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Distribution Pattern of Acetylcholinesterase in the Caudal Rhombencephalic Nuclei of an air breathing Teleost, *Heteropneustes fossilis*

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ABSTRACT

Teleosts which represent the most prominent and diversified group among actinopterygians, are interesting in many features particularly in their complex nervous system in terms of cytoarchitecture, hodology and number of neurons. Present study has been carried out to histoenzymologically map the caudal rhombencephalic nuclei of Heteropneustis fossilis by employing a modified histochemical technique to visualize acetylcholinesterase containing neurons. It is interesting to mention that in the present investigation, most of the nuclei of medulla oblongata including cranial motor nuclei, reticular and raphe nuclei showed strong activity for acetylcholinesterase. The present investigation has also been compared with that of other vertebrates including fishes studied earlier to set a homology among different rhombencephalic nuclei in the light of recent cytoarchitectural & connectional studies.

Key words: *Acetylcholinesterase, Reticular Nuclei, Medulla Oblongata, Neuropil.*

INTRODUCTION

Rhombencephalon or the hind brain of teleosts presents a typical vertebrate pattern and consists of metencephalon or cerebellum and myelencephalon or medulla oblongata respectively. Anatomical distinction between rhombencephalic regions is particularly difficult in teleosts due to poor cytological differentiation. Acetylcholinesterase (AChE) activity in the present study is differentially expressed in most of the rhombencephalic neurons & neuropil areas which clearly demarcates cell populations and regions. The distribution of cholinesterases has been carried out in the brain of several mammalian[1-5], avian[6-9], and reptilian[10-13] species. Data available on enzyme localization in the brain of fishes[14-16] particularly Indian teleosts is inadequate and scattered. In addition, the cholinergic[17] and non cholinergic[18-21] roles of acetylcholinesterase, which have been elucidated recently, provide adequate base to functionally correlate its variable distribution in the different rhombencephalic regions. Keeping in view these facts, present histochemical study has been carried out in the caudal rhombencephalic centres of *Heteropneustes fossilis.*

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MATERIALS AND METHODS

Ten adult male *Heteropneustes fossilis* weighing 35-40 gm and ranging in length between16-20 cm were used in the present study. The animals were maintained in a 12 hours light: 12 hours darkness cycle in tanks at a constant temperature of 28^0 C before sacrificing. Experimental procedures were performed according to the guidelines of the Institutional Animal Ethics Committee (IAEC) of Ranchi University. The fish were anesthetized with MS-222 (Sigma, St. Lovis, MO) and brains were dissected out by decapitation method. Brains were then post fixed in 0.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1M phosphate buffer for 6 hours at 4^0 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 Cryocut (A O Histostat) at - 22^0C and stored serially in 0.1 M phosphate buffer. AChE histochemistry was carried out by using a modified histochemical technique[22]. After washing in 0.1 M acetate buffer, pH 6.0, sections were incubated at room temperature for 30 minutes in an incubating medium made up of 25 mg acetylthiocholine iodide as substrate for AChE, 32.5 ml 0.1 M acetate buffer (pH 6.0), 2 ml 0.1M sodium citrate, 5 ml 0.03 M cupric sulphate, 9.5 ml double distilled water, 1 ml 0.005 M potassium ferricynide and 0.2 m M ethopropazine (sigma) as an inhibitor of non specific esterases. After incubation, sections were given five changes of acetate buffer (pH 6.0) then treated with 1% ammonium sulphide. Sections were then given five changes of 0.1 M sodium nitrate then exposed to 0.1% silver nitrate followed by five changes of 0.1 M sodium nitrate again. Sections were then rinsed in acetate buffer and mounted in glycerine. The dark brown coloured patches appeared in sections which designated AChE activity. Omission of the substrate acetylthiocholine iodide was carried out as control for the AChE histochemistry and no residual activity was observed.

RESULTS AND DISCUSSION

Caudal rhombencephalon in the presently studied fish comprises the motor nuclei of cranial nerves, nuclei of octavo-lateral area, raphe and reticular nuclei. All these are the part of medulla oblongata and most of the nucle showed AChE positive neurons. Reticular formation presented very large nuclear area which is rostrocaudally extended adjacent to medial longitudinal fascicle (MLF) (Fig 1-4). It comprises reticular nuclei, raphe nuclei and the mauthner cells. Intermediate reticular nucleus (ImRN) showed very large sized, round or ovoid somata with high dendritic processes extending almost in all adjacent areas including octavolateral area and mauthner cells. (Fig 1,7B) This nucleus showed very high intensity. Inferior reticular nucleus (IRN) showed intense activity within its cell bodies, dendrites and neuropil.(Fig 4). Mauthner cells also showed strong activity, though their lateral and ventral dendrites were moderately stained (Fig 7B). The medial longitudinal fascicle (MLF) was totally devoid of AChE in entire rostrocaudal extensions (Fig 2-6). Octavolateral area of caudal rhombencephalon showed one of the highest densities of AChE positive neurons (Fig 1). Magnocellular octaval nucleus (MaON) which is located dorsolateral to IRN and ventrolateral to lobus caudales (LCa) and corpus cerebella (CC) showed large sized AChE positive neurons and highly ramified axonal and dendritic processes extended to other nuclei of medulla oblongata (Fig 2,7C). 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 3). Posterior octaval nucleus (POC) which appears in caudal parts and is located ventrolateral to vagal lobe and vagal motor nuleus showed intense AChE activity (Fig 5). Facial motor nerve (NVIIm) which is located ventral to secondary octaval nucleus in caudal sections showed medium sized somata with ventrally and ventrolaterally oriented dendritic

processes. This nucleus demonstrated very high activity for AChE (Fig 2-3). Caudal to the octavolateral area and adjacent to intermediate reticular nucleus, secondary gustatory tract (SGT) nuclei are located which showed very large sized somata extended ventrolatrarally in rostrocaudal parts (Fig 2-3) These nuclei showed very high intensity for AChE particularly in their neuropil and somata (Fig 7C, 7D). Fascial lobes (FL) showed very high intensity with small sized diffused somata (Fig 1-2, 7A). Lateral line lobes showed scattered medium sized cell bodies with high AChE intensity (Fig 1) Vagal Lobe (Lx) which is located in caudal sections also showed very high intensity for AChE (Fig 6, 7F). Vagal motor nucleus (Xm) and vagal reticular region (Xr) which are ventrally located to vagal lobe also showed very high intensity for AChE (Fig 4, 7F). Medial funiculus nucleus (MFN) which is ventrally placed to vagal lobe also showed intense activity for AChE along with ventral horn which also demonstrated intense reaction for AChE (Fig 6).

However, present observations regarding AChE histochemistry are comparable to that of other vertebrate groups from the view point of discussion. In the medulla oblongata, motor neurons of fascial nucleus which showed strong activity for AChE are reported to be cholinergic in lampreys[23], elasmobranch[24], teleosts[25-28], amphibians[29], reptiles[30-31], birds[8-9], and mammals[32-34]. It is suggested therefore that motoneurons of cranial nerves are cholinergic throughout vertebrate phylogeny. The superior reticular nucleus also showed very strong AChE activity. Acetylcholinesterase positive cells were also observed in the superior reticular nucleus of zebrafish[25], which also showed ChAT (choline-acetyl transferase) positive cells in the same nuclei. These findings are in agreement with the observations described in other teleosts[25-28]. This nucleus projects to the superficial pretectal nucleus, contralateral preoptic nucleus and optic tectum in other cyprinids[27], and these nuclei and regions are reported to be AChE positive in present investigations. Intermediate and inferior reticular nuclei displayed very strong AChE activity in our study. Similar results were obtained in zebrafish[25] but no ChAT immunoreactive cells were detected. In cyprinids the afferents from optic tectum are reported in these two nuclei[35-36]. These two reticular nuclei also receive afferents from cerebellum[37]. Cholinergic cells are reported to be present in intermediate and inferior reticular nuclei in lampreys[23], elasmobranch[24], teleosts[27-28], amphibians[29], reptiles[30-31], birds[8-9] and mammals[38-39]. It is presumed therefore that these two nuclei are cholinergic in nature. Mauthner cells which are located in the rostral octavolateral region, showed intense AChE activity in the fish studied. They mediate fast escape motor responses, important in predator avoidance after reception of unexpected vibrational/visual stimuli[40]. In zebrafish, mauthner cells showed AChE positively but ChAT negativity[25]. It may be due to alternate multiple roles of AChE other than cholinergic function[18, 21].

In our results most of the nuclei of octavolateral area except descending octaval nucleus and anterior octavolateral nucleus, showed strong activity for AChE. Thus AChE positive cells were detected in the medial and posterior octavolateral nuclei, secondary octavolateral nucleus and anterior, magnocellular octavolateral nuclei. Nuclei within the ocavolateral area receive profuse ChAT immunoreactive innervations which could mediate the cholinoceptive nature of the AChE positive neurons within the aforesaid nuclei[25]. In the teleosts, studied hitherto, cholinergic cells in the octavolateral area are absent or poorly developed[25-27]. Nonetheless the octavolateral area contains abundant cholinergic cells in dog fish[24]. In other vertebrate groups cholinergic cells appear in very concrete regions[8-9, 29, 33-34]. 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[25]. Among the other

caudal rhombencephalic nuclei, fascial lobes, vagal labes and lateral line lobes, vagal motor nucleus, vagal reticular nucleus and medial funiculus nucleus showed very strong AChE activity in the present study. The presence of cholinergic cells in the visceromotor column is common in all vertebrates studied todate[23-28]. ChAT immunoreactive motoneurons of the nucleus of the spinooccipital nerve in Zebrafish overlap rostrally with the caudal most portion of the ChAT immunoreactive vagal motor nucleus[25]. This nucleus shows cholinergic neurons in other fish[23-24, 28,] and [41]. It is suggested therefore that most of the caudal rhombencephalic motor nuclei are cholinergic in function.

In addition to the main role of AChE i.e. hydrolysis of acetylcholine in to choline and acetate[17], many other findings have shown that AChE hydrolyses substance P, met and leu-enkephalin and could degrade other neuropeptides as well[18-19].Moreover AChE can facilitate neurite growth during embryogenesis[20]. It also acts as neuronal adhesion protein[21].These facts could explain the very wide spread staining, observed in different rhombencephalic nuclei which may be noncholinergic or cholinoceptive in nature.

Table: Acetylcholinesterase Activity in the caudal rhombencephalic nuclei of *H. fossilis*

S. No.	Name of Nuclei	Abbreviation	AChE - activity	Fig. No.
1.	Fascial motor nucleus	NVIIm	+ + + +	2,3
2.	Intermediate reticular nucleus	ImRN	+ + + +	1,7B
3.	Inferior reticular nucleus	IRN	+ + +	2,3
4.	Mauthner cells	MA	+ + +	1,7B
5.	Magnocelluler octaval nucleus	MaON	+ + +	2,7B
6.	Descending octaval nucleus	DON	+ +	3
7.	Secondary octaval nucleus	SO	+ + +	7C
8.	Posterior octaval Nucleus	POC	+ + +	5
9.	Secondary gustatory tract	SGT	+ + + +	1-3 ,7C,7D
10.	Fascial lobes (1-5)	FL 1-5	+ + +	1,7A
11.	Lateral line lobes	LLB	+ + +	1
12.	Vagal lobe	LX	+ + + +	5-6,7F
13.	Vagal motor nucleus	Xm	+ + +	4,7F
14.	Vagal reticular nucleus	Xn	+ + +	4
15.	Medial funiculus nucleus	MFN	+ + +	6

Note: ++++ = Very Intense, +++ = Intense, ++ = Moderate

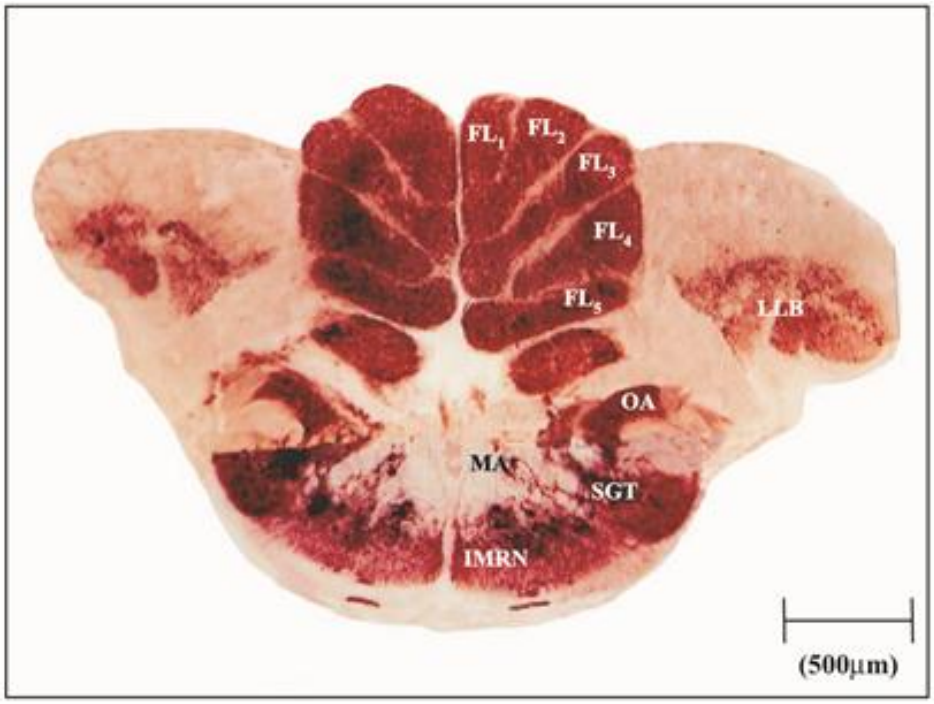

Fig. 1

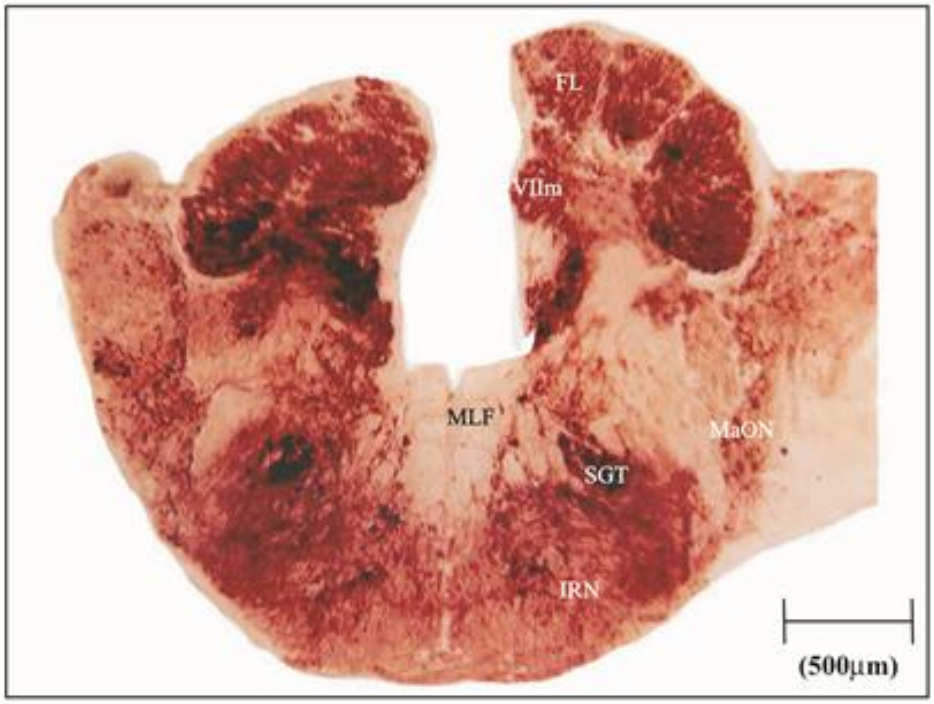

Fig. 2

Fig. 1-2: Photomicrographs of 30 µm thick cryocut transverse sections passing through rostrocaudal region of rhombencephalon. 4X

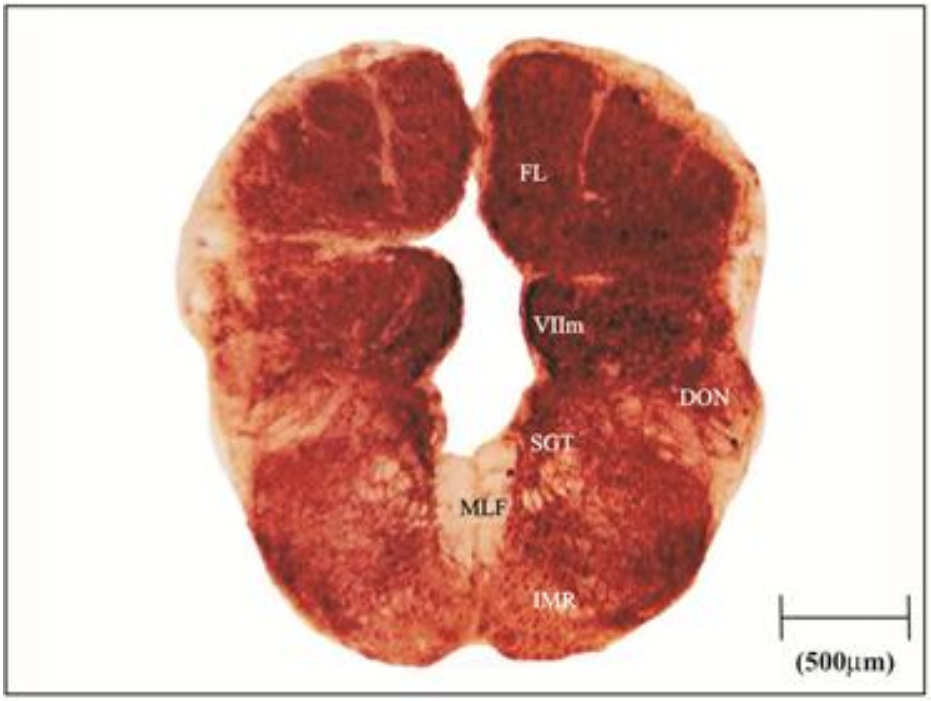

Fig. 3

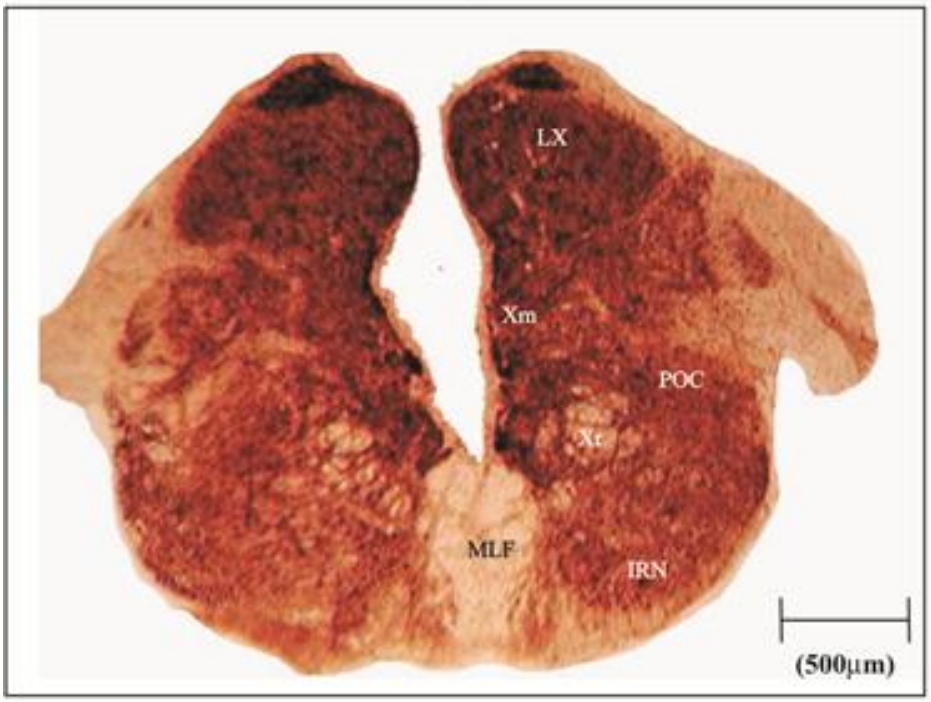

Fig. 4

Fig. 3-4: Photomicrographs of 30 µm thick cryocut transverse sections passing through middle region of rhombencephalon. 4X

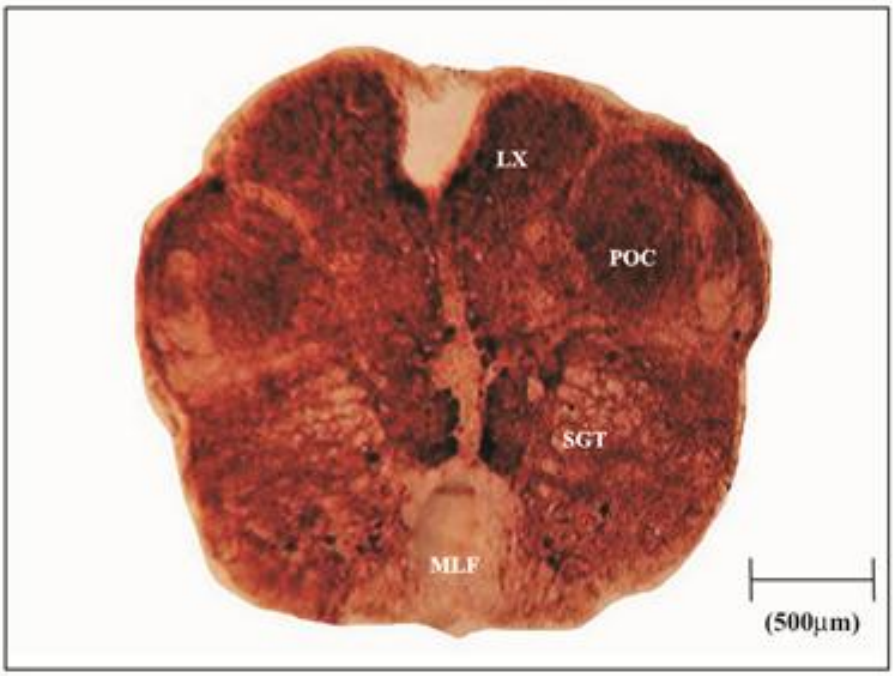

Fig. 5

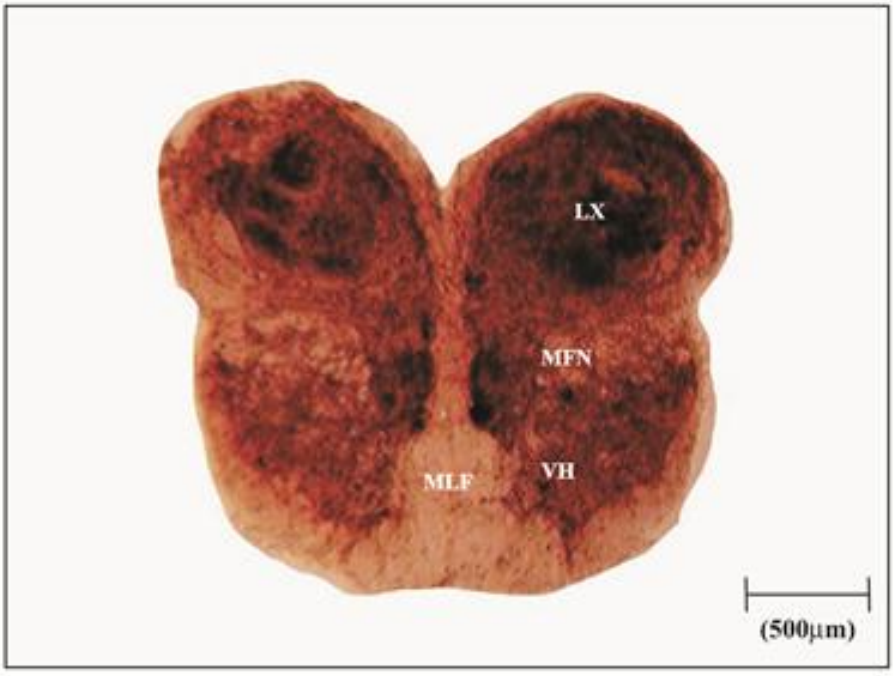

Fig. 6

Fig. 5-6: Photomicrographs of 30 µm thick cryocut transverse sections passing through caudal region of rhombencephalon. 4X

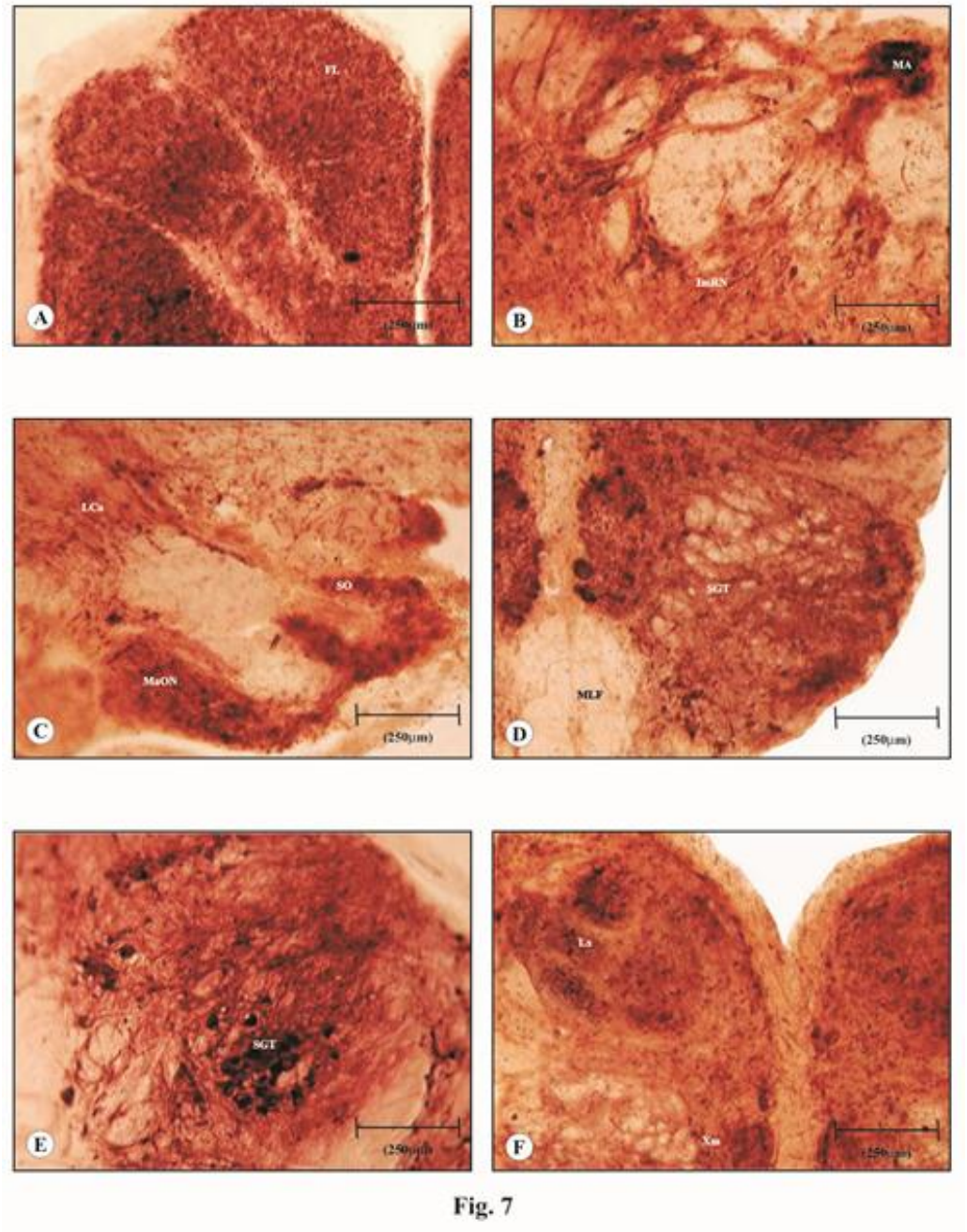

Fig. 7

Fig. 7(A-F): Photomicrographs of 30 µm thick cryocut transverse sections passing through different rhombencephalic areas showing AChE stained nuclei and neuropil. 10X

CONCLUSION

In the present investigations acetylcholinesterase showed a very wide spread intensity in most of the caudal rhombencephalic nuclei from their perykarya, dendrites to the neuropil also. But it was poor to negligible in tracts and commissures. It is interesting to mention that most of the cranial motor nuclei of caudal rhombencephalon are intensly AChE positive as in case of other vertebrates also hence they are considered to be cholinergic in nature. However few nuclei of octavolateral area showed intense activity in our study like in dog fish also which was contrary to other vertebrates where this area is reported to be non cholinergic thus it may be a primitive feature of vertebrates. In addition, some nuclei in our study were AChE positive unlike other vertebrates; it may be attributed to their cholinoceptive nature having innervations from other cholinergic centres.

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