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# Histoenzymological distribution of acetylcholinesterase in the Corpus cerebelli of two Indian air breathing teleosts.

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**Abstract :** The present study describes the distribution of acetylcholinesterase enzyme (AChE) in the various layers of corpus cerebella of brain of two Indian air breathing teleost, *Heteropneustes fossilis* and *Channa punctatus*. The distribution pattern of enzyme showed uneven distribution of enzyme in all the three layers of corpus cerebelli. The outermost molecular layer (ML) in *Heteropneustes fossilis* showed fainted activity for AChE, while the Purkinje cells (PC) present in the intermediate layer exhibit strong cholinesterase positive reaction in their cell bodies. The inner granular layer (GL) also demonstrated intense reaction to AChE. However, the molecular layer (ML) and the inner granular layer of corpus cerebelli of *Channa punctatus* also showed strong intense reaction in comparison to *Heteropneustes fossilis*. The Purkinje cells in the intermediate layer exhibit mild or no activity. It is suggested that the variability of AChE distribution may perhaps deals with noncholinergic role in addition to its main cholinergic role.

**Key Words :** Acetylcholinesterase, Purkinje cell, Granular layer, Cerebellum, Brain.

## 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 at the neuromuscular junction ( Appleyard and Johnson, 1992; Soreq & Seidmann, 2001; Kumar and Tembhre, 2010). Medically the acetylcholinesterase is an important for the diagnosis and treatment of neurodegenerative diseases such as cardiac and Alzheimer's diseases (Praveen & Kumar, 2005). The role of acetylcholinestrase is also used a biomarker in environmental biomonitoring by Lionetto *et al.* (2010)

AChE also plays some non-cholinergic function apart from its main cholinergic role. It plays a role in cell- adhesion, cell differentiation and neurogenesis ( Silman & Sussman, 2005; Downes & Granto, 2004; Chub *et al*., 1980, 1982; Genever *et al*., 1999). Several studies on brain displaying AD lesions have shown changes in the expression and distribution of AChE (Talesa, 2001). These wide roles of AChE provide adequate base in functionally correlating it with its variable histochemical distribution.

The histochemical distribution of cholinesterases in the avian and mammalian brains has been well documented in the literature (Whittaker, 1953; Shute & Lewis, 1963; Krnjevic & Silver, 1964; Cookson *et.al*., 1996 ; Ishii & Friede, 1967; Bhatt & Tewari, 1978; Giris, 1980). The histology of the cholinergic nerves in the reptilian brains was studied by numerous investigators (Sethi & Tewari, 1976, 1977; Srivastava & Tripathi, 2007; Tripathi & Srivastava, 2007; Maurya and Srivastava, 2012. The distribution of AChE in fish brain has been studies by Contestebile & Zanoni (1975), Contestebile (1975) and Sood & Sinha (1983). Demble *et al*. (2000) studied the concentration effects of exposure of selected insecticideson the brain AChE in the common carp, *Cyprinus carpio L*.

## Materials and Methods

Five 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 transcordially with 500 ml solution of 0.5% paraformaldehyde and 1.5% gluteraldehyde 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 sliced at 30 ?m thickness on cryocut at 22°C. Serial sections were then processed for AChE staining described by Hedreen *et al.* (1985). Suitable controls were also maintained.

## Results

Cerebellum of teleosts consists of three parts, the vestibulolateralis lobe, the corpus cerebelli and valvula cerebelli. Corpus cerebelli lies on the top of rostral rhombencephalon comprised the wide outer molecular layer (ML), middle thin intermediate layer and inner granular layer (GL) (Figs. 1 - 2).

The histological preparations of the corpus cerebelli of *Heteropneustes fossilis* shows that the outermost thick molecular layer (ML) showed fainted activity for AChE (Fig. 1). The irregularly arranged Purkinje cells (PC) were either ovoid or pyriform in shape were placed in the in the intermediate layer. The cell bodies exhibit deep strong reaction. The inner granular layer (GL) also showed intense reaction for AChE (Table - 1). In the corpus cerebelli of *Channa punctatus*, the molecular layer (ML) and inner granular layer both take deeper reaction along. The Purkinje cells present in the intermediate layer showed mild or no AChE activity (Fig. 2, Table - 1).

## Discussion

Wulliman M.F. ( 1998) and Kumar and Tembhre (2010) described the anatomy and histology of the brain of fishes. In the present study,the cerebellum of these fishes also consists of three parts, the vestibulolateralis lobe, the corpus cerebelli and valvula cerebelli. Corpus cerebelli lies on the top of rostral rhombencephalon comprised the wide outer molecular layer (ML), middle thin intermediate layer and inner granular layer.

AChE activity in the different layers of corpus cerebelli demonstrated variability in both the species examined The presence of AChE in Purkinje cells in different fish species have been reported in *Salmo* ,*Poecilia* , *Carassius*, *Phoxinus*, *Porichtys* , *Acipenser*, *Scyliorhinus* and *Danio* ( Contestebile & Zanoni (1975); Contestebile *et al.*, 1977, 2004; Ekstrom, 1987; Brantley and Bass, 1988; Adrio *et al*., 2000; Anadon *et al*., 2000; Perez *et al*., 2000). In the present investigation the cholinergic Pukinje cells were not observed in the cerebellum of teleosts as noticed by Clemente *et al*. (2004). AChE positive granule cells were also observed in several fish species (Centestebile and Zannoni, 1975; Contestebile *et al*., 1977;) and in other vertebrates (Contestebile and Tabanelli, 1977; Willani et al. 1977; Kusunoki et al. 1987; Robertson & Roman, 1989). However. The results of the present investigations are in conformity to the above referred authors who have reported cholinergic Purkinje cells. The cerebellum has been described as one region where AChE exists beyond the requirements or in the absence of cholinergic

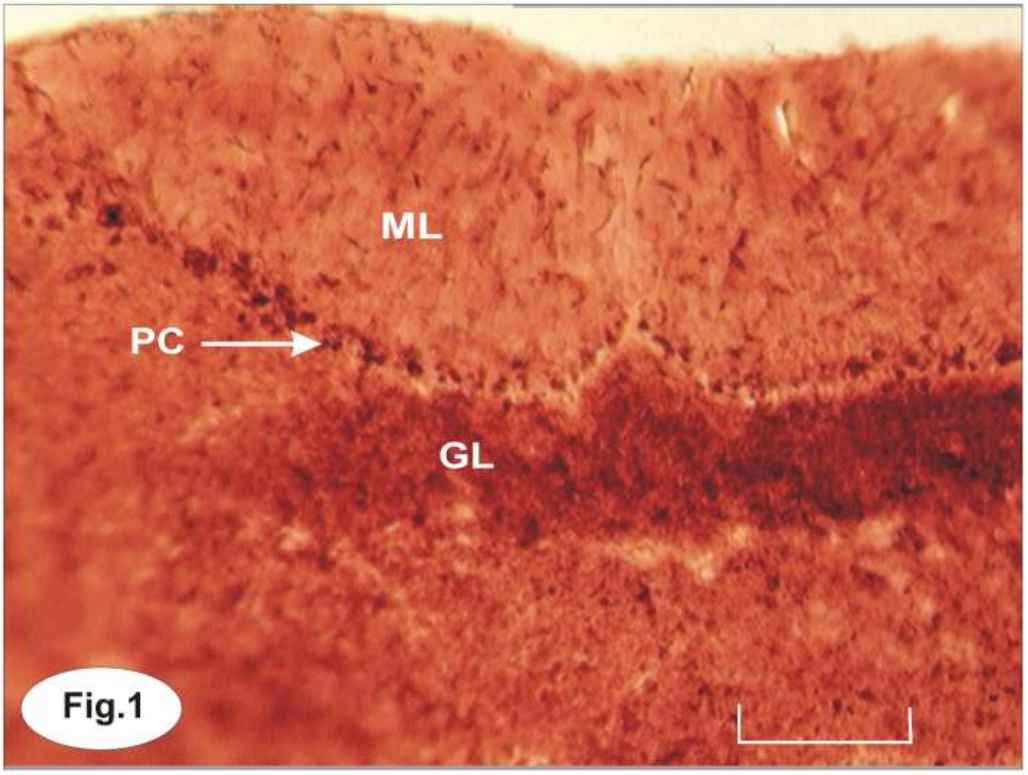


**Fig. 1 :** 30 micron transverse cryocut section passing through carpus cerebelli of *Heteropneustes fossilis* showing AChE activity in different layers (10 X) (Scale Bar - 100 micron).

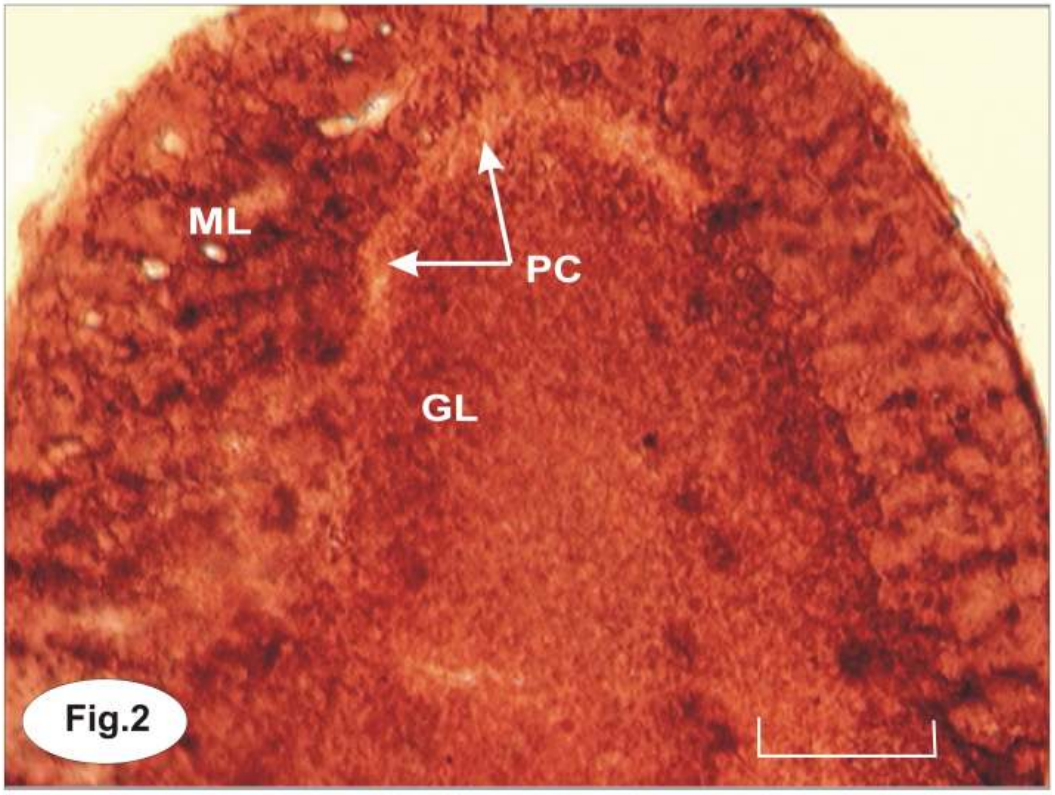


**Fig. 2 :** 30 micron transverse cryocut section passing through carpus cerebelli of *Channa punctatus* showing AChE activity in different layers (10 X) (Scale Bar - 100micron).

**Table 1**

| Sl. No. | Layers | Abbreviations | AChE Activity | |
|---|---|---|---|---|
| | | | ***Heteropneustes*** | ***Channa*** |
| 1. | Molecular Layer | ML | +— | +++ |
| 2. | Intermediate Layer of Purkinje Cell | PC | +++ | +— |
| 3. | Granular Layer | GL | ++ | ++ |

**Notation :** Very Intense : +++, Intense : ++,
Moderate/mild : +—. Negative : ——

transmission in mammals. It may be presumed therefore that Purkinje cells have similar role in the presently studied fishes, because it is also reported earlier that fish granule cells use the excitatory neurotransmitter glutamate in their synapses with purkinje cells.

The cytoarchitectonic properties of the teleostean cerebellar cortex and its input-output characterstics are so similar to other vertebrates that it probably subserves functions in motor learning and coordination as well (Wullimann, 1998).

However, the presence of AChE in the different layers of corpus cerebelli may be attributed to its non-cholinergic roles which have been explored recently. AChE plays role in synaptogenesis, morphometric processes, cell differentiation along nervous system (Silman, & Sussman, 2005). Furthermore AChE hydrolyses substance P, met - leu-enkephalin, and neuropeptides as well (Chub et al., 1980, 1982).

Thus the essence of discussion is that the different layers of corpus cerebelli in the presently

studied fishes as a whole reflect the configuration of the neurons exhibiting AChE activity in their parikarya, plasma membrane & synapses with its non classical roles. And in totality these layers are helping in the transmission of non cholinergic nerve impulses and playing significant role in physiological and metabolic processes.

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