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# Species variation in the localisation of esterases in the cerebellar cortex of mouse and bat

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**Abstract.** A comparative study of the distribution of a simple esterase and acetylcholinesterase in the cerebellar cortex of mouse and bat has been made. The Purkinje layer is intensely positive for simple esterase in both species. The granular and molecular layers showed mild to moderate activity in mouse and intense activity in bat. Acetylcholinesterase in cerebellar layers of bat is more intense than in mouse. In bat cerebellum, acetylcholinesterase is observed in the dendrites of Purkinje cells, but not in their cell bodies. Acetylcholinesterase was not found in Purkinje cells of mouse.

**Keywords.** Simple esterase; acetylcholinesterase; cerebellar cortex; mouse; bat.

## Introduction

A number of histochemical studies on the distribution of acetylcholinesterase (AChE) in the cerebellar cortex of rat (Tewari and Bourne, 1962; Brown and Palay, 1972), squirrel monkey (Shantha *et al.*, 1967), cat (Kasa *et al.*, 1965), man, cow, rabbit, guinea pig, pigeon, hamster, parakeet (Friede and Flemming, 1964) and squirrel (Tewari and Bhatt, 1978) have been made. Much species variation in the pattern of distribution has been noted (Friede, 1966; Sood and Bohra, 1977) and this has been correlated with the brain size.

The bat has an arboreal mode of life and hence the body balancing system must be better developed than mouse, a burrowing animal. The vestibular nuclei, which receive the statical impulses from the ampullae of semicircular canals and redirect them to the cerebellum, are better developed in microchiropteran bat than mouse. It is therefore of interest to study the distribution of AChE, an enzyme associated with impulse transmission in bat and mouse cerebellar cortex. The distribution of simple esterase (SE) in the different layers of cerebral cortex of mouse and bat was also studied for comparison.

## Materials and methods

Young, unanaesthetised, locally collected mice (*Mus. musculus*) and bats (*Taphozous melanopogan*) of both sexes were sacrificed by decapitation and the cerebellar cortex

Abbreviations used : acetyl choline esterase, AchE ; simple esterase, SE.

was quickly removed and fixed in 10% formalin chilled at 4°C, 30 $\mu$ thick sections, cut on freezing microtome, were washed thoroughly in distilled water, and processed for acetylcholinesterase (Gomori, 1952) and simple esterase (Barka and Anderson, 1963). Brief incubation periods of 3 h for acetylcholinesterase and 5 to 10 min for simple esterase were used. The controls were prepared simultaneously by incubating the sections in substrate-free media, by boiling the sections in distilled water for 5 min before incubation and by using appropriate inhibitors as recommended in the techniques.

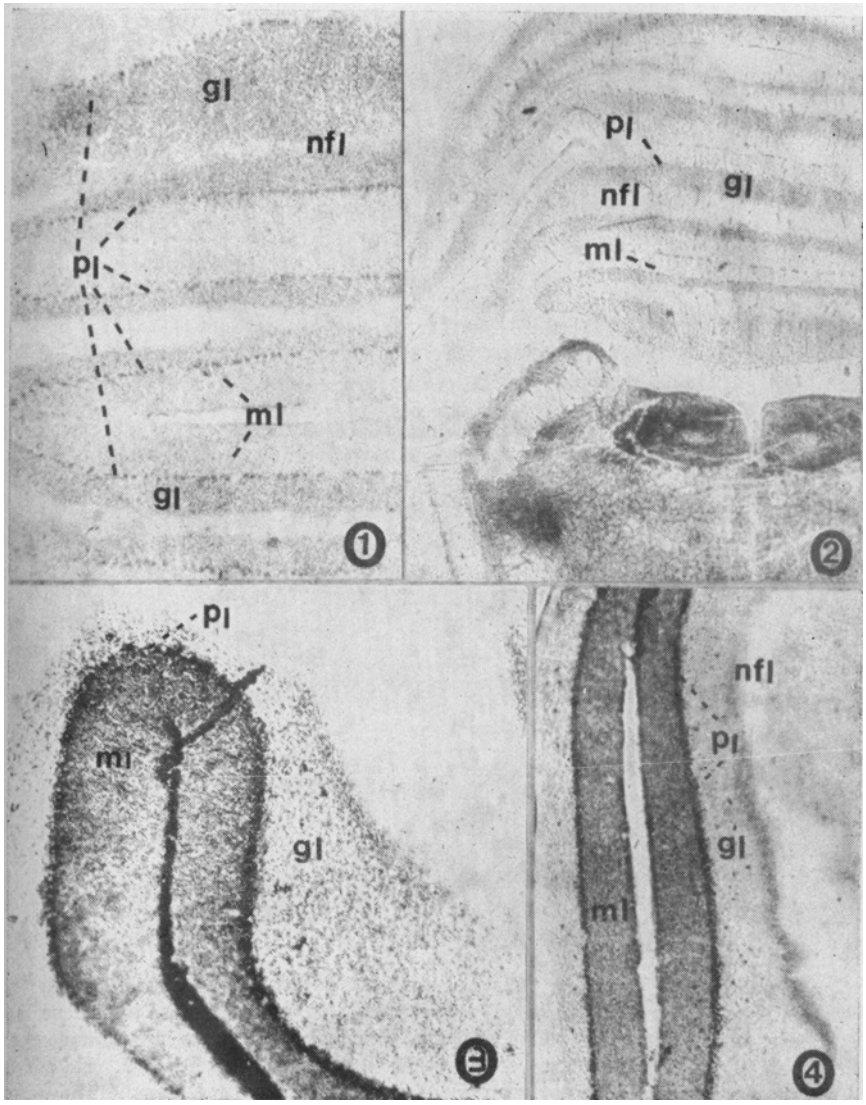
## Results

In AChE preparations, the Purkinje and molecular layers in mouse cerebellar cortex show complete lack of enzymatic activity, whereas the granular layer (figure 2) exhibits a mild diffused reaction. In contrast, bat cerebellar cortex showed more intense activity (figures 5 and 6). Though the Purkinje cells of bat are negative for AChE (figures 5 and 6), their processes, running in the molecular layer, demonstrate intense beaded reaction (arrows; figure 6). Hence, the molecular layer becomes moderately positive (figures 5 and 6) though its cells are completely negative for the enzyme. The granular layer shows intense activity (figures 5 and 6). The layer of nerve fibres is completely negative in both the animals (figures 2 and 5).

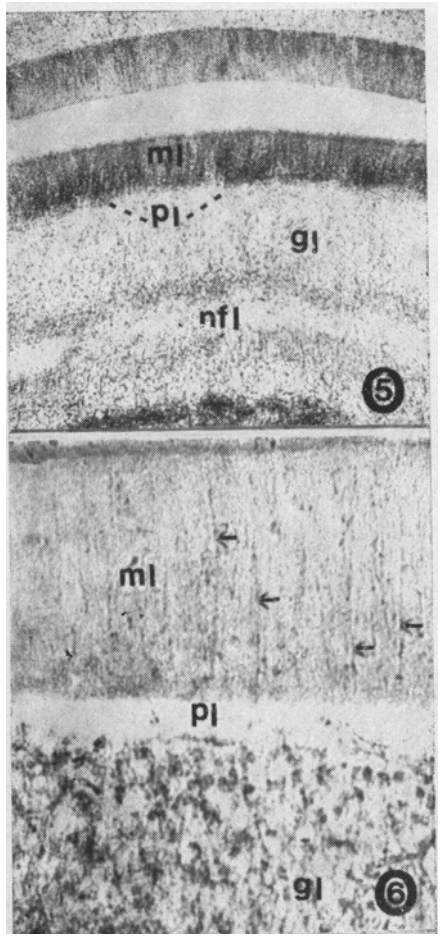
In simple esterase preparations, the Purkinje layers are clearly marked out as these cells are intensely positive in both the animals (figures 1, 3 and 4) . However, the activity of this enzyme in Purkinje cells of bat is more intense than mouse (compare figures 1 and 3) . The molecular layer is intensely positive in bat and mildly positive in mouse. A similar difference is also seen in the granular layers of these animals. The nerve fibre layers in both the animals are free from SE activity (figures 1 and 4).

## Discussion

Literature reveals much species variation of AChE in the different layers of cerebellar cortex (Friede, 1966; Arvy, 1966) but its functional significance is not known. While comparing the localisation of esterases in cerebellar cortex of the mouse and bat a number of differences have been observed. Thus in molecular and granular layers of the cerebellar cortex of mouse, the distribution of AChE differs from man, guinea pig, pigeon, parakeet, monkey and squirrel, where mild to intense activity of the enzyme is observed either in one of these layers or in both the layers (Friede, 1966). In contrast, there is little variation in AChE activity in the Purkinje layer in most of the mammals, including mouse and the layer shows complete absence of AChE (Tewari and Bourne, 1962; Shantha *et al.,* 1967). However, though the Purkinje cell bodies of bat are devoid of enzymatic activity, yet their processes demonstrate intense activity. Since the enzyme is absent in perikarya of Purkinje cells, it may be synthesised in the processes. This view is also shared by Koenig (1965) and Tennyson *et al.* (1967). The presence of AChE in the processes indicate that in the bat cerebellar cortex the Purkinje cells are cholinergic.




**Figures 1–4. 1.** Transverse section of cerebellar cortex of mouse demonstrating SE. Note intense activity in Purkinje layer (pl), moderate activity in molecular layer (ml). The nerve fibre layer is completely negative (nfl). × 60. **2.** Transverse section of cerebellar cortex of mouse demonstrating AChE. Note mild reaction in granular layer (gl) and negative molecular (ml), Purkinge (pl) and nerve fibre (nfl) layers. × 45. **3, 4.** Transverse section of cerebellar cortex of bat demonstrating intense activity of SE in the Purkinje (pl), molecular (ml) and granular (gl) layers. The nerve fibre layer is completely negative. × 60 and 50.




**Figures 5–6. 5.** Transverse section of cerebellar cortex of bat. Note intense activity of AChE in the granular layer (gl) and moderate to intense activity in the molecular layer (ml). The nerve fibre (nfl) and Purkinje (pl) layers are completely free from enzymatic activity. × 50. **6.** High magnification of figure 5. Note intense granular activity in the processes in the molecular layers (arrows). The granular layer is intensely positive, whereas the Purkinje layer is completely free from the enzymatic activity. × 110.

AChE activity in the cerebellar cortex of bat was observed to be higher than in mouse. In a recent study on the hind brain of a microchiropteran bat (*Taphozous melanopogon*), the vestibular nuclei showed strong AChE activity (Hafiza, 1979). In comparison, the same area in mouse brain was earlier shown to have less AChE activity (Sood and Bohra, 1977). The vestibular nuclei receive statical impulses from the ampullae of semicircular canals and sacculus and project into the cerebellar cortex, and since these nuclei and cerebellar cortex of bat in general show good activity of enzyme, it appears that in bat, statical system is better developed than in mouse. The vestibular nuclei of bat also demonstrate intense activity of ATPase and succinic dehydrogenase (Hafiza, 1979).

The most intense reaction of simple esterase in the cerebellar cortex of mouse and bat is seen in Purkinje layers. The molecular and granular layers are mild to moderately positive. The enzyme in these layers is observed in the perikarya, neuropil, nerve fibres and their synapses. Earlier, Tewari and Bourne (1962) reported a similar activity of this enzyme in cerebellum of rat. However, the function of simple esterase in nervous system is not known.

## References

Arvy, L. (1966) in *International review of cytology,* eds. G. H. Bourne and J. F. Danielli (London and New York : Academic Press), **20,** 277.

Barka, T. and Anderson, P. J. (1963) *Histochemistry—theory, practice and bibliography* (New York : Harper and Row).

Brown, W. J. and Palay, S. L. (1972) *Z. Anat. Entwickl. Gesch.* **137** 317.

Friede, R. L. and Flemming, L. M (1964) *J. Neurochem.,* **11,** 1.

Friede, R. L. (1966) *Topographic brain chemistry* (New York and London: Academic Press).

Gomori, G. (1952) *Microscopic histochemistry* (Chicago : University Press).

Hafiza, B. (1979) *Histochemical studies of certain enzymes in the central nervous system of bat* (*microchiroptera*) *with special reference to medulla oblongata and pons,* Ph.D. thesis, submitted to Department of Zoology, University of Udaipur.

Kasa, P., Joo, F. and Csillik, B. (1965) *J. Neurochem.,* **12,** 31.

Koenig, E. (1965) *J. Neurochem.,* **12,** 343.

Myers, D. K. and Mendal, B. (1953) *Biochem. J.,* **53,** 16.

Shantha, T. R., Iijima, K. and Bourne, G. H. (1967) *Acta Histochem.,* **27,** 129.

Sood, P. P. and Bohra, M H. (1977) *J. Hirnforsch.,* **18,** 75.

Tennyson, V. M., Brzin, M. and Duffy, P. E. (1967) *J. Neuropathol. Exp. Neurol.,* **26,** 136.

Tewari, H. B. and Bhatt, D. K. (1978) *Zool J. Anat.* (in press).

Tewari, H. B. and Bourne, G. H. (1962) *Acta Anat.,* **51,** 349.