateral optic tract, we conclude that the nucleus of the torus lateralis is not a retinofugal target.

In conclusion, the pattern of retinofugal terminal fields in the pretectum of *Anguilla* reveals that the small and, in cross-section, nonpleated parvocellular SPN is retinorecipient, and that both the magnocellular SPN and the pretectal cell plate are not retinorecipient. This retinofugal pattern clearly supports the hypothesis of a reduced pretectum.

In the moray eel, *Gymnothorax funebris*, the parvocellular SPN has even been reported to be entirely absent in a silver degeneration study [Ebbesson, 1968]. Additional studies on retinofugal projections in muraenids using HRP confirm this contention [M.H. Hofmann and D.L. Meyer, unpubl. observ.]. These findings indicate that, within anguilliforms, muraenids reduced parts of the pretectum even more than anguillids.

AChE Histochemistry

The restricted AChE distribution within the dorsolateral portion of the dorsal accessory optic nucleus in *Anguilla* was not observed in three other teleost species [Wullimann and Meyer, 1990]. However, its identity as the dorsal accessory optic nucleus is considered unquestioned since, in contrast to the posterior pretectal nucleus, it receives heavy retinal input via the ventrolateral optic tract (fig. 4b). As in other teleost species [Wullimann and Meyer, 1990], the neuropils of the parvocellular SPN, magnocellular SPN and the small posterior pretectal nucleus are highly AChE-positive in *Anguilla*. Neurons in the magnocellular SPN were also labeled. Furthermore, in *Anguilla* (present study), in the cichlid *Hemichromis* [Wullimann and Meyer, 1990], and in the cyprinid *Carassius* [Wullimann and Meyer, 1990] the nucleus of the torus lateralis and, at least, portions of the diffuse nucleus of the inferior lobe were labeled. However, there was no AChE positivity in the large pretectal neurons forming the cell plate at the base of the optic tectum in

Anguilla (fig. 5a, b), nor was there any positivity in the neuropil of the ovoid preglomerular cell aggregate (fig. 5c, d). In contrast, strong AChE positivity has been observed in neurons of nucleus corticalis and in the neuropil of the posterior pretectal nucleus/nucleus glomerulosus in other teleost species [Wullimann and Meyer, 1990]. These data render it unlikely that the pretectal cell plate and the ovoid preglomerular nucleus represent a nucleus corticalis and a posterior pretectal nucleus, respectively. Rather, the AChE distribution clearly supports the hypothesis of a reduced pretectal pattern in *Anguilla*.

The pretectal cell plate in *Anguilla* more likely represents a unique specialization (autapomorphy) of some anguillids. The huge ovoid portion of the preglomerular cell masses is superficially similar in appearance to the nucleus glomerulosus of percomorphs (or the posterior pretectal nucleus) and has been interpreted as such [Ekström, 1982]. We have argued [Northcutt and Wullimann, 1988; Wullimann and Meyer, 1990] that a glomerular nucleus exists only in paracanthopterygians and acanthopterygians and that a histologically simpler posterior pretectal nucleus is its homologue in more ancestral teleosts [Wullimann and Meyer, 1990; Wullimann and Northcutt, 1989]. The posterior pretectal and glomerular nuclei are both strongly positive for AChE. The large 'glomerular nucleus' in *Anguilla* is not. It is therefore unlikely that this nucleus is even a posterior pretectal nucleus. A small AChE-positive posterior pretectal nucleus, however, was found more dorsally in *Anguilla* in the present study. The ovoid preglomerular nucleus, therefore, most likely represents a hypertrophied lateral preglomerular nucleus, which is related to ascending mechanosensory information in other ray-finned fishes [McCormick, 1989]. The lateral preglomerular nucleus is also free of AChE positivity in other teleosts [Wullimann and Meyer, 1990]. Moreover, the primary and secondary mechanoreceptive centers in the brain stem (medial octavolateralis nucleus, eminentia granularis; Alnaes, 1973a, b; Meredith et al., 1987] and mesencephalon [lateral torus semicircularis, M.F. Wullimann, pers. observ.], respectively, are also well developed, which indicates a hypertrophy of this sensory system in *Anguilla*. This interpretation of the large ovoid preglomerular nucleus must still be confirmed, as the ascending mechanosensory pathways have been described only as far as the torus semicircularis in *Anguilla* [Meredith and Roberts, 1986].

Alternatively, the ovoid preglomerular cell aggregate may be a tertiary gustatory nucleus, which is similarly enlarged in *Carassius* [Northcutt and Wullimann, 1988; Wullimann, 1988]. If so, the hypertrophy of the tertiary gustatory nucleus in both *Anguilla* and *Carassius* would represent a case of parallelism. If the ovoid preglomerular nucleus in *Anguilla* turns out to be the mechanosensory preglomerular center, its hypertrophy is convergent with the large tertiary gustatory preglomerular nucleus in *Carassius*.

Conclusions

Many characteristics of pretectal organization in *Anguilla* resemble the simple pretectal pattern described in *Carassius* [Wullimann and Meyer, 1990]: 1) the parvocellular SPN is represented by a thin nucleus appearing nonfolded in cross-section and containing a central neuropil which is retinorecipient, AChE-positive, and is sandwiched by two layers of small neurons. 2) Medial to the parvocellular SPN, there is a magnocellular SPN which is not retinorecipient but is AChE-positive. 3) Medioventral to the magnocellular SPN, a minute AChE-positive posterior pretectal nucleus can be found. 4) Although, unlike in *Carassius*, there are loosely scattered large neurons at the base of the tectum in *Anguilla*, these neurons are not AChE-positive and retinorecipient. Therefore, *Anguilla* probably lacks a nucleus corticalis, as does *Carassius*. (5) *Carassius* retains only a minute ventral accessory optic nucleus, which has been argued to be ancestrally present in ray-finned fishes [Northcutt and Wullimann, 1988; Wullimann and Northcutt, 1988]. Similarly, in *Anguilla* this nucleus can not be observed in either retinal preparations or in normal material. However, we did not look at specimens in the migratory silver-eel stage. There is evidence that eels in that stage develop a ventral accessory optic nucleus (nucleus opticus accessorius of Medina et al., 1990].

These features comprising the simple pattern of pretectal organization have so far been described only in some ostariophysan cyprinids [Ekström, 1987; Northcutt and Wullimann, 1988; Wullimann and Meyer, 1990], in addition to the elopomorph *Anguilla* in the present study. In *Elops saurus*, another elopomorph, a small nucleus corticalis may be present [Butler et al., 1991], and a pleated parvocellular SPN [Northcutt and Wullimann, 1988] and a posterior pretectal nucleus which is slightly larger compared to the one found in *Anguilla*, can be observed [Butler et al., 1991]. The pretectum of *Elops*, therefore, exhibits a mixture of features characteristic of the (derived) simple or the (ancestral) intermediately complex pretectal patterns, and, therefore, appears to be less reduced than that of *Anguilla*. Moreover, the interme-

diately complex pattern (pleated parvocellular SPN, large posterior pretectal nucleus and presence of nucleus corticalis) can be observed in most osteoglossomorphs and in many other teleosts [Wullimann and Meyer, 1990; Butler et al., 1991] and has been argued to be plesiomorphic for teleosts [Wullimann and Meyer, 1990]. This taxonomic distribution of pretectal patterns strongly suggests that anguillids underwent a reduction of homologous nuclei parallel to that in cyprinids.

Unfortunately, the efferent projections of the small posterior pretectal nucleus are not known in either *Carassius* or in *Anguilla*. It is therefore unclear whether or not the posterior pretectal nucleus looses its heavy projection to the inferior lobe, together with its morphological reduction, in one or both species. Preliminary results from injections of the fluorescent carbocyanine neuronal tracer DiI into the inferior lobe of *Anguilla* in our laboratory showed no retrogradely labeled cells in the ovoid preglomerular cell aggregate, although labeled fiber bundles traverse it. This is consistent with our interpretation that this large nucleus is neither the posterior pretectal nucleus nor nucleus glomerulosus as present in other teleosts. However, we did not positively recognize labeled neurons in the small posterior pretectal nucleus of *Anguilla*.

An independent reductive process leading to morphologically similar simplifications of the pretectum in *Anguilla* and *Carassius* could be due to similar environmental pressures or conditions. The otherwise widely divergent general morphology (including that of the brain) and life habits of these two teleosts suggests that an alternative, non-adaptationist explanation [Northcutt, 1988] is more likely: structural or genetic constraints may channel reductive processes in limiting ways, allowing some, but not other, morphological changes.

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