



Distribution of Acetylcholinesterase in the Octavolateral Area of *Heteropneustes fossilis*

Anurag Tripathi

*Department of Zoology, Kulbhaskar Ashram P G College, Prayagraj,
Corresponding author: rahulzau@rediffmail.com*

ABSTRACT

Octavolateral area is a significant component of rhombencephalon in teleosts which shows a fine cytoarchitecture and comprises many intermingled nuclear groups. In the present study, distribution pattern of enzyme acetylcholinesterase has been carried out by employing a modified histochemical technique to visualize acetylcholine containing neurons described by Hedreen, J.C. et.al. (1985).

Acetylcholinesterase is an effective marker of cholinergic-cholinoceptive neurons since it hydrolyses acetylcholine in to choline and acetate at synaptic clefts. Present histochemical results exhibited a widespread distribution of acetylcholinesterase in the different nuclei of octavolateral area of *Heteropneustes*. Octavolateral efferent nucleus, medial octavolateral nucleus, magnocellular and posterior octaval nucleus demonstrated intense activity for acetylcholinesterase, while anterior octavolateral nucleus and descending octaval nucleus exhibited moderate staining. The distribution of acetylcholinesterase was homogenous in the whole rostro-caudal and lateral extension of this nucleus.

Thus in overall picture, octavolateral nucleus contains abundant cholinergic cells in the brain of *Heteropneustes*. In conclusion, the abundance of cholinergic innervations in this area is a well conserved characteristic among vertebrates which has been discussed from phylogenetic perspective.

Keywords: Cholinergic, Acetylcholinesterase, Octavolateral nucleus, Magnocellular Octaval Nucleus.

INTRODUCTION:

Teleosts which represent the most prominent and diversified group among actinopterygians are interesting in many features particularly in their complex social and territorial behaviours. Teleosts exhibit a complex nervous system in terms of cyto-architecture, hodology and number of neurons. This is also a fascinating group because other terrestrial vertebrates radiated from this. Acetylcholinesterase which is an enzyme of hydrolase group and it splits the neurotransmitter acetylcholine in to choline and acetate (Soreq and Seidman, 2001) thus it is an effective marker of cholinergic and cholinoceptive neurons. In the present study a modified histochemical technique to visualize acetylcholinesterase containing

neurons has been employed (Hedreen et.al.,1985).

The distribution of cholinesterases has been carried out in the brain of several mammalian (Krnjevic and Silver,1964; Bennet et.al., 1966; Ishii and Friede,1967; Bhatt and Tewari,1978; Giris,1980), avian (Cavaunagh and Lolland,1961; Zuschratter and Scheih,1990; Cookson et.al.,1996; Sadananda, 2004), reptilian (Sethi and Tewari,1976; Sethi and Tewari,1977; Subhedar and Rama Krishna,1990) species. Data available on enzyme localization in the brain of fishes (Contestabile and Zannoni,1975; Northcut and Butler,1993; Contestabile,et. al.,2013; Tripathi et.al.,2013; Ttripathi and Rahman,2014; Medina and Reiner,1994,), particularly on Indian teleosts is inadequate and scattered. For these reasons a

histochemical study was carried out of AChE distribution in the octavolateral area of the hindbrain of *Heteropneustes* which was far from detailed study, though few studies are reported (Perez et al., 2000; Ekstrom, 1987; Brantley and Bass, 1988; Marin and Gonzalez, 1997; Anadon et al., 2000; Tago, et al., 1989).

MATERIALS AND METHODS

Five adult *Heteropneustes fossilis* weighing 40-45 gram and length between 18-22 centimeter were used in the present study. The animals were acclimatized according to laboratory conditions before sacrificing them. Experimental procedures were performed according to the guidelines of the Institutional Animal Ethics Committee (IAEC). The fish were anesthetized with MS-222 (Sigma, St. Louis, MO) and decapitated. Brains were fixed in the solution of 0.5% Paraformaldehyde and 1.5% Gluteraldehyde in 0.1 M phosphate buffer (pH 7.4) for 6 hours at 4°C. The tissue was then given 2-3 changes in 15% sucrose solution in 0.1M phosphate buffer and stored in the same solution for 2-3 days. 30 micron thick frozen sections were cut by A O Histostat at -22°C and stored serially in 0.1 M phosphate buffer. AChE histochemistry was carried out by using a modified histochemical technique (Kimura et al., 1990). The dark brown coloured patches appeared in sections which designated AChE activity. Omission of the substrate acetylthiocholine iodide from the incubating mixture was carried out as control for the AChE histochemistry and no residual activity was observed in controlled experiment.

Results and discussion :

The octavolateral area of rhombencephalon showed one of the highest densities of AChE positive neurons within *H. fossilis* (Fig. 1-2). Octavolateral efferent nucleus (OEN) showed numerous and intensely stained pyriform neurons. These cells showed ventrally or ventrolaterally oriented long dendrites (Fig. 2). AChE positive neurons of small size were observed in medial octavolateral nucleus (MON). AChE distribution was homogenous in the whole rostrocaudal and mediolateral extension of the nucleus (Fig. 1-2). A group of AChE positive cells were observed in the central portion of the anterior octave nucleus (AON) (Fig. 1, 4).

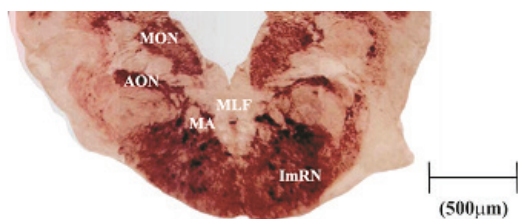


Figure-1: Rostral rhombencephalic area of *H. fossilis* showing AChE activity in octavolateral nuclei.

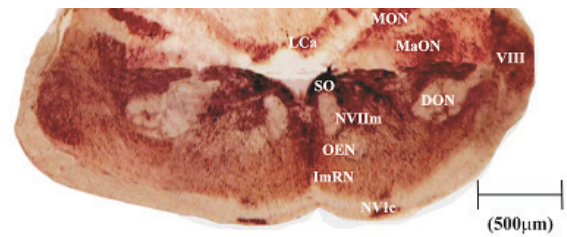


Figure-2: middle rhombencephalic area of *H. fossilis* showing AChE activity in octavolateral nuclei.

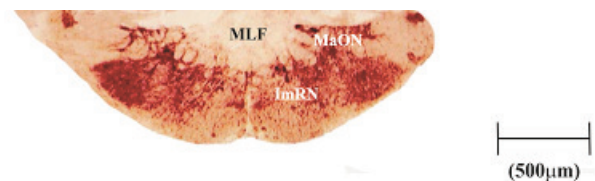


Figure-3: Caudal rhombencephalic area of *H. fossilis* showing AChE activity in octavolateral nuclei.

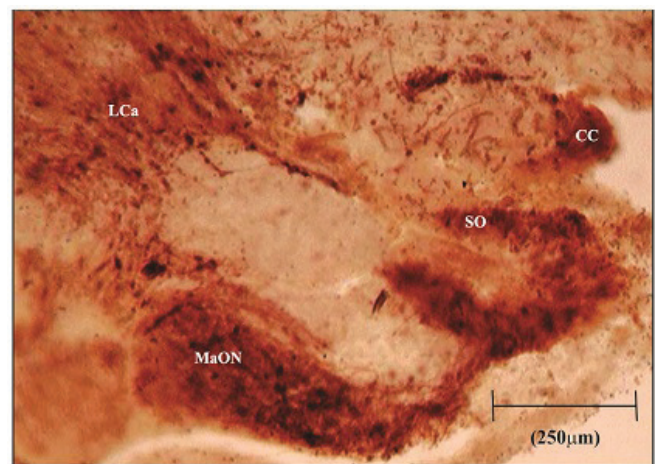


Figure-4: Rostral rhombencephalic area of *H. fossilis* showing AChE activity in octavolateral nuclei (Magnified).

In the middle rhombencephalic parts, magnocellular octaval nucleus (MaON) appears which showed large sized AChE positive neurons and highly ramified axonal and dendritic processes extended to other nuclei of medulla oblongata (Fig. 2-3). Descending octaval nucleus (DON) which is located ventral to MaON showed moderate intensity for AChE (Fig. 3), but it received dendritic processes from secondary octaval nucleus (SO) and magnocellular octaval nucleus (MaON) which were AChE positive (Fig. 2). In Caudal sections DON showed intense activity (Fig. 3). Secondary octaval nucleus (SO) showed intense activity at all levels (Fig. 2-3). These AChE positive neurons displayed round or ovoid medium somata (Fig. 4). A summary of the acetylcholinesterase activity has been given in table-1.

Table-1: Acetylcholinesterase Activity in the octavolateral area of the rhombencephalon of *H. fossilis*

Sl. No.	Name of Nuclei	Abbreviation	AChE -activity	Fig. No.
1.	Octavolateral efferent nucleus	OEN	+++	2
2.	Medial octavolateral nucleus	MON	+++	2-3
3.	Anterior octavolateral nucleus	AON	++	1
4.	Magnocellular octaval nucleus	MaON	+++	2-3
5.	Secondary octaval nucleus	SO	+++	2-4

Notation :

++++ = Very Intense +++ = Intense

++ = Moderate +— = Mild; — — = Negative

Among the teleosts, studied hitherto, cholinergic cells in the octavolateral area are absent or poorly developed (Clemente et.al.,2004; Perez et.al.,2000; Ekstrom,1987; Brantley and Bass,1988). Nonetheless the octavolateral area contains abundant cholinergic cells in dog fish (Anadon, et. al., 2000). In other vertebrate groups cholinergic cells appear in very concrete regions(Tago et. al.,1989; Kimura et.al.,1984; Carpenter,et.al.;1990; Barmack et.al.;1992). It is suggested therefore that the presence of cholinergic cells in the octaval region may be a primitive feature of vertebrates. A reduction of these populations is observed in tetrapods whereas teleosts may have lost these populations secondarily(Clemente et.al.,2004). On the other hand AChE activity was displayed throughout the rostrocaudal octavolateral area. Thus AChE positive cells were detected in the medial and posterior octavolateral nuclei, secondary octavolateral nucleus and anterior, magnocellular octavolateral nuclei. Nuclei within the octavolateral area receive profuse ChAT immunoreactive innervations which could mediate the cholinceptive nature of the AChE positive neurons within the aforesaid nuclei(Clemente et.al.,2004).

ACKNOWLEDGEMENTS:

Thanks are due to my supervisor, Prof U C Srivastava, FNASc, Zoology Department, Allahabad University for his constant support and encouragements. My sincere thanks are also due to the Principal, K A Degree College, Prayagraj for providing me required laboratory facilities.

Declaration: We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.

REFERENCES:

- Anadon R., Molist P., Rodriguez-Moldes I, Lopez JM, Quintela I, Cervino M.C., Barja P., Gonzalez A. (2000). Distribution of choline acetyltransferase immunoreactivity in the brain of an elasmobranch, the lesser spotted dogfish (*Scyliorhinus canicula*). *J. Comp. Neurol.* **420**: 139-170.
- Barmack, N.H., Baughman, R.W., Eckenstein, F.P. (1992). Cholinergic innervation of the cerebellum of rat, rabbit, cat, and monkey as revealed by choline acetyltransferase activity and immunohistochemistry. *J. Comp. Neurol.* **317**: 233-249
- Bennet E.L., Diamond M.C., Morimoto H, et.al (1966). Acetylcholinesterase activity and weight measures in fifteen brain areas from six lines of rats. *J Neurochem.***3**:563-572.
- Bhatt, D.K., and Tewari, H.B.(1978). Histochemical mapping of acetylcholinesterase and butyrylcholinesterase in the medulla oblongata and pons of squirrel. *J Neurosis Res.* **3(5-6)**:419-439.
- Brantley, R.K., Bass, A.H. (1988). Cholinergic neurons in the brain of a teleost fish (*Porichthys notatus*) located with a monoclonal antibody to choline acetyltransferase. *J. Comp. Neurol.* **275**: 87-105.
- Carpenter M.B., Huang Y., Pereira A.B., Hersh L.B. (1990). Immunocytochemical features of the vestibular nuclei in the monkey and cat. *J. Hirnforsch* **31**:585-599.
- Cavanagh JB, Lolland P.(1961). Cholinesterase in the chicken nervous system. *Nature.* **190**:735-336.
- Clemente, D., Porteres, A., Weruaga, E., Alonso, J.R., Arenzana, E.J., Aikjon, J. and Arevalo, R. (2004). Cholinergic elements in the Zebra fish central, nervous system. Histochemical and immunocytochemical analysis. *J. Comp. Neurol.* **474**: 75-107.
- Contestabile A, Zannoni N.(1975). Histochemical location of acetylcholinesterase in the cerebellum and optic tectum of four fresh water teleosts. *Histochemistry.* **45**:279-288.
- Cookson, K.K., Hall, W.S., Heaton, J.T.(1996). Distribution of choline acetyltransferase and acetylcholinesterase in vocal control nuclei of the budgerigar (*Melopsittacus undulatos*). *J Comp Neurol.* **369**:220-235.
- Ekstrom, P. (1987). Distribution of choline acetyltransferase-immunoreactive neurons in the brain of a cyprinid teleost (*Phoxinus phoxinus* L.). *J. Comp. Neurol.* **256**: 494-515.
- Giris, M.(1980). Acetylcholinesterase enzyme localization in the amygdala: A comparative histochemical and ultrastructural study. *Acta Anat .* **106(2)**:192-202.

- Hedreen, J.C., Bacon, S.J., Price, D.L.(1985). A modified histochemical technique to visualize acetylcholinesterase-containing axon. *J. Histochem Cytochem.* **33**: 134-140.
- Ishii, T., Friede, R.L.(1967). A comparative histochemical mapping of the distribution of acetyl cholinesterase and nicotinamide adenine di nucleotide diphorase activities in the human brain. *Inter Rev Neurobiol.* **10**:231-275.
- Kimura, H., McGeer, P.L., Peng, J.-H., McGeer, E.G. (1984). Choline acetyltransferase-containing neurons in the rat brain. In: Bjorklund A., Hokfelt T., Kuhar M.J., editors. Handbook of chemical neuroanatomy. Amsterdam: Elsevier. p. 51-67.
- Krnjevic, K., Silver, A.(1964). The development of acetylcholinesterase staining in forebrain of the cat. *J. physiol Lond.* **175**:22-23.
- Marin, O., Smeets, W.J.A.J., Gonzalez, A. (1997). Distribution of Choline acetyltransferase immunoreactivity in the brain of anuran (*Rana Perezi*, *Xenopus Locevis*) and urodele (*Pleurodeles walt*) amphibians. *J. Comp. Neurol.*, **382**: 499-534.
- Medina, L. and Reiner, A. (1994). Distribution of choline acetyl transferase immunoreactivity in the pigeon brain, *J.Comp. Neurol*, **342**: 497-537.
- Northcut, R.G., Butler, A.B.(1993). The diencephalen and optic tectum of the Longnose gar, *Lepisosteus osseus* : Cytoarchitectonics and distribution of acetylcholinesterase. *Brain Behav Evol.* **41**:57-81.
- Perez, S.E., Yanez, J., Marin, O., Anadon, R., Gonzalez, A., Rodriguez-Moldes, I. (2000). Distribution of choline acetyltransferas (ChAT) immunoreactivity in the brain of the adult trout and tract-tracing observations on the connections to the nuclei of the isthmus. *J. Comp. Neurol.* **428**: 450-474.
- Sadananda, M.(2004). Acetylcholinesterase in central vocal control nuclei of the zebra finch (*Taeniopygia guttata*). *J Biosci.* **29(2)**:189-200.
- Sethi, J.S., Tewari, H.B.(1977). Histochemical mapping of acetylcholinesterase in the cerebral hemispheres of *Uromastix hardwickii*. *Cell and Molec Biol.* **2**:263-275.
- Sethi, J.S., Tewari, H.B.(1976). Histoenzymological mapping of acetylcholinesterase and butyrylcholinesterase in the diencephalon and mesencephalon of *Uromastix hardwickii*. *J Hirnforsch.* **17(4)**:335-349.
- Soreq, H., Seidman, S.(2001). Acetylcholinesterase New role for an old actor. *Natl Rev Neurosci.* **2**:294-302.
- Subhedar, N.K., Rama Krishna, N.S.(1990). Pattern of acetylcholinesterase activity in the hypothalamus of the Cobra, *Naja naja*. *J Hirnforsch.* **31(1)**:41-49.
- Tago, H., McGeer, P.L., McGeer, E.G., Akiyama, H., Hersh, L.B.(1989). Distribution of choline acetyltransferase immuno-positive structures in the rat brainstem. *Brain Res.* **495**: 271-297.
- Tripathi, A., Rahman, M., Chakraborty, B.(2013). Distribution pattern of acetylcholinesterase in the optic tectum of two Indian air breathing teleosts. *Annals Neurosci.* **20 (1)**:7-9.
- Tripathi, A., Rahman, M.(2014). Distribution of acetylcholinesterase in the telencephalon of an Indian air breathing teleost *Heteropneustes fossilis*: A histochemical analysis. *IJBPR.* **5(2)**:179-185.
- Tripathi, A., Srivastava, U.C.(2007). Histoenzymological distribution of acetylcholinesterase in the cerebral hemispheres of Indian wall lizard, *Hemidactylus flaviviridis*. *Annals Neurosci.* **14**:64-71.
- Zuschratter, W., Scheich, H.(1990). Distribution of choline-acetyltransferase and acetylcholinesterase in the vocal motor system of zebra finch (*Taeniopygia guttata*) . *Brain Res.* **513**:193-201.