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The segmental distribution of acetyl cholinesterase in the cat spinal cord

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INTRODUCTION

The histochemical method for the localization of acetyl cholinesterase activity has been applied to many parts of the central and peripheral nervous system with a view to correlating function and structure. The spinal cord has thus far remained largely uncharted, with the exception of data on selected segments of the upper cervical cord in the rat and in other species by Koelle (1954), Giacobini & Holmsted (1957), Gerebtzoff (1959) and Snell (1961). The present study provides a complete survey of the distribution of acetyl cholinesterase in the segments of the feline spinal cord. The data show that the various nuclear groups of the spinal grey matter differ characteristically in their patterns of enzyme activity. Indeed, the histochemical method delineates nuclear groups more clearly than do conventional methods and can be recommended as a convenient and reliable tool for neuroanatomical studies of the cord.

MATERIAL AND METHODS

Six male cats weighing 2000–2500 g were sacrificed by giving overdoses of barbiturate, and the spinal cords were removed in *toto*. Immediately upon removal the cords were fixed in neutral formalin at 5 °C for 24 h. The segments of the cord were then blocked individually and 30 μ frozen sections were cut from each block. The sections were washed in distilled water for 3 h; then three to four sections from each block were incubated in separate containers for 90–150 min in a water-bath shaker at 38 °C. The incubation medium consisted of 132 ml of 0·2M Trismaleate buffer at pH 6·6; 3 ml of 3·75 % glycine; 3 ml of 0·1M cupric acetate, and 12 ml of acetylthiocholine iodide. The substrate was prepared immediately before use in the following proportions: 180 mg of acetylthiocholine iodide; 9·36 ml of glass-distilled water; and 3·12 ml of 0·1 M copper acetate. The solution was centrifuged for 15 min at 3500 to 4000 rev min. Approximately 5 ml of incubation medium was used for each three to four sections. After incubation the medium was poured off and the sections were immersed with rapid motion in glass-distilled water, then in light yellow ammonium sulphide for exactly 20 s, and then washed off in three changes of glass-distilled water. The sections were dehydrated and mounted in Permount. Additional sections were cut from selected blocks of one spinal cord for inhibitor controls. The inhibitors used were eserine sulphate,* 10^{-5}M; BW,* 10^{-5}M; Iso-OMPA* 10^{-6}M. The sections were incubated in inhibitor solutions for thirty min prior to incubation in media containing the inhibitors.

* Eserine sulphate, Malenckrodt Co. BW 284c51, Burroughs-Wellcome Co. Tetraisopropylpyrophosphoramide (Iso-OMPA), K and K Co.

RESULTS

General distribution patterns

Acetyl cholinesterase (AChE) activity was generally limited to neuronal cell bodies and their processes. Thus the staining in the white matter consisted chiefly of axonal activity. The large axons showed evenly distributed high activity, but smaller fibres were difficult to distinguish. One reason for this might be their smaller volume, but it is also possible that many of the smaller fibres lacked enzyme activity.

In the grey matter the neuronal perikarya and the large dendrites and axons stood

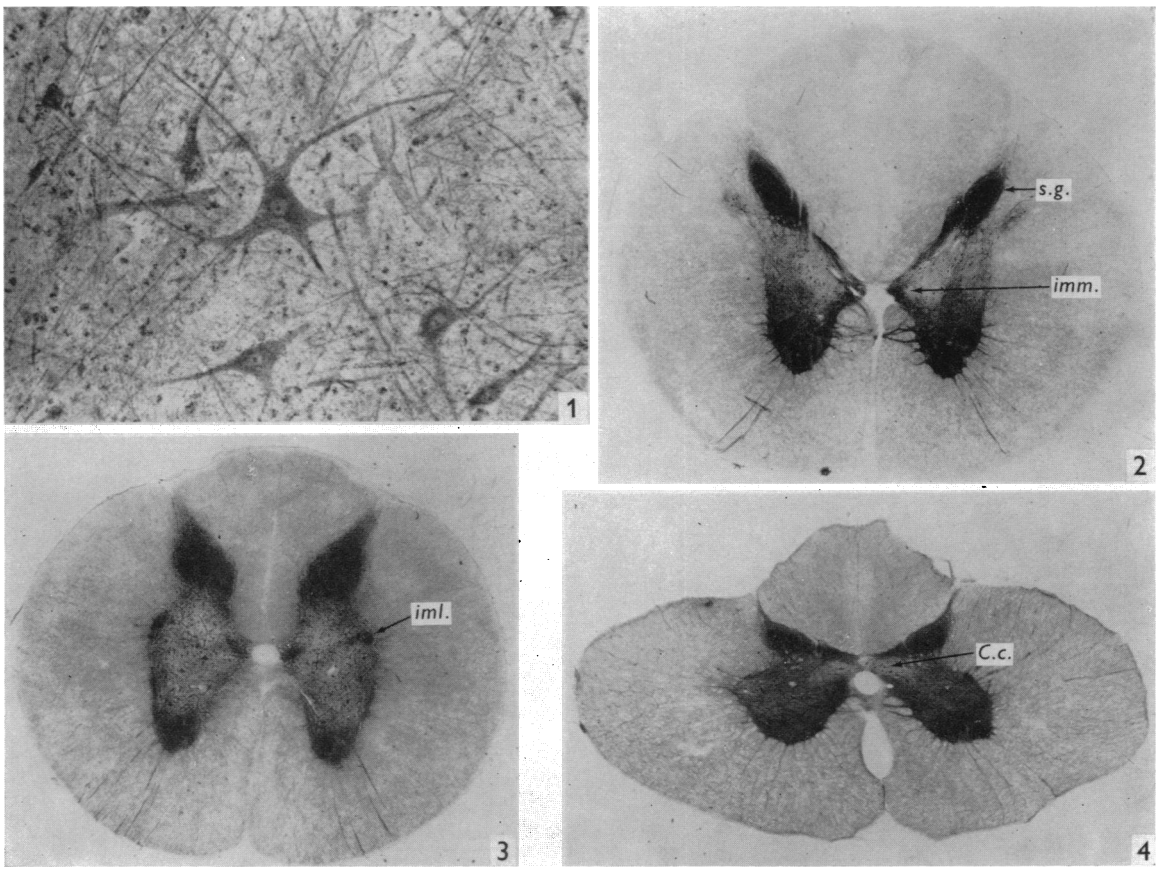


Fig. 1. Large neurons with medium acetyl cholinesterase activity from the central portion of the ventral column in the eighth cervical segment. Uniform distribution of the activity is found throughout the perikaryon and the dendrites; several axons are seen in the field.

Fig. 2. Second cervical segment. Intense activity is noted in the three groups of motoneurons of the ventral column, the intermediomedial nucleus (*imm.*), and the substantia gelatinosa (s.g.).

Fig. 3. First thoracic segment: the intermediolateral column (*iml.*) appears as a strongly active group of cells.

Fig. 4. Thirteenth thoracic segment. At T 13 Clarke's column (*C.c.*) is the most conspicuous feature. The pallor of its neuropil is in contrast with the enzymatic activity of its large neurons.

out from the lighter-staining neuropil. In the neurons AChE activity seemed evenly distributed throughout the perikaryon, but the perinuclear region (seemingly nuclear membrane) and the nucleolus showed somewhat higher activity (Fig. 1). The nucleoplasm itself appeared pale. AChE activity varied markedly among neurons. No neurons lacking the ability to hydrolyse acetylcholin were found in the cat spinal cord.

Maximum AChE activity was found in the motoneurons of the *ventral grey column*, and these cells stained intensely at all levels with short incubation periods (60–75 min). The motoneurons were surrounded by smaller cells that showed almost equal activity. Large and medium-size cells with lower activity occurred mostly in the central portion of the column. The neurons of the *intermediate grey column* showed uniformly low activity throughout; the neurons of the *intermediomedial nucleus* in the thoracic segments of the central grey column, however, stood out by showing rather intense activity. The cell groups of the *intermediolateral column* showed marked activity that clearly delineated them from the fibre tracts.

In the *dorsal grey* column no activity occurred in the marginal zone. Both the small and the large cells of the posterior marginal nucleus hydrolysed acetylcholine slowly, and neuropil of the nucleus appeared very pale, especially in the upper cervical and lower lumbar and sacral segments. High AChE activity occurred in the perikarya of substantia gelatinosa, nearly equal to that in the motoneurons in the ventral horns. In the remainder of the dorsal grey column, cells with moderate activity varied little among each other.

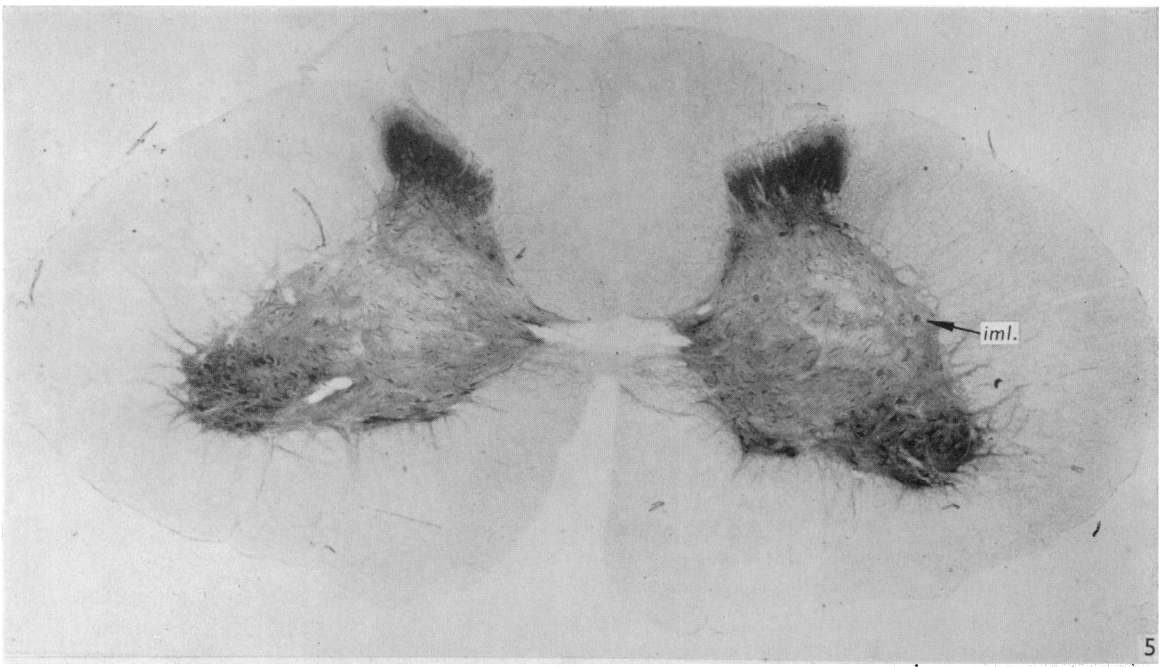
The most striking appearance was that of the *column of Clarke*. Neuronal AChE activity was moderate to high, but the neuropil surrounding the cells seemed to lack activity completely, so that Clarke's column appeared as a sharply delineated light area in the grey matter.

Segmental distribution patterns

The segmental variation in the distribution patterns of AChE activity corresponded perfectly to the segmental variation in cytoarchitecture described by Rexed (1954).

Cervical cord. The cervical cord (C 1–8) showed its characteristic enlargement which in the case of the cat begins at C 5, reaches a maximum size at C 6, and then tapers off rapidly. The upper segments (Fig. 2) contained three groups of motoneurons with high AChE activity in the ventral column; at C 5 a fourth group appeared and, at the level of maximum enlargement, six cell groups could be distinguished, although the boundaries were not very distinct. These six groups showed roughly equal activity. The intermediomedial nucleus consisted of medium and small-sized cells, showing intense activity uniformly throughout the entire spinal cord. Stilling's nucleus was not distinct from the remainder of the dorsal column.

Thoracic segments. The thoracic segments (1–13) displayed only minimal variations in size and shape. At T 1 (Fig. 3) the ventral grey column had already attained the small size characteristic of the thoracic segments, and only two groups of motoneurons could be distinguished by their AChE activity throughout the thoracic segments. The intermediolateral column appeared constantly at the level of T 1. It consisted of medium-sized cells with very intense AChE activity comparable to that in the motoneurons. The neuropil surrounding these cells also showed marked



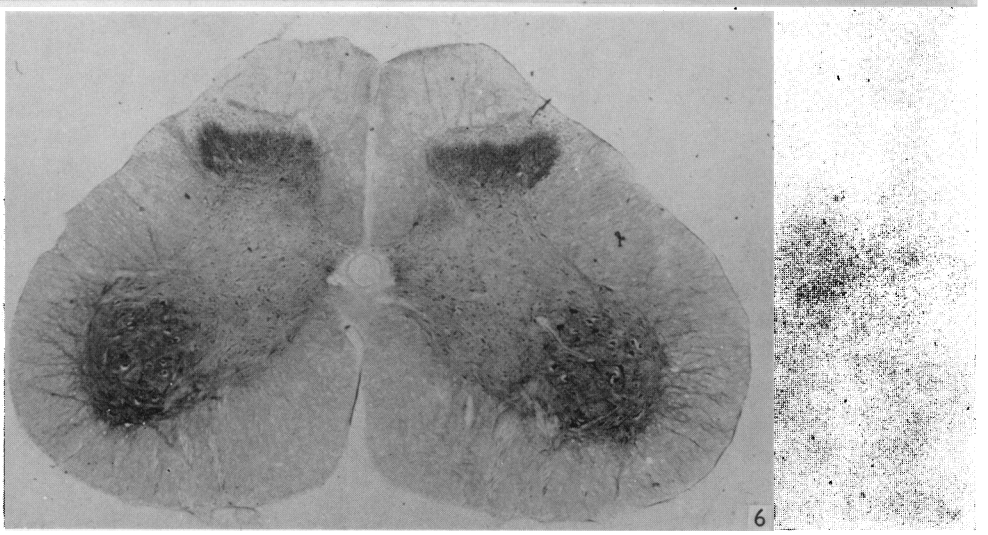


Fig. 5. Fourth lumbar segment. The intermediolateral column (*iml.*) does not show as a compact nucleus at this level, but can be distinguished by a few highly active cells in the lateral eminence. The grouping of the motoneurons in the ventral column is not distinct.

Fig. 6. First sacral segment. The motoneurons form a single group in the ventral column at the first sacral segment. The neuropil of the apical nucleus is very pale. No intermediolateral nucleus is found at this level.

activity; projections of this neuropil extended for considerable distances between the fibre bundles of the lateral funiculus. The size of the column and the numbers of the cells in it varied minimally, with only a slight increase at the cranial end. Clarke's column extended from the second or third thoracic segment to the third lumbar segment. Although the neurons showed moderate activity, none occurred in the surrounding neuropil, in sharp contrast to the observations in the remainder of the dorsal column (Fig. 4). These features were the same throughout the thoracic cord. A longitudinal fibre tract running within the column could give it such an appearance, but none could be detected; the striking delineation of Clarke's column is probably due to a lack of AChE activity in the dendritic processes of the neurons of the column.

Lumbar segments. The seven lumbar segments showed greater variability. The lumbar enlargement commenced at the first segment and reached a maximum size at L 6. The number of cell groups in the anterior column increased, but the boundaries were not so distinct as those for the groups in the cervical segments. Clarke's column—delineated by the lack of activity in the neuropil—extended to the third lumbar segment, where it ended quite abruptly. The intermediolateral column, outlined by high activity, was seen down to the fourth lumbar segment (Fig. 5).

Sacral segments: In the three sacral segments the spinal cord narrowed rapidly; the white matter was very thin, and the grey matter occupied most of the tissue (Fig. 6). The motoneurons in the ventral column were arranged in three groups in the first segment, and formed only one group from the third sacral segment on. In the first two segments, scattered individual cells were found in the intermediolateral column, but only at S3 was there a distinct group of cells to be seen, all showing the high enzyme activity characteristic of the intermediolateral column.

Controls

Eserine is a long-known inhibitor of all cholinesterases, but it does not inhibit aliesterases. In our material it completely inhibited all enzyme activity in the tissue, giving uniformly blank sections throughout the entire spinal cord.

BW is a selective, reversible inhibitor of acetyl cholinesterase (Austin & Berry, 1953). Sections incubated with BW showed only very faint background staining and faint outlines of neurons that were better defined along the dendritic branches. Thus, visual estimation agrees with biochemical data (Burgen & Chipman 1952) in showing that non-specific cholinesterase activity accounts for only a minimal fraction of the acetyl cholinesterase reaction in the neurons.

Iso-OMPA, which is a specific inhibitor for non-specific or butyryl cholinesterase, produced no detectable change in the distribution and intensity of enzyme activity in this material.

DISCUSSION

The present description of the general features of the intracellular distribution of acetyl cholinesterase activity is in agreement with previous histochemical and biochemical observations, with one exception. Giacobini (1959), using the Cartesian-diver technique on microdissected parts of neurons, found no activity in the nucleolus;

in our preparation the nucleoli appeared distinctly stained. The reason for this disparity is not clear.

Acetyl cholinesterase activity varies in feline nerve cells, the highest activity occurring in the motoneurons of the ventral grey column and successively lower levels of activity evident in the intermediolateral column, the small cells of the substantia gelatinosa, and the large cells of Clarke's column. The remaining cells of the dorsal and the ventral columns show the least activity. Giacobini & Holmsted (1958) measured the AChE activity in individual cells of the ventral column and were able to show two classes of cells for which enzyme activity differed by a factor of four. They did not demonstrate any activity in the cells of the dorsal column. It may be possible, however, that there were more than two classes of neurons in spinal grey matter in terms of their AChE activity.

The cells of the intermediolateral and intermediomedial columns give origin to autonomic nerve fibres. These cell groups show high AChE activity; however, the same cell groups have been reported to contain marked monoamine oxidase activity (Hashimoto, Maeda, Torii & Shimizu, 1962). Admittedly the demonstration of these enzymes does not prove that their substrates are active in the transmission of impulses at these sites, but the findings show that the metabolic pathways for these substrates are available. Apparently, it is impossible to distinguish sympathetic or parasympathetic cells in the spinal cord by their histochemical features.

The strikingly low acetyl cholinesterase activity in the neuropil of Clarke's column apparently has not been noted before. The dendritic processes of these cells form halo-like structures around the perikarya and do not extend beyond the borders of the column (Cajal, 1909). Apparently these dendrites are devoid of any AChE activity which may suggest that the mode of transmission of impulses in Clarke's column differs, in principle, from that in the rest of the dorsal column.

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