neuroReport

NeuroReport 3, 33–35 (1992)

Is the nucleus corticalis of teleosts a new cholinergic central nervous system for vertebrates?

Mario F. Wullimann[CA] and Gerhard Roth

Brain Research Institute, University of Bremen, P.O. Box 330440, W-2800 Bremen 33, Germany

[CA] Corresponding Author

ACETYLCHOLINESTERASE histochemistry suggests that the pretectal nucleus corticalis of teleost fish may be cholinergic. Because immunohistochemical analysis with antibodies 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 *Hemichromis guttatus*, 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; Cholinergic system; Immunohistochemistry; Nucleus corticalis; Pretectum; Teleost fish; Visual system

Introduction

The cholinergic system is best understood in mammals, where it comprises three major innervation subsystems: The basal forebrain system, the mesopontine tegmental system and the classical motor system of the spinal cord and brain stem.[1-3] In addition, intrinsic cholinergic neurons exist in the cerebral cortex and parts of the striatum. Some minor neuronal populations are likewise cholinergic in mammals[1] (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,[4,5] cholinergic neurons have never been reported in the pretectum of any mammal.[1,5] In teleost fish, however, acetylcholinesterase (AChE) histochemistry suggests that the pretectal nucleus corticalis may be cholinergic.[6] 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, *Hemichromis lifalili* and *Hemichromis guttatus*, were deeply anesthetized with tricaine (methanesulfonate, 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 cryoprotected 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-lysin (MW 70 000–150 000, Sigma). The immunohistochemical 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 goat serum; (4) polyclonal primary rabbit anti-chicken ChAT antibody (courtesy of Dr Miles Epstein, University of Wisconsin) at dilutions ranging from 1:500 to 1:2000 overnight at 4°C; (5) biotinylated secondary goat and anti-rabbit antibody; (6) avidin-biotin-complex; (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 commercially 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[7] and used successfully in a variety of vertebrate species,[7-9] including mammals, birds and teleosts.

A silver-stained cross section series of the brain of *Hemichromis lifalili* prepared according to the Bodian method[6] was available for cytoarchitectonic analysis.

Results

In our experiments, many well-known cholinergic nuclei in the brain of *Hemichromis* (Fig. 1) were visualized 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 brainstem, 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

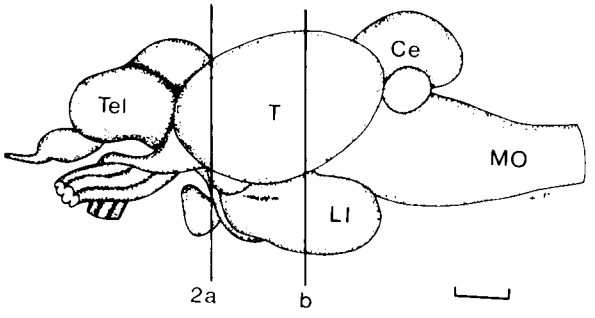

FIG. 1. Lateral view of the brain of the cichlid fish *Hemichromis*. Ce: cerebellum, LI: lobus inferior hypothalami, MO: medulla oblongata, T: tectum opticum, Tel: telencephalon. Levels of cross sections shown in Fig. 2 are indicated. Scale bar = 1 mm.

the prominent, histologically well characterized nucleus corticalis in teleosts has recently been documented.[10] 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*.[6] Similarly, some neurons of the magnocellular superficial pretectal nucleus and most neurons of the nucleus corticalis showed immunoreactivity for antibodies against ChAT (Fig. 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.[6]

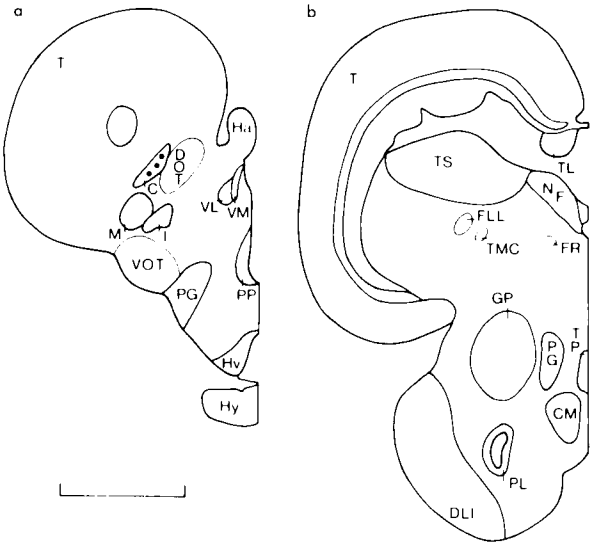

FIG. 2. Camera lucida drawings of cross sections through the diencephalon and mesencephalon of *Hemichromis*. (a) Neurons of the nucleus corticalis are indicated by black circles. (b) Glomeruli within nucleus glomerulosus, the projection site of the nucleus corticalis neurons, are indicated with clusters of dots. C: nucleus corticalis, CM: corpus mammillare, DLI: diffuse nucleus of the inferior lobe, DOT: dorsomedial optic tract, FLL: fasciculus longitudinalis lateralis, FR: fasciculus retroflexus, GP: nucleus glomerulosus pars posterior, Ha: habenula, Hv: ventral periventricular hypothalamus, Hy: hypophysis, I: intermediate superficial pretectal nucleus, M: magnocellular superficial pretectal nucleus, NF: nucleus of medial longitudinal fascicle, PG: preglomerular complex, PL: periventricular nucleus of the inferior lobe, PP: parvocellular preoptic nucleus, T: tectum opticum, TL: torus longitudinalis, TMC: tractus mesencephalocerebellaris, TP: posterior tuberal nucleus, TS: torus semicircularis, VL: ventrolateral thalamic nucleus, VM: ventromedial thalamic nucleus, VOT: ventrolateral optic tract. Scale bar = 1 mm.

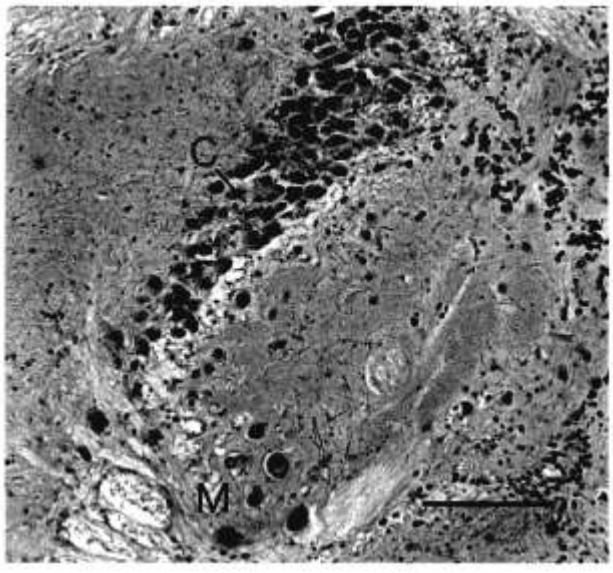

FIG. 3. Photomicrograph of a silver-stained cross section through the pretectum of *Hemichronis* showing the morphology of the nucleus corticalis (C) and the magnocellular superficial pretectal nucleus (M). Level is slightly caudal to that of Fig. 2a. Scale bar = 0.1 mm.

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).[11,12] Bands of AChE

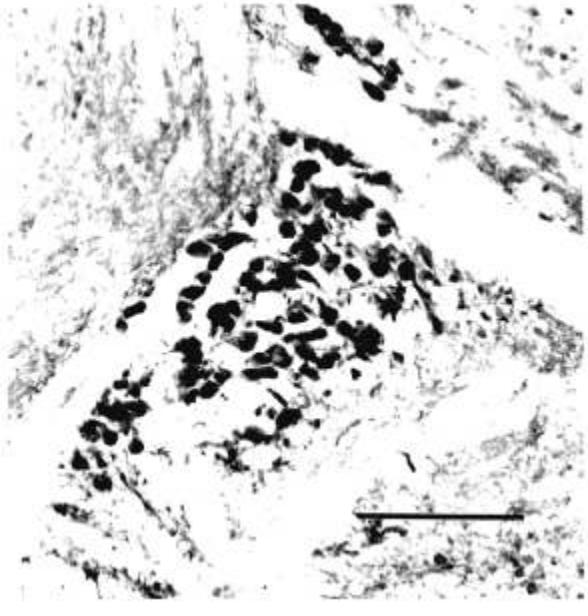

FIG. 4. Photomicrograph of a cross section through the pretectum of *Hemichromis* showing the nucleus corticalis immunohistochemically stained for antibodies against ChAT. Note that the massive fascicle (containing retinal fibers on their way to the optic tectum) which traverses the nucleus is not immunoreactive. Scale bar = 0.1 mm.

activity and ChAT immunoreactivity are co-localized with laminae of retinal terminals in these vertebrates.[13-15] However, more recent evidence suggests that these retinotectal terminals are cholinoceptive rather than cholinergic. First, a probably exclusive external source of such cholinergic input to these terminals can be localized in nucleus isthmi[16,17] (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,[18] indicating that these acetylcholine receptors are presynaptic rather than postsynaptic at the retinotectal synapses.

Recently, the morphologically elaborate pretectum 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 pretectal nuclei and of the nucleus corticalis.[6] While the magnocellular superficial pretectal nucleus is not a retinofugal target, the parvocellular superficial pretectal nucleus and the nucleus corticalis of teleosts are.[19] 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.[20] Within the nucleus glomerulosus, no other neural elements than the glomeruli show intense AChE activity.[6] Neurons of the nucleus gloromerulosus project, in turn, to the inferior lobe of the hypothalamus.[20] 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.[21] Thus, a conclusion with respect to cholinergic circuitry in the pretectum could not be made in the previous study.[6]

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

Because it is established that (1) neurons of the nucleus corticalis project into the glomeruli of the nucleus glomerulosus,[20] and that (2) AChE is present in neurons of the nucleus corticalis and the glomeruli of nucleus glomerulosus,[6] 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 together cholinoceptive 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.[22] Intracellular recordings show that neurons of the nucleus corticalis respond best to small spots moving on a contrasting background[23] and the nucleus has been interpreted to be involved in predation.[23] 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

1. Semba K, Fibiger HC. Organization of central cholinergic systems. In: Nordberg A, Fuxe K, Holmstedt B, Sundwall A, eds. *Nicotinic receptors in the CNS. Progress in Brain Research Vol 79*. Amsterdam: Elsevier, 1989: 37–63.
2. Kimura H, McGeer PL, Peng JH *et al. J Comp Neurol* **200**, 151–201 (1981).
3. Kimura H, McGeer PL, Peng JH. Choline acetyltransferase containing neurons in the rat brain. In: Björklund A, Hökfelt T, Kuhar MJ, eds. *Handbook of Chemical Neuroanatomy, Vol. 3: Classical Transmitters and Transmitter Receptors in the CNS, Part II*. Amsterdam: Elsevier, 1984: 51–67.
4. Satoh K, Fibiger HC. *J Comp Neurol* **253**, 277–302 (1986).
5. Woolf NJ, Butcher LL. *Brain Res Bull* **16**, 603–637 (1986).
6. Wullimann MF, Meyer DL. *Brain Behav Evol* **36**, 14–29 (1990).
7. Johnson CD, Epstein ML. *J Neurochem* **46**, 968–976 (1986).
8. Tumosa N, Stell WK, Johnson CD *et al. Brain Res* **370**, 365–369 (1986).
9. Zuschratter W, Scheich H. *Brain Res* **513**, 193–201 (1990).
10. Butler AB, Wullimann MF, Northcutt RG. *Brain Behav Evol* **38**, 92–114 (1991).
11. Oswald RE, Freeman JA. *Life Sciences* **27**, 527–533 (1980).
12. Contestabile A, Migani P, Poli A *et al. Adv Physiol Sci* **31**, 75–94 (1980).
13. Zottoli SJ, Rhodes KJ, Mufson EJ. *Brain Behav Evol* **30**, 143–159 (1987).
14. Oswald RE, Schmidt DE, Freeman JA. *Neuroscience* **4**, 1129–1136 (1979).
15. Schmidt A, Roth G, Ernst M. *J comp Neurol* **288**, 123–135 (1989).
16. Zottoli SJ, Rhodes KJ, Corrodi JG *et al. J Comp Neurol* **273**, 385–398 (1988).
17. Wallace MT, Ricciuti AJ, Gruberg ER. *Neuroscience* **35**, 627–636 (1990).
18. Henley JM, Lindstrom JM, Oswald RE. *Science* **232**, 1627–1629 (1986).
19. Northcutt RG, Wullimann MF. The visual system in teleost fish: morphological patterns and trends. In: Atema J, Fay RR, Popper AN, Tavolga, WN, eds. *Sensory Biology of Aquatic Animals*, New York: Springer, 1988: 515–552.
20. Sakomoto N, Ito H. *J comp Neurol* **205**, 291–298 (1982).
21. Greenfield SA. *Cellular and Molecular Neurobiol* **11**, 55–77 (1991).
22. Fryer G, Iles TD. *The cichlid fishes of the great lakes of Africa*. Edinburgh: Oliver and Boyd, 1972: 641.
23. Rowe JE, Beauchamp RD. *Brain Res* **236**, 205–209 (1982).

ACKNOWLEDGEMENTS: We thank Dr Miles Epstein, University of Wisconsin, Madison, for generously providing the primary ChAT antibody, and Dr Andrea Schmidt for reading the manuscript. This study was supported by the Swiss National Science Foundation and the Janggen-Pöhn Stiftung.

Received 7 October 1991; resubmitted 24 October 1991; accepted 31 October 1991