

Distribution pattern of acetylcholinesterase in the optic tectum of two Indian air breathing teleosts

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KEY WORDS

Acetylcholinesterase
Optic tectum
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Stratum marginale
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ABSTRACT

Background: A histoenzymological study has been carried out on the distribution of enzyme acetylcholinesterase in the optic tectum of two Indian air breathing teleosts by employing a histochemical technique to visualize acetylcholinesterase containing neurons described by Hedreen, JC (1985). **Purpose:** Data available on enzyme localization in the brain of fishes, particularly Indian teleosts is inadequate and scattered. **Methods:** AChE distribution in the optic tectum shows a prevalent pattern characterized by precise laminar distribution of enzyme which shows alternatively strong, weak or negative reaction in the different layers. **Results:** Layers with maximum enzyme activity most likely correspond to areas where cholinergic mechanism is prevailing whereas layers with mild activity may be considered to be non-cholinergic/cholinergic having some cholinergic innervations from other layers. **Conclusion:** The present investigation suggests some possible connections between enzyme localization and functional and anatomical organization of optic tectum.

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Introduction

Acetylcholinesterase (AChE) is a lytic enzyme belonging to the family of type B carboxylesterase that hydrolyses the neurotransmitter acetylcholine (ACh) in to choline and acetate.¹ This enzyme is in abundance in the synapse of cholinergic neurons, playing a key role in the cholinergic neurotransmission. Recently, additional non cholinergic functions of AChE have been elucidated.²⁻⁴ The two roles of AChE (Cholinergic and non-cholinergic) provide adequate ground to functionally correlate its distribution in the different brain centers.

The distribution of cholinesterases has been carried out in the brain of several mammalian,⁵⁻¹⁰ avian,¹¹⁻¹⁵ and reptilian¹⁶⁻¹⁹ species. Data available on enzyme localization in the brain of fishes,²⁰⁻²² particularly Indian teleosts is inadequate and scattered.

For this reason, a study was initiated on the enzymatic distribution pattern in some brain regions of two Indian air breathing teleosts (*Channa punctatus* and *Heteropneustes fossilis*). Our research starts with histochemical distribution of AChE in the optic tectum (OT) of above mentioned species based on recent cytoarchitectonic and hodological studies. The neuroanatomical nomenclature of optic tectum follows that of Meek and Schettart, 1978²³ as adopted by Castro *et al*, 2006.²⁴

Methods

Ten adult males of *Heteropneustes fossilis* (Length 16 ± 18 cm, weight 35 ± 40 gm) and *Channa punctatus* (length 15 ± 17 cm, weight 45 ± 50 gm) were collected from the natural habitat of Ranchi district and acclimatized for laboratory. All the experiments were carried out according to ethical guidelines of Ranchi University, Ranchi.

Animals were anesthetized with 0.2% 2-phenoxy ethanol. Fishes were perfused transcardially with 500 ml solution of 0.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Brain was dissected out and post fixed in the same solution for six hours. Brain was then given 2,3 changes in 15% sucrose solution in 0.1 M phosphate buffer and stored in the same solution for 1-3 days. Brain was sectioned at 30 μm

thickness on cryotome at 22°C. Serial sections were then processed for AChE staining described by Hedreen. JC *et al*, 1985.²⁵ Suitable controls were also maintained.

Results

Optic tectum (OT) in presently studied specimens, which constitutes the dorsal part of mesencephalon, is a bilobed structure. This is composed of six stratified zones of differently shaped and differently sized neurons. From the ependyma to the outer surface, the following strata are considered. The stratum periventriculare (SPV) in the inner region, the stratum album centrale (SAC), the Stratum griseum centrale (SGC) the stratum fibrosum et griseum superficiale (SFGS), the stratum opticum (SO), and the stratum marginale (SM) (Figs. 1, 2).

In *Channa punctatus* the outermost SM comprises two sub-zones based on AChE intensity the outer most sub-zone exhibited moderate reaction while the inner sub-zone showed intense activity. Next to SM is the SO which is wide in comparison to its adjacent zones. This layer demonstrated strong activity in the central zone while moderate reaction in the outer and inner sub-zones, SFGS showed very strong activity almost in all parts. In contrast SGC showed mild activity for AChE preparations while next to SGC, SAC exhibited no reaction, however few innervations of AChE positive somata of adjacent layers are present in SGC and SAC both. Interestingly the inner most SPV which comprises large sized somata demonstrated very high intensity (Fig. 1).

In the optic tectum of *Heteropneustes fossilis*, the distribution of AChE is less complex and does not correspond so precisely to histologic lamination of the nervous system. Only five zones are apparent in the present investigation. The outer most SM and next to SM, SO demonstrated intense activity while SFGS and next layer showed moderate activity. The inner most SPV, exhibited strong reaction like that of *Channa punctatus* (Fig. 2).

Details are given in Table 1 and Figs. 1, 2.

Discussion

The laminar distribution pattern of AChE in the optic tectum of both the species outlines the presence of cholinergic

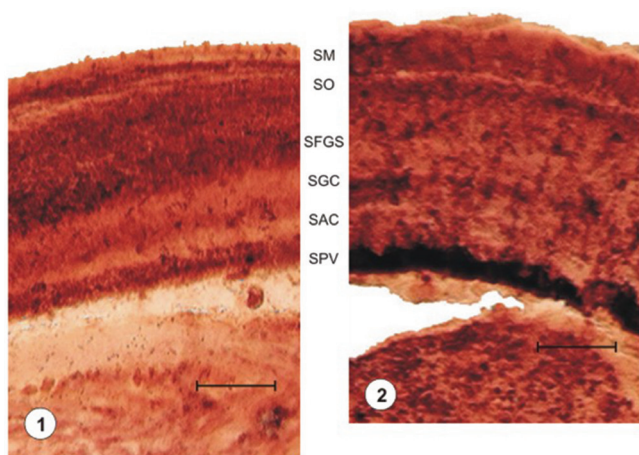


Fig. 1, 2: Microphotographs of 30 μm thick cryocut transverse sections passing through OT of 1 - Channa Punctatus 2 - Heteropneustes fossilis showing laminar AChE distribution IOX (Scale bar - 100 μm)

Table 1: AChE intensity in different layers/strata of Optic Tectum of both the fish species

Sl. No.	Layers/Strata	Abbreviations	AChE Activity	
			Channa	Heteropneustes
1.	Stratum periven-triculare	SPV	+++	+++
2.	Stratum album centrale	SAC	--	+ -
3.	Stratum griseum centrale	SGC	+ -	+ -
4.	Stratum fibrosum et griseum super-ficiale	SFGS	+++	+ -
5.	Stratum opticum	SO	++	++
6.	Straum marginale	SM	++	++

Notation: Very Intense: +++
Intense: ++
Moderate/mild: + -
Negative: --

synapses and uneven distribution of cholinergic, cholinceptive and non-cholinergic neurons. Cells within the SPV were found to be AChE positive and similar results have been obtained in previously studied species.²⁶⁻²⁸ Thus this receptive layer appears to be a cholinergic area. Retinal afferents discharge in this layer and many of these synapses are probably cholinergic.²⁹ The intense activity observed in SO points out the possible function of the most superficial neurons in OT. Dendritic branches of these marginal neurons ramify in the SO and SM and receive many synapses at this level.^{29, 30}

SM contains fine non myelinated axons in abundance which synapse on spines of thick dendrites of pyramidal cells of SO in teleosts.³⁰ Our study thus suggests that SM and SO have cholinergic synapses in abundance, though cholinergic somata may be comparatively less in number. This investigation is also

in consonance with previous report³¹ which suggested that unmyelinated nerves have higher concentration of AChE than myelinated nerves.

In SFGS, characteristic terminals belonging to optic nerve are large and irregular having pale mitochondria and abundant round vesicles as suggested by EM study.³² We therefore suggest that the difference in the intensity of AChE in this layer in the currently studied specimens may be due to irregularity of optic nerve terminals. SAC and SGC layers showing mild or negative reactions are non-cholinergic which may have some cholinergic innervations of the dendrites and axons of other layers. The other ultrastructural analyses on AChE in the optic tectum of the gold fish and cat fish³² revealed that AChE is synthesised by all neuronal types present in layers. The fine localization of AChE is the result of its synthesis in cell bodies, its storage and transport along dendrites and its release in extra cellular space. The difference in AChE localization between two teleosts examined, mainly derive from differential enzyme release in corresponding layers.

Recently, certain non-classical functions of AChE²⁻⁴ have been elucidated that can be independent of its role in hydrolysing acetylcholine. AChE can facilitate neurite growth,² it also acts as neuronal adhesion protein³ and can degrade few neuropeptides as well.⁴ These functions explain the very wide spread staining observed in different layers which may be non-cholinergic.

Thus the essence of present discussion is that highly intense nuclei/strata may be cholinergic or cholinceptive while moderately stained layers may be cholinceptive merely. The mild or negatively stained layers are non cholinergic and in totality such areas are helping in the transmission of nerve impulses and playing roles in physiological and metabolic processes.

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