ACETYLCHOLINESTERASE histochemistry suggests that
the pretectal nucleus corticalis of teleost fish may be cholin-
ergic. Because immunohistochemical analysis with anti-
bodies against choline acetyltransferase provides a more
reliable means of recognizing cholinergic central nervous
structures, we investigated transverse brain sections of
the African cichlid fish, *Hemichromis lifalili* and *Hem-
ichromis gattatus*, with standard immunohistochemical
techniques and found the nucleus corticalis to contain
choline acetyltransferase. This supports the hypothesis
that teleosts (unlike all other known vertebrates) have a
cholinergic second-order sensory (i.e. visual) circuit.

**Key words**: Acetylcholine; Choline acetyltransferase; Choli-
ergic system; Immunohistochemistry; Nucleus corticalis;
Pretectum; Teleost fish; Visual system

**Introduction**

The cholinergic system is best understood in mam-
imals, where it comprises three major innervation sub-
systems: The basal forebrain system, the mesopontine
tegmental system and the classical motor system of the
spinal cord and brain stem. In addition, intrinsic
cholinergic neurons exist in the cerebral cortex and
parts of the striatum. Some minor neuronal popula-
tions are likewise cholinergic in mammals (e.g. in the
habenula or the parabigeminal nucleus).

Although the mammalian pretectum has been reported to receive a cholinergic innervation from the mesopontine tegmental system, cholinergic neurons have never been reported in the pretectum of any mammal. In teleost fish, however, acetylcholineste-
erase (AChE) histochemistry suggests that the pretectal
nucleus corticalis may be cholinergic. In the present
study, we investigated this hypothesis with antibodies
against the final acetylcholine synthesizing enzyme,
choline acetyltransferase (ChAT), because AChE
alone is not a reliable marker for cholinergic structures.

**Materials and Methods**

Specimens of the African cichlid teleosts, *Hemi-
chromis lifalili* and *Hemichromis gattatus*, were deeply
anesthetized with tricaine (methanesulphonate, Sigma)
before they were transcardially perfused with cold
0.1 M phosphate buffer (PB; pH 7.4), followed by
4% paraformaldehyde in PB. The brains were taken out
of the crania and postfixed in the same fixative
plus 30% sucrose for 60–75 min before they were
cyroprotected overnight in PB containing 30%
sucrose. The brains were then cut on a cryostat at
20 μm and mounted on slides covered with poly-L-lys
(MW 70 000–150 000, Sigma). The immunohistochemi-
ical staining protocol included the following steps
which, except in between steps 2/3 and 3/4, alternated

with washing steps in PB: (1) 1% peroxide and 0.2%
Triton-X-100 (T) in PB; (2) 3% bovine albumine
(Sigma) and 0.2% T in PB; (3) blocking with normal
goose serum; (4) polyclonal primary rabbit anti-chicken
ChAT antibody (courtesy of Dr Miles Epstein, Uni-
versity of Wisconsin) at dilutions ranging from 1:500
1:2000 overnight at 4°C; (5) biotinylated secondary
goat and anti-rabbit antibody; (6) avidin-biotin-com-
plex; (7) 0.025% diaminobenzidine (Sigma) in 200 ml
PB containing 4 ml 1% nickel-sulfate, 4 ml 1% cobalt-
chloride and 2 ml dimethylsulfoxide (Merck). Then
100–200 μl of 30% peroxide was added to this solution.
Steps 3, 5 and 6 were carried out using the com-
mercially available ABC staining kit (VECTOR
Laboratories, Burlingame, CA). Controls consisted of
alternative sections prepared identically, but omitting
the primary antibody. The present ChAT antibody
has been characterized previously and used success-
fully in a variety of vertebrate species, including
mammals, birds and teleosts.

A silver-stained cross section series of the brain of
*Hemichromis lifalili* prepared according to the Bodian
method was available for cytostarchitectonic analysis.

**Results**

In our experiments, many well-known cholinergic
nuclei in the brain of *Hemichromis* (Fig. 1) were visual-
ized with the ChAT antibody. These areas included
the motor nuclei of cranial nerves (e.g. oculomotor,
trochlear, abducens and trigeminal nerves), efferent
neurons of the lateral-line system located in the brain-
stem, neurons of the nucleus isthmi, and neurons in the
periventricular gray zone of the optic tectum. Instead
of giving a full account on these cholinergic systems,
we highlight here the unusual finding of ChAT
immunoreactive neurons in the nucleus corticalis of
*Hemichromis*.

The morphology and phylogenetic distribution of

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the prominent, histologically well characterized nucleus corticalis in teleosts has recently been documented. The nucleus forms a cell plate, composed of very large and tightly packed neurons, which lies in the ventral continuation of the central zone of the optic tectum (Figs 2 and 3). The somewhat even larger and more loosely arranged neurons of the magnocellular superficial pretectal nucleus are located ventrolateral to the nucleus corticalis (Figs 2 and 3). Neurons of both nuclei display acetylcholinesterase histochemical staining in Hemichromis. Similarly, some neurons of the magnocellular superficial pretectal nucleus and most neurons of the nucleus corticalis showed immunoreactivity for antibodies against ChAT (Fig.


FIG. 3. Photomicrograph of a silver-stained cross section through the pretectum of Hemichromis showing the morphology of the nucleus corticalis (C) and the magnocellular superficial pretectal nucleus (M). Level is slightly caudal to that of Fig. 2a. Scale bar = 0.1 mm.

4). Somewhat surprisingly, immunoreactivity could not be observed in neurons of the parvocellular superficial pretectal nucleus, although at least some of these neurons were shown to be AChE-active.

Discussion

More than a decade ago, a debate emerged whether or not amphibians and bony fish use acetylcholine, at least partially, in primary visual projections to the optic tectum (superior colliculus). Bands of AChE
activity and ChAT immunoreactivity are co-localized with laminae of retinal terminals in these vertebrates. However, more recent evidence suggests that these retinotectal terminals are cholinceptive rather than cholinergic. First, a probably exclusive external source of such cholinergic input to these terminals can be localized in nucleus isthmi (parabigeminal nucleus of mammals). Secondly, acetylcholine receptors have been demonstrated to be synthesized in ganglion cells of the eye and to be transported to their site of action in the goldfish optic tectum, indicating that these acetylcholine receptors are presynaptic rather than postsynaptic at the retinotectal synapses.

Recently, the morphologically elaborate prepectum of predominantly visual percomorph teleost fish, such as the African cichlid *Hemichromis*, has been demonstrated to contain strong AChE-activity, e.g. in neurons on the parvo- and magnocellular superficial prepectal nuclei and of the nucleus corticalis. While the magnocellular superficial prepectal nucleus is not a retinofugal target, the parvo-cellular superficial prepectal nucleus and the nucleus corticalis of teleosts are. Furthermore, the nucleus corticalis is known to project to the conspicuous nucleus glomerulosus, where axonal arborizations of the nucleus corticalis neurons participate in forming glomeruli. Within the nucleus glomerulosus, no other neural elements than the glomeruli show intense AChE-activity. Neurons of the nucleus glomerulosus project, in turn, to the inferior lobe of the hypothalamus. These hodological and AChE-histochemical data indicate that cholinergic circuitry may be involved in this multisynaptic retinopretecto-hypothalamic pathway. However, AChE, the acetylcholine degrading enzyme, can occur in brain structures which are not cholinergic. Thus, a conclusion with respect to cholinergic circuitry in the prepectum could not be made in the previous study.

As reported above, neurons on the prepectal nucleus corticalis in *Hemichromis* showed strong ChAT-immunoreactivity. Also, whereas some neurons of the magnocellular superficial prepectal nucleus were immunoreactive, the presence of such reactivity in neurons of the parvo-cellular superficial prepectal nucleus remains doubtful in our preparations. This indicates that only the (immunoreactive and histochemically stained) neurons of the magnocellular superficial prepectal nucleus may be cholinergic, and that the solely histochemically stained parvo-cellular superficial prepectal neurons are cholinceptive.

Because it is established that (1) neurons of the nucleus corticalis project into the glomeruli of the nucleus glomerulosus, and that (2) AChE is present in neurons of the nucleus corticalis and the glomeruli of nucleus glomerulosus, the herein reported presence of the final acetylcholine-synthesizing enzyme ChAT in neurons of the nucleus corticalis strongly supports the hypothesis that this segment of the outlined retinopretecto-hypothalamic visual pathway is cholinergic.

However, the unlikely possibility that the neuronal somata and the terminal arborizations of the neurons of nucleus corticalis are both cholinceptive needs further investigation.

As discussed earlier, primary retinal projections do not appear to be cholinergic in fish, whereas secondary visual projections originating in the nucleus corticalis of teleosts, likely are. If confirmed, it represents, to our knowledge, the first example of a second-order cholinergic sensory (i.e. visual) projection in any vertebrate. Percomorph teleosts, such as African cichlids, display a most complex, visually guided behavior. Intra-cellular recordings show that neurons of the nucleus corticalis respond best to small spots moving on a contrasting background and the nucleus has been interpreted to be involved in predation. Depending on its further confirmation as being (at least partially) cholinergic, the projection from nucleus corticalis to nucleus glomerulosus may represent a model to study the involvement of acetylcholine in brain functions (such as object recognition) much closer to the sensory periphery (i.e. more accessible) than in any other known vertebrate cholinergic system.

**Conclusion**

The present ChAT immunohistochemical findings corroborate previous AChE histochemical results and support the hypothesis that most neurons of the nucleus corticalis of teleosts are cholinergic.

**References**


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