The Scientific Temper (2025) Vol. 16 (11): 5014-5022
E-ISSN: 2231-6396, ISSN: 0976-8653
Doi: 10.58414/SCIENTIFICTEMPER.2025.16.11.05
https://scientifictemper.com/

RESEARCH ARTICLE

Distribution pattern of acetylcholinesterase in the Diencephalic nuclei of *Hemidactylus flaviviridis*

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Abstract

Diencephalon is the part of forebrain which comprises epithalamic, dorsal and ventral thalamic and hypothalamic parts. Neuroanatomically diencephalon is very significant comprising a large area and plenty of nuclear groups. In the present study, distribution pattern of enzyme acetylcholinesterase (AChE) has been carried out in the diencephalon of Indian wall lizard Hemidactylus flaviviridis, by employing a modified histochemical technique to visualize AChE stained nuclei described by Hedreen, J.C. et.al. (1985).

Acetylcholinesterase is an effective marker of cholinergic and cholinoceptive neurons since it hydrolyses neurotransmitter acetylcholine in to choline and acetate at synaptic clefts. Present histochemical results exhibited a widespread distribution of acetylcholinesterase in the different diencephalic nuclei of Hemidactylus flaviviridis from rostral to caudal sections in a mosaic pattern. Most of the thalamic nuclei demonstrated intense activity for acetylcholinesterase. Among the dorsal thalamic nuclei, nucleus dorsolateralis thalami, nucleus medialis thalami and dorsal corpus geniculatum nucleus exhibited intense activity. Among the ventral thalamic nuclei, nucleus ventromedialis thalami, nucleus ventrolateralis thalami, nucleus supra-peduncularis showed intense reaction for AChE. In hypothalamus, magnocellular nuclei demonstrated very high intensity while parvocellular nuclei showed moderate to intense activity at different levels.

Present study establishes that thalamic nuclei are intricately connected to other brain centres with cholinergic involvements and hypothalamus being a neurosecretory part shows mosaic pattern of AChE activity.

In conclusion, the abundance of cholinergic innervations in the thalamic and hypothalamic areas, is a well conserved feature of vertebrates particularly among tetrapods. Present investigation has been compared with the homologous nuclei of other vertebrates studied earlier.

Key words: Cholinergic, Acetylcholinesterase, Parvocellular, Magnocellular, supraoptic nucleus, Nucleus arcuatus.
关键词：胆碱能，乙酰胆碱酯酶，小细胞，大细胞，视上核，弓状核。

Introduction

Diencephalon in vertebrates including presently studied animal can be subdivided in to following distinct regions:

- Epithalamus
- Dorsal thalamus
- Ventral thalamus
- Hypothalamus

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How to cite this article: Tripathi, A. (2025). Distribution pattern of acetylcholinesterase in the Diencephalic nuclei of *Hemidactylus flaviviridis*. The Scientific Temper, **16**(11):5014-5022.

Doi: 10.58414/SCIENTIFICTEMPER.2025.16.11.05

Source of support: Nil

Conflict of interest: None.

Above mentioned diencephalic regions are further divisible in to many nuclear subregions with distinct cyto-architecture, hodology and number of neurons. 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,1989 ; Tripathi and Srivastava, 2010) and fishes brains (Contestebile and Zannoni, 1975; Tago, *et. al.,*1989 ; Northcut and Butler, 1993; Medina and Reiner, 1994, Marin and Gonzalez, 1997; Perez *et.al.,* 2000 ; Anadon *et. al.,* 2000; ; Tripathi *et.*

Received: 28/10/2025 **Accepted:** 16/11/2025 **Published:** 22/11/2025

al., 2013; Tripathi and Rahman,2014; Tripathi, 2021,2022). Present study is the continuation of previous studies by the same author who studied the distribution pattern of AChE in the cerebral hemispheres and medulla oblongata of *Hemidactylus flaviviridis* (Tripathi and Srivastava, 2007; Srivastava and Tripathi,2007). A vast and detailed study on the neurochemistry of the diencephalon of reptiles is still inadequate and scattered. For these reasons a detailed histochemical study was carried out on the distribution of AChE in the different diencephalic nuclear areas.

Materials and Methods

Four adults *Hemidactylus flaviviridis* were sacrificed in the present study. Experimental procedures were performed according to the guidelines of the Institutional Animal Ethics Committee (IAEC). The wall lizard was anesthetized with MS-222 (Sigma, St. Lovis, MO) and decapitated immediately. Brains were taken out and 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 4^0C. 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. 40-micron thick frozen sections were cut by 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 (Hedreen, J.C.,1985) 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

Epithalamus

It consists of the epiphysis,havenular ganglia and the Havinular commissure. Havenular ganglia are located in the dorsomedial corner of the diencephalon. It can be divided in to medial and lateral parts. Lateral havenular nucleus shows moderate activity in all regions (Fig. 7-8; 7A,8A). Medial havenular nucleusshows intense activity in anterior diencephalic region (Fig 5-6, 5A-6A) while it shows mild reaction in caudal region for acetylcholinesterase (AChE) (Fig. 8,8A) AChE activity in the different epithalamic and thalamic nuclei has been summarised in Table 1.

Dorsal thalamus

Dorsal thalamus is occupied by six nuclear groups In the presently studied animal :

- Nucleus dorsomedialis thalami
- Nucleus dorsolateralis thalami
- Nucleus rotundus
- Nucleus medialis thalami
- Corpus geniculum laterale pars dorsalis
- Nucleus medialis posterior

Nucleus dorsomedialis thalami (Dm) is periventricular in position and extends rostro-caudally from the level of the posterior pallial commissure to mid rotundal level (Fig.5-6; 5A-6A). In anterior level it shows moderate reaction but in posterior level it demonstrates intense activity for AChE. Nucleus dorsolateralis thalami(Dl) lies ventrolateral to dorsomedial nucleus. It can be divided in to smaller celled part (Dls) and larger celled part (Dll). For AChE preparations both the parts demonstrated high intensity (Fig.3-4;3A-4A.). Nucleus rotundus (Rot) is located at mid diencephalic level. Rostrally, the nucleus rotundus is separated from the ventricle by the dorsomedial and dorsolateral nuclei; at caudal level it lies immediately adjacent to the ventricular surface (Fig.8,8A). At the rostral level Rot exhibits intense reaction which gradually transforms in to highly intense reaction in caudal levels (Fig.6-7;6A-7A.). Nucleus medialis thalami (Mt) lies ventral to the caudal half of the nucleus rotundus,occupying a periventricular position (Fig.6-7;6A-7A). It shows moderate reaction in rostral region while in caudal regions it demonstrated intense reaction (Fig.6-7). Corpus geniculum laterale pars dorsalis (Cgld)lies dorsolateral to the nucleus dorsolateralis and Rot (Fig.4A,5A). It consists of a sphere of cells with a central neuropil. The neuropil as well as perikaryal demonstrate very high intensity for AChE (Fig.4-5). Nucleus medialis posterior (Mp) constitutes the most caudal cell group of dorsal thalamus. Rostrally the nucleus lies lateral to the medial thalamic nucleus whereas caudally it assumes a periventricular position. This nucleus shows intense reaction for AChE (Fig. 4-5; 4 A-5A)

Ventral thalamus

It is considerably smaller than the dorsal thalamus and its nuclei are less distinct. However, eight nuclei could be recognized in the present study. Nucleus Interstialis (I) lies in the rostral part of ventral thalamus and consists of smaller cells. Caudally this nucleus is replaced by the dorsolateral nucleus of the hypothalamus and it demonstrated moderate activity for AChE (Fig. 3, 3A)

Area triangularis (At) contains larger cells than those of nucleus interstitialis. It lies between septo-hypothalamic tract and lateral fore-brain bundle in rostral parts. It demonstrated negative reaction for AChE (Fig. 3, 3A).

Nucleus ovalis (O) is located in the caudal area triangularis which is made up of the densely packed cells. It showed mild activity. Nucleus ventromedialis thalami (Vmt) lies in the mid thalamic region situated dorsolateral to the septo-hypothalamic tract. It demonstrated very intense reaction (Fig.5, 5A).

Corpus geniculatum laterale pars ventralis (Cglv) is composed of a medial cell plate and a lateral neuropil. It revealed very high intensity for AChE (Fig.4-5;4A-5A). Nucleus supra peduncularis (Sped) can be traced throughout almost the entire thalamic region .it demonstrated very high intensity for AChE (Fig.4,8;4A,8A). Nucleus entopeduncularis (Ent) can be recognized at rostral thalamic level and consists of cells intermingling with the fibres of Lateral fore bundle (LFB) it showed mild reaction (Fig.4,4A).

Nucleus ventrolateralis (Vltd) is located at the ventrolateral position of thalamus. It consists of dorsal and ventral subdivisions that have medial and ventromedial position with respect to the Cglv. Both the nuclei revealed very high intensity for AChE preparation (Fig. 7-8).

Hypothalamus

The largest portion of the diencephalon in the Hemidactylus is composed of hypothalamic nuclei. It is rostrally continuous with the preoptic area. The lateral wall of the diencephalon is thick which forms optic thalami and floor of the diencephalon is the hypothalamus on the ventral side of hypothalamus, two optic nerves cross each other forming optic chiasma. Laterally hypothalamus is bound by medial and lateral forebrain bundles. Almost, all the hypothalamic nuclei are paired and they are classified in to magnocellular and parvocellular regions. AChE activity in the various hypothalamic nuclei has been summarised in Table 2.

Magnocellular region

It consists of three nuclei; Nucleus supraopticus (SON) is the largest group of magnocellular cells. This nucleus is conspicuous and begins anteriorly above optic chiasma. It demonstrated very high intensity for AChE from rostral to caudal regions (Fig. 1-2). Nucleus paraventricularis (PVN) is located in either side of the third ventricle. The paraventricular neurons are generally than those of SON. This nucleus shows moderate activity for AChE (Fig.1,1A). Bridge cells (B) are visible in the area between SON and PVN and show moderate activity for AChE (Fig.1-2).

Parvocellular neuronal complexes

It comprises a large number of nuclei. Nucleus supra chiasmaticus (SCN), is located dorsal to optic chiasma and lateral to the ventral margin of the preoptic recess and sub ventricular gray. It demonstrated moderate activity (Fig.1-2). Nucleus commissure anterioris (NAC) is demarcated ventral to the anterior commissure and dorsomedial to the third ventricle. It showed intense activity for AChE (Fig.1-2). Lateral preoptic area (LPA) is bordered laterally by forebrain bundle and medially by anterior and posterior periventricular nuclei. It revealed mild action in anterior sections while moderate activity in posterior sections(Fig.1-2). Nucleus periventricularis anterior (APV) forms one of the largest nuclei of the preoptic area. Its anterior margin is bordered medially by the nucleus preopticus periventricularis and laterally by LPA. It demonstrated moderate activity(Fig.2,2A). Nucleus periventricularis posterior (PPV) forms the second largest nuclear area of the preoptic region. It is bordered laterally by the LPA and medially by nucleus hypothalamicus periventriclaris and third ventricle. It demonstrated moderate action for AChE preparations(Fig.2). Nnucleus hypothalamicus periventricularis (HPE) is bilaterally compressed nucleus disposed dorsoventrally to the third ventricle. It is bordered laterally by nucleus periventricularis. It demonstrated moderate action(Fig.2). Nucleus arcuatus (A) is the largest hypothalamic nucleus. It gets visible dorsally at the level of periventricular nucleus and extends caudally as per as the rostral end of the median eminence. Rostrally, it shows mild activity but interestingly it demonstrated negativity for AChE caudally (Fig.3,8).

Nucleus hypothalamicus ventromedialis (VMH) occupies the area between nucleus arcuatus and the ventricular region rostrally but its caudal end protrudes in to an area dorsal to 'A'. It exhibited mild activity (Fig.3,3A). Nucleus hypo-thalamicus dorso-medialis (DMH) is situated dorso-medially to the third ventricle and dorsally to the nucleus periventricular organ (PVN). It showed moderate activity (Fig.5,5A). Nucleus hypothalamicus lateralis (LH) is demarcated dorsomedially to the 'A' medially to the VMH. It showed negative activity in anterior region while mild action in caudal regions for AChE preparations (Fig.5,8). Nucleus ventralis tuberis (VT) is a rod shaped bifurcated nuclear entity located in the infundibular floor ventral to the third ventricle. It showed moderate activity (Fig.4). Paraventricular organ (PVO) is flanked by the nucleus of the paraventricular organ (NPVO). Its anterior portion extends rostral to preoptic area. In rostro-caudal direction it fans out to the level marked ventrally by the caudal end of the 'A'. It showed negative reaction for AChE (Fig.4). Nucleus sub-fornicales (SF) is small oblong in shape and bordered dorsally by the anterior periventricular nucleus and ventrally by the posterior ventricular nucleus. It showed moderate activity for AChE (Fig.2). Nucleus hypothalamicus posterior (HP) occupies the area between the 'A' and the ventricular border. It is negative for AChE rostrally but it showed mild activity for AChE (Fig.5,8). Nucleus preopticus peri-ventricularis (NPP) commences at the level marked dorsally by the anterior commissure and terminates caudally slightly rostral to the nucleus para-ventricularis. It showed moderate activity for AChE (Fig.1). Nucleus of the preoptic recess (NPOR) occupies the area dorsal to the SCN and extends medially to the preoptic recess. It demonstrated moderate activity (Fig.2). Nucleus of the subventricular gray (SVG) is composed of scattered neurons located slight ventral to the preoptic recess. Rostrally it is located dorsal to OC. Rostrally it is moderately positive but caudally it showed mild activity (Fig.2). Median eminence (ME) is located in the caudal most regions of hypothalamus ventral to ventricle. It demonstrated mild activity for AChE (Fig.7-8). However, few additional nuclear groups present on the comparable regions of diencephalon in turtle

Table 1: Various diencephalic nuclei showing AChE activity

S. No.	*Name of Nuclei/Fibres*	*AChE Activity*	*Abbreviation*	*Fig. No.*
Epithalamus & Dorsal Thalamus				
1.	Lateral habenular nucleus	+–	Hbl	7,8
2.	Medial habenular nucleus	++	Hbm	6-8
3.	Nucleus dorsomedialis thalami	++	Dm	5-6
4.	Nucleus dorsolateralis thalami	++	Dl	3
5.	Nucleus rotundus	+++	Rot	6-8
6.	Nucleus medialis thalami	+++	Mt	6-7
7.	Corpus geniculatum laterale pars dorsalis	+++	Cgld	4-5
8.	Nucleus medialis posterior	+++	Mp	4-5
Ventral thalamus				
7.	Nucleus interstitialis	+–	I	3
8.	Area triangularis	– –	At	3
9.	Nucleus ovalis	+–	O	4
10.	Nucleus ventromedialis thalami	+++	Vmt	5
11.	Nucleus ventrolateralis thalami pars dorsalis	+++	Vltd	7-8
12.	Nucleus ventrolateralis thalami pars ventralis	+++	Vltv	8
13.	Nucleus suprapeduncularis	+++	Sped	4,8
14.	Nucleus entopeduncularis	+ –	Ent	4
15.	Corpus geniculatum laterale pars ventralis	+++	Cglv	4-5

Table 2: Hypothalamic nuclei showing AChE activity

Sl. No.	*Name of Nuclei/Fibres*	*AChE Activity*	*Abbreviation*	*Fig. No.*
Hypothalamus				
1.	Nucleus supraopticus	++++	SON	1
2.	Nucleus paraventricularis	++	PVN	1
3.	Bridge cells	++	B	1
4.	Nucleus Suprachiasmaticus	++	SCN	1-2
5.	Nucleus commissure anterioris	+++	NAC	1-2
6.	Lateral preoptic area	+–	LPA	1-2
7.	Nnucleus periventricularis anterior	++	APV	2
8.	Nucleus periventricularis posterior	++	PPV	2
9.	Nucleus hypothalamicus periventricularis	+–	HPE	2
10.	Nucleus arcuatus	+–	A	3,8
11.	Nucleus hypothalamicus ventromedialis	+–	VMH	3
12.	Nucleus hypothalamicus dorsomedialis	++	DMH	5
13.	Nucleus hypothalamicus lateralis	++	LH	5,8
14.	Nucleus ventralis tuberis	++	VT	4
15.	Paraventricular organ	– –	PVO	4
16.	Nucleus subfornicales	++	SF	2
17.	Nucleus hypothamicus posterior	– –/ + –	HP	5,8
18.	Nucleus preopticus periventricularis	++	NPP	1
19.	Nucleus of the preoptic recess	++	NPOR	2
20.	Nucleus of the subventricular gray	++	SVG	2
21.	Median eminence	+–	ME	7-8

Notation:
++++ = Very Intense +++ = Intense ++ = Moderate +– = Mild — — = Negative

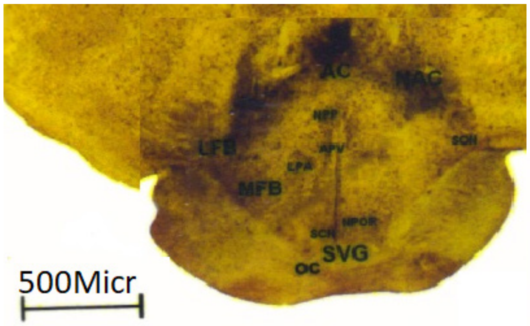

Fig.1

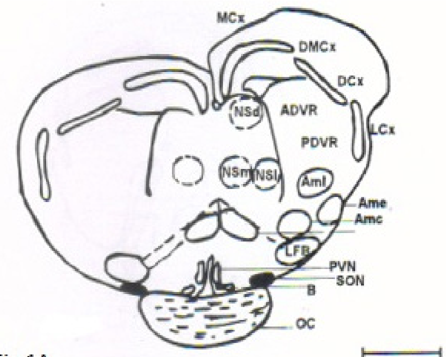

Fig.1A

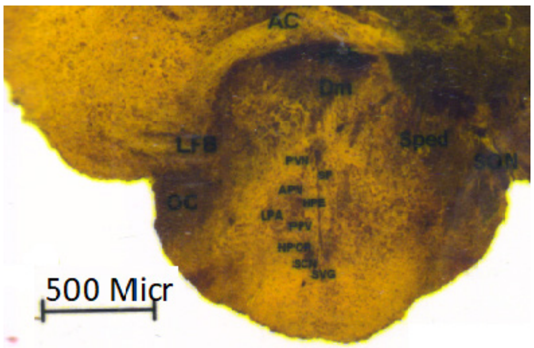

Fig.2

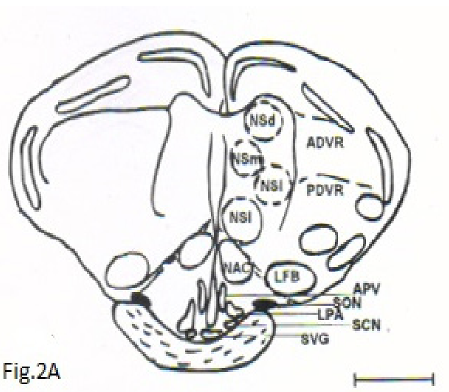

Fig.2A

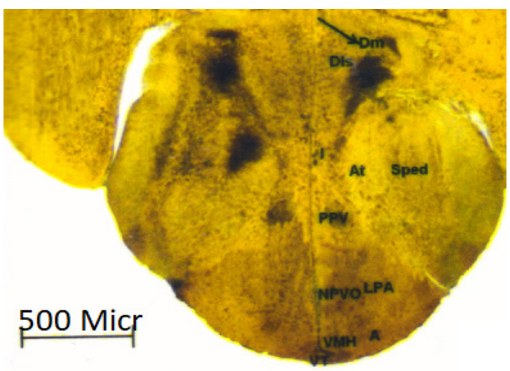

Fig.3

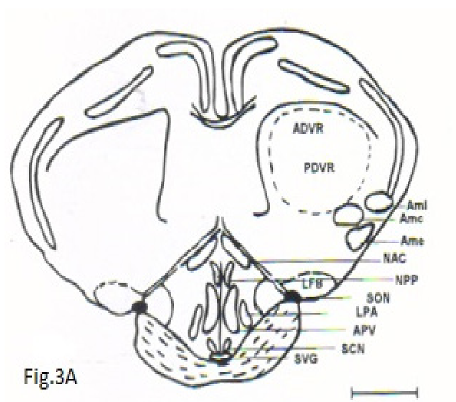

Fig.3A

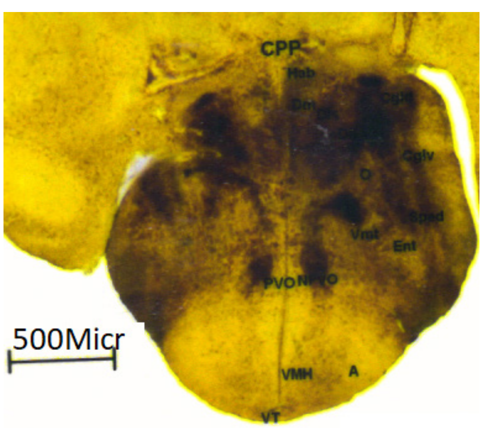

Fig.4

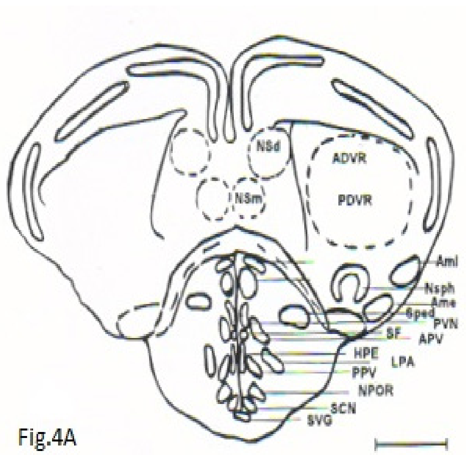

Fig.4A

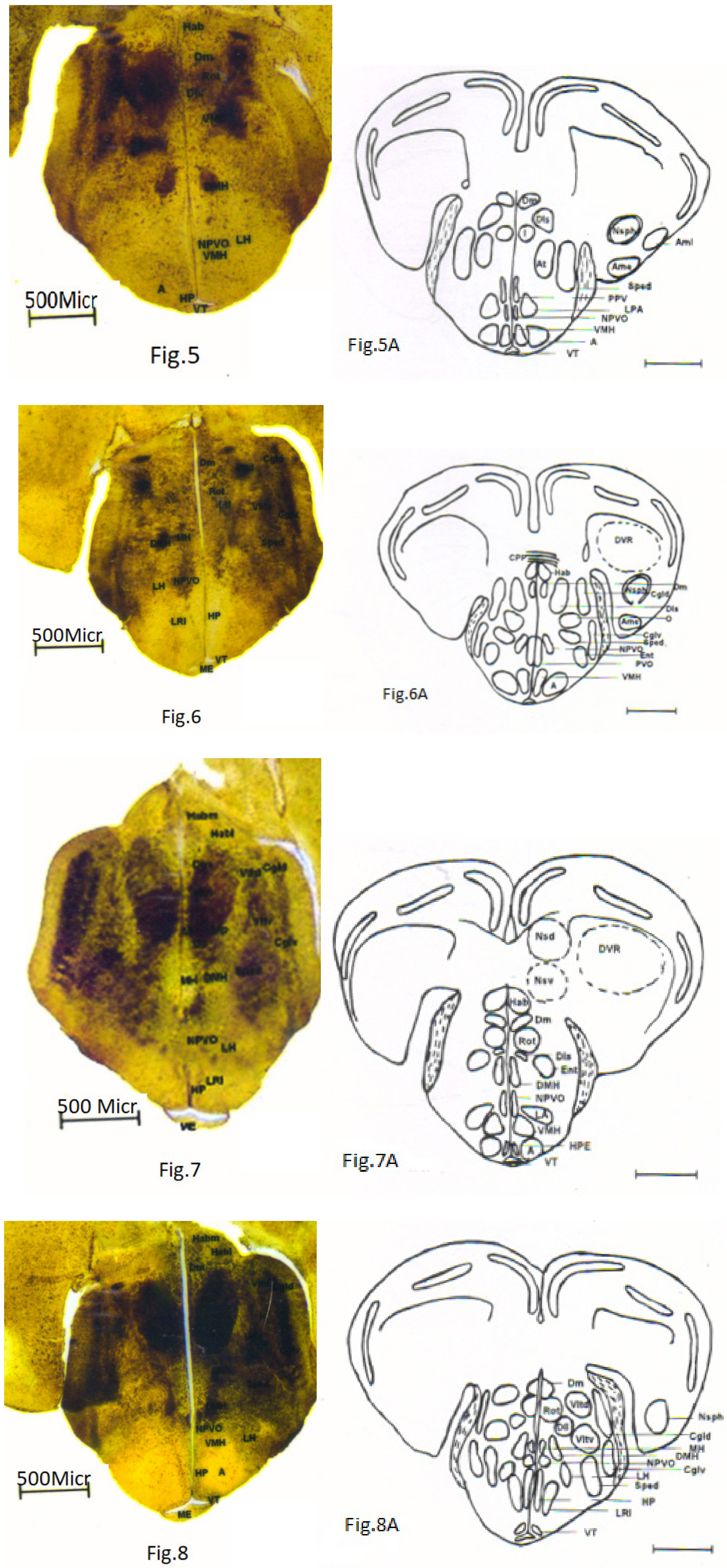

Fig. 1-8: Photomicrographs of Acetylcholinesterase positive nuclei in the different diencephalic centres from rostral to caudal regions (Fig.1A-8A, diagrammatic representation of diencephalic nuclei)

and other reptiles are obscure and need more precise investigations.

Discussion

In the diencephalic region ten thalamic nuclei (Dls, Rot, Mt, Cgld,Mp, Vmt, Vltd, Vltv, Spedm, Cglv) demonstrated very high intensity for AChE. It might be due to excess and intricate connections of thalamic nuclei with the other forebrain and hindbrain centres involved in higher brain processing and controlling the cognitive and emotional behaviour of animals (Bartus, 1982) with deep involvement of cholinergic neurotransmission. Interestingly commissures and fibre tracts demonstrated negativity for AChE. These findings are in tune with the findings of Sethi and Tewari (1976). It is therefore suggested that positive staining in the nuclei at the gross level may have been contributed by neurons and glial cells. The fibre tracts and commissures have no part in the overall picture of histo-enzymological map. All the nuclei whether they are collective or discharge centres are intensely positive. Among the hypothalamic nuclei magnocellular neurosecretory centres of the most of vertebrates showed hito-chemically detectable AChE activity (amphibians, Shen et al.,1955; birds, Kobayashi and Farner,1964; Follet et al., 1966; Kusunoki, 1969; mammals, Cottle and Silver, 1970a; Parent and Butcher, 1976; Parent et al., 1977,1979).With particular reference to reptiles, in turtle *Chrysemys picta*, perikarya of the supraoptic nuclei showed strong AChE activity (Parent, 1979). In cobra all the magnocellular subdivisions viz., SON, C, PVN, RCN, showed intense activity (Subhedar and Rama Krishna, 1989). In present study SON and PVN showed intense activity. In addition, the bridge cells extended between SON and PVN as reported by Haider and Sathyanesan (1974) also showed strong activity. The well-developed AChE positive magnocellular system in cobra and *Hemidactylus* may be a reflection of their terrestrial mode of life. Friede (1966) reported increased AChE activity in the perikaryal of SON in rats subjected to high salt diet. Besides, possibilities that AChE may be involved in the neurosecretory activity with the release of neurohormones (Alexendrava et al.,1987). In the absence of any concrete evidence, studies based on AChE histochemistry may be useful in tracing interspecific homologies of nuclear groups.

Analysis of the available data on AChE in different vertebrates including the present study reveals the widespread similarities between putative homologous nuclear groups. As in case of *Hemidactylus*, AChE containing cells were also absent in the medial preoptic area of monkey (Parent and Oliver, 1970) and cobra (Subhedar and Ram Krishna, 1989). However, scattered reactive neurons were observed in both cobra as well as wall lizard in the POA. The neurons of SCN in cobra (Subhedar and Ramakrishna, 1989) and presently studied animal were weekly stained reflecting the similarity between these two. In mammals, the neurons of lateral hypothalamus and nucleus dorso-medialis were positive for AChE (Jacobowitz and Pulcovits,1974; Parent and Olivier,1970; Parent et al., 1979), whereas the nuclei occupying corresponding positions in cobra (Subhedar and Rama Krishna,1989) showed intense reaction while the nucleus hypothalamicus dorsomedialis showed weak to moderate activity. Inour investigation both these nuclei demonstrated moderate activity. In the monkey (Parent et al., 1979) nucleus ventromedialis was negative to AChE and in cobra (Subhedar and Rama Krishna ,1989), it was weakly stained. In our findings also, this nucleus showed mild activity.

The results on the nucleus arcuatus in different forms are somewhat at various. In rat, ‘A’ is composed of mixed cell population with most neurons stained moderately for AChE but a few showed strong reaction (Parent and Olivier,1970). The homologous nucleus infundibularis in the white crowned sparrow (*Zonotrichia leucophyris gambelli*) also showed the presence of AChE (Kobayashi and Farner,1964). In guinea pig, this nucleus displayed varying degree of AChE activity which could be correlated to reproductive stage of animal (Cottle and Silver, 1970a). In cobra the nucleus arcuatus showed very intense reaction (Subhedar and Rama Krishna,1989). In presently studied animal this nucleus demonstrated mild reaction in rostral sections while negative reaction in caudal sections. In cobra PVO showed intense activity (Subhedar and Rama Krishna, 1989) but in our study this nucleus in wall lizard demonstrated mild activity. Interestingly in zebra fish hypothalamus no cell somata is reported to be positive for AChE and the presence of cholinergic and cholinoceptive cell group is poorly represented in most teleosts (Clement et al., 2004).

In overview of the wall lizard hypothalamus stained with AChE revealed a distinct mosaic pattern with some nuclei being totally negative, some moderate, some intense, while others displaying either the intermediate or the heterogenous reaction of the perikarya particularly in the tuberal region nucleus arcuatus showed mild to negative reaction. Whereas the nucleus hypothalamicus posterior and nucleus paraventricular organ demonstrated total negativity.

On the lateral aspect, the anterior and posterior subdivisions of hypothalamicus lateralis demonstrated mild activity. VMH located rostrally to nucleus arcuatus showed weak reaction. The cells of DMH were moderately stained. Nucleus peri-ventricularis anterior as well as peri-ventricularis posterior demonstrated intense reaction. The occurrence of cell clusters of different characteristics and the zone of AChE positive and negative neuropil, the wall lizard hypothalamus appears as a progressive neuroanatomical structure to subserve elaborate neuroendocrine functions. The significance of the discrete cell groups interacting in the reptilian hypothalamus has already been emphasised by Oksche (1978a, b). The ME of mammals demonstrate negativity for AChE (Parent *et al*., 1979). Cobra ME cells demonstrated intense activity (Subhedar and Rama Krishna, 1989) as also revealed by present investigation. The functional significance of AChE containing cells in ME of wall lizard is not well known however a considerably important role for the enzyme in the release of neurosecretory material may be suspected. In this connection the suggestion that AChE may be involved in the production, transport or mobilization of neurosecretory material including releasing factors is noteworthy (Cottle and Silver, 1970 b). With reference to wall lizard, it may be interesting to find out if, the AChE distributed in a well-defined pattern in magnocellular and parvocellular systems, neuropil zones and in ME, subserves a more dominant role in neuroendocrine regulation.

Acknowledgements

This work was supported by the CSIR fellowship F. No. 2-56/2002(I) EU and U.P. Govt (Higher Education Department) sponsored Research Project dated 28/12/21. Author acknowledges, his supervisor, Prof. U. C. Srivastava, Ex. Professor, Department of Zoology, University of Allahabad for his constant guidance and support. Author is also thankful to Head Department of Zoology, University of Allahabad for providing equipment facilities.

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