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DISTRIBUTION PATTERN OF NEUROENZYME ACETYLCHOLINESTERASE IN THE OLFACTORY BULB OF AN INDIAN AIR BREATHING TELEOST *CHANNA PUNCTATUS*

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ABSTRACT
The present work deals with the histoenzymological distribution pattern of enzyme acetylcholinesterase (E.C.3.1.1.7) in the different layers of olfactory bulb of *Channa punctatus* by employing a modified technique to visualize acetylcholinesterase containing neurons. Acetylcholinesterases distribution in the olfactory bulb shows a precise laminar distribution of enzyme which is alternatively mild, strong, weak or negative in the different layers. Layers with maximum enzyme reaction most likely correspond to areas with prevalence of cholinergic mechanism, whereas layers with mild or negative reaction may be considered to be non cholinergic/cholinoceptive.

INTRODUCTION

Acetylcholinesterase (AChE) is a hydrolytic enzyme belonging to the family of type B carboxylesterase which hydrolyses the neurotransmitter acetylcholine in to choline and acetate (Soreq and Seidman, 2001).

The histochemical distribution of cholinesterases in mammalian brains (Shute and Lewis, 1963; Krnjevic and Silver, 1964; Bennet *et al*., 1966; Ishii and Friede, 1967; Bhatt and Tewari, 1978; Giris, 1980), avian brains (Cookson *et al*., 1996; Sadananda, 2004) and reptilian brains (Sethi and Tewari, 1976; Srivastava and Tripathi, 2007; Tripathi and Srivastava, 2010) have already attracted much attention, studies on the distribution of AChE in pieces brain (Contestebile and Zanoni, 1975; Contestebile, 1975; Sood and Sinha, 1983) particularly Indian teleosts is inadequate and scattered.

In the light of the facts stated above a histoenzymological study has been carried on the distribution pattern of AChE in the olfactory bulb of Indian air breathing teleost *Channa punctatus*. The functional significance of enzyme regarding its variable distribution has also been discussed. The nomenclature of layers is based on the previous cytoarchitectonic studies (Khan *et al.,* 1999; Sarkar and Subhedar, 2001; Gaikwad *et al.,* 2004).

MATERIALS AND METHODS

Ten adult males of *Channa punctatus* (length 15 ± 17 cm, weight 45 ± 50g) 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 transcordially with 500mL solution of 0.5% paraformaldehyde and 1.5% gluteraldehyde in 0.1M phosphate buffer (pH 7.4). Brain was dissected out and post fixed in the same solution for six houres. 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 sliced at 30 μm thickness on cryocut at — 22ºC. Serial sections were then processed for AChE staining described by Hedreen *et al.*, 1985.

RESULTS

The olfactory bulb in the presently studied teleost is a paired structure located anterior and adjacent to cerebral hemispheres. In the transverse section four layers are visible, which from outer to inner side are olfactory nerve layer (ONL), the glomerular layer (GL), the mitral all layer (MCL), and the inner most granule cell layer (GCL) (Fig. 1 and 2).

In the histoenzymological demonstrations for AChE activity, the outer most ONL showed moderate activity while the GL exhibited mild to negative activity. The third, MCL however demonstrated vary intense activity for AChE preparations. In contrast the GCL showed moderate to intense AChE staining in their large sized somata (Fig. 2; Table 1).

Table 1: AChE activity in different layers

Layers	Abbreviations	AChE Activity
Olfactory nerve layer	ONL	+ +
Glomerular layer	GL	+ -
Mitral cell layer	MCL	+ + +
Granular cell layer	GCL	+ +

+ + + = Very intense; + + = Intense; + - = Moderate

DISCUSSION

The variable distribution pattern of AChE in the olfactory bulb of *Channa punctatus* outlines the presence of cholinergic synapses and uneven distribution of cholinergic, cholinoceptive and non-cholinergic neurons. Cells within ONL were found to be positive and similar result has been found out in previously studied species (Clement *et al*., 2004). The highly intense MCL layer appears to be cholinergic area with bulk of synapses from other cell layers in the light of earlier findings (Lewis and Shute, 1959). Layers with low AChE activity reflect the non-cholinergic nature of neurons. The cholinoceptive cells may often have AChE on their cell membrane even being noncholinergic (Hebb, 1961).

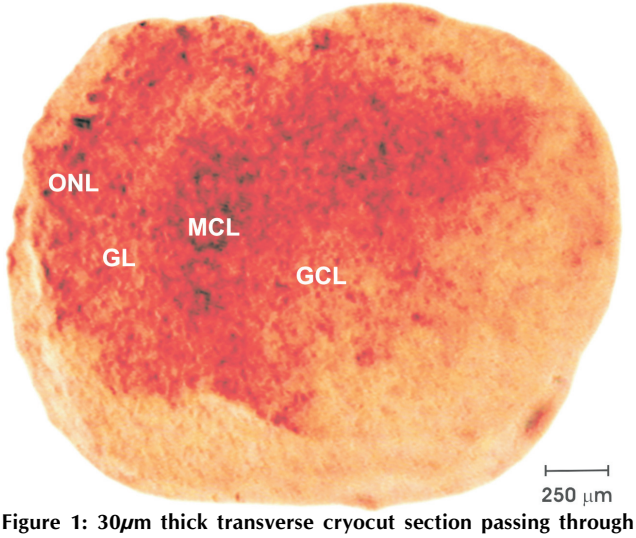

Figure 1: 30µm thick transverse cryocut section passing through olfactory bulb of *Channa punctatus* showing AChE activity in different layers (4X)

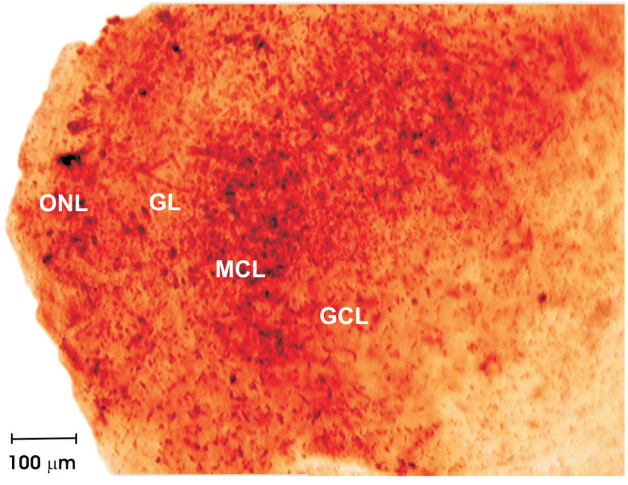

Figure 2: 30µm thick transverse cryocut section passing through olfactory bulb of *Channa punctatus* showing AChE activity in different layers (10X)

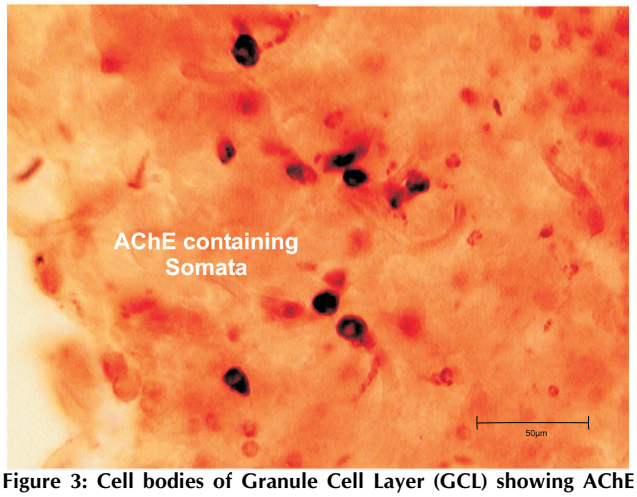

Figure 3: Cell bodies of Granule Cell Layer (GCL) showing AChE activity (40X)

Recently certain non classical functions of AChE have been elucidated (Chub *et al*., 1980; Downes and Granto, 2004; Silman and Sussmen, 2005) that can be independent of its role in hydrolyzing acetyl Choline. AChE can facilitate neurite growth (Downes and Granto, 2004). It acts as neuronal adhasion protein (Silman and Sussman, 2005) and can degrade few neuropeptides like, substance P, met, leu-enkephalin as well (Chub *et al.,* 1980) 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/layer may be cholinergic or cholinoceptive while moderately stained layers may be cholinoceptive. 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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