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Histochemically distinct compartments in the striatum of human, monkey, and cat demonstrated by acetylthiocholinesterase staining

(basal ganglia/caudate nucleus/cholinergic transmission/acetylcholine/acetylcholinesterase, EC 3.1.1.7)

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ABSTRACT **We here report observations on the distribution of acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) in the striatum of the adult human, the rhesus monkey, and the cat. By the histochemical staining methods of Geneser-Jensen and Blackstad and of Karnovsky and Roots, compartments of low cholinesterase activity were identified in parts of the striatum in all three species. In frontal sections, these enzyme-poor zones appeared as a variable number of weakly stained ≈0.5-mm-wide zones embedded in a darkly stained background. The zones varied in cross-sectional shape from round to elongated and were sometimes branched. They were most prominent in the head of the caudate nucleus. Three-dimensional reconstructions of serial sections through the caudate nucleus in the human and cat suggest that over distances of at least several millimeters, the zones of low enzyme activity form nearly continuous labyrinths.**

The mammalian striatum is characterized by one of the highest concentrations of acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) in the brain. The presence of this enzyme is not in itself sufficient to establish the existence of cholinergic transmission in the striatum, but the caudoputamen has also been shown to contain cholinergic receptors (1) and the synthetic enzyme of the cholinergic mechanism, choline acetyltransferase (2). The histochemical demonstration of acetylcholinesterase has therefore come to be regarded as a useful indirect indicator of cholinergic activity in the striatum and has further attracted interest because of the remarkable similarity in distribution of dopamine histofluorescence and acetylcholinesterase activity in the caudoputamen and nucleus accumbens. We report here observations on the distribution of striatal acetylcholinesterase in the human, together with parallel observations in cat and monkey. The main finding of this study is that the cholinesterase of the adult striatum in all three species is characterized by a complex intrinsic architecture that can be visualized by the histochemical methods of Karnovsky and Roots (3) and Geneser-Jensen and Blackstad (4). These findings strongly suggest that in the human, as in the cat and monkey, the contrast between an architecturally highly differentiated cerebral cortex on the one hand and a structurally homogeneous striatum on the other has been overdrawn. When considered in the context of other recent experimental studies of the mature and developing striatum (5–13), the present findings support the concept of a fundamental subdivision of the striatum into a mosaic of at least partially segregated, histochemically distinct units.

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MATERIALS AND METHODS

This report is based on a study of postmortem striatal tissue from the brains of three humans, two monkeys, seven cats, and three kittens.

Human autopsy material was obtained 11–26 hr after death from two men (H1 and H2, ages 72 and 74 years) and one woman (H3, age 76 years). Tissue blocks containing part of the head of the caudate nucleus or lentiform nucleus and control blocks from the brainstem were immersed for 4–20 hr in a solution of fixative containing 10% formalin, 0.9% sodium chloride, 3% sucrose, and 10 or 20% dimethyl sulfoxide. Each block was cut in the frontal plane at 75 μm on a freezing microtome.

Tissue from the experimental animals was fixed by transcardial perfusion with 10% formol/saline or an aldehyde mixture containing 1% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer. Blocks were washed in a 5–15% sucrose/phosphate buffer for up to 18 hr and were then cut at 50 μm.

In all cases, sections were stained for acetylthiocholinesterase according to the protocols of Geneser-Jensen and Blackstad (4) or Karnovsky and Roots (3). Trial sections were developed at frequent intervals to determine the optimal incubation period for each tissue block. In several series of sections from the kitten brains, the method of Hardy *et al.* (14) was used to intensify the stain. Controls for specificity of the esterase stain were carried out on selected sections from some of the cat brains and one human brain (H3). These controls included: (*i*) omission of substrate; (*ii*) incubation with butyrylthiocholine iodide; (*iii*) incubation with, respectively, acetylthiocholine iodide and butyrylthiocholine iodide in the presence of 0.1 mM eserine sulfate; and (*iv*) omission of the pseudocholinesterase inhibitor ethopropazine from the incubation step of Geneser-Jensen and Blackstad's method. Sections were mounted in serial order and coverslipped without counterstain.

RESULTS

Marked nonuniformities in acetylthiocholinesterase staining were present in the striatum of all brains examined. These inhomogeneities were most prominent in the anterior part of the caudate nucleus where, in each species, the dense background matrix of cholinesterase stain was repeatedly and abruptly interrupted by a variable number of macroscopically visible zones that were much less densely stained.

Figs. 1–4 illustrate this pattern of staining in frontal sections passing through the caudate nucleus of the cat (Fig. 1), monkey (Fig. 2), and human (Figs. 3 and 4). Typically, 6 to 12 distinct regions of low enzyme activity appeared in the caudate nucleus in single sections near the levels illustrated. The average transverse diameter of the zones was 0.3–0.5 mm in the cat and

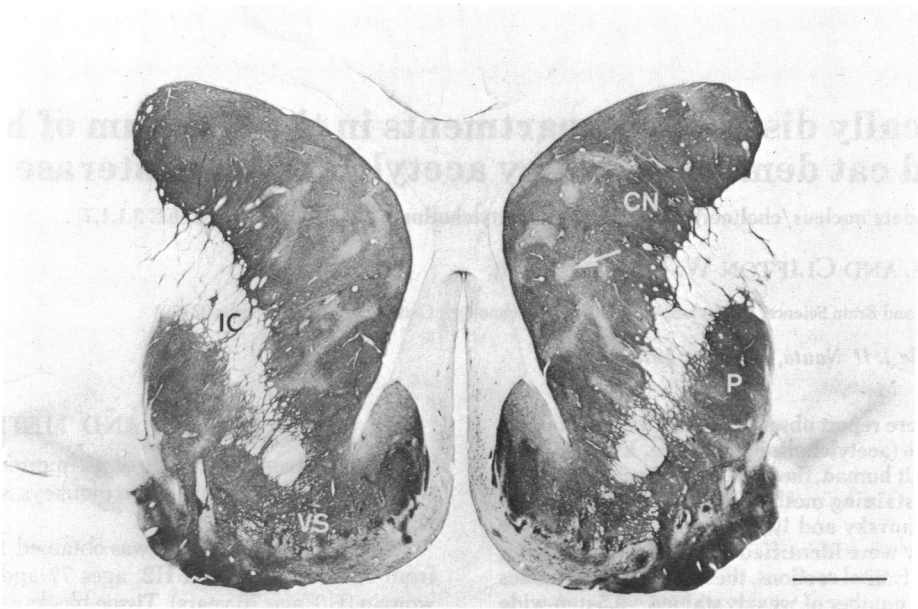

FIG. 1. Transverse section (50 μm) through the striatum of the adult cat, stained by acetylthiocholinesterase method of Geneser-Jensen and Blackstad (4). Caudate nucleus (CN), putamen (P), and ventral striatum (nucleus accumbens-olfactory tubercle, VS) all show dense cholinesterase-positive stain (dark). Fibers of the internal capsule (IC) are not stained. In the caudate nucleus, the Swiss-cheese pattern of staining results from the presence of a number of very weakly stained zones of variable shape. One such cholinesterase-poor zone is marked by the arrow on right. A tendency toward diagonal alignment of the pale zones, evident on the left, appeared frequently. Bar indicates 2 mm.

monkey and slightly larger (0.5–0.8 mm) in the human. As shown in Figs. 1–4, the pale zones were rather widely spaced and varied in cross-sectional shape from circles, ellipses, or chevrons to more complicated, elongated forms with one or several side branches. It is interesting that, despite the sometimes abrupt changes in size or shape of the zones, their contours were nearly always smooth and rounded.

Even in the most briefly incubated sections, the pale zones never appeared completely free of enzyme activity and exhibited at least a weak cholinesterase reaction that made it easy to distinguish them from outlying fiber bundles of the internal capsule (and from profiles of large blood vessels). The borders of the pale zones were not crisp, either in the perfusion-fixed cat and monkey or in immersion-fixed human material. Occasionally, however, the background stain at the borders of a pale zone appeared especially dense, as though a rim of more concentrated enzyme surrounded the region of low activity. In some sections, in the dorsolateral part of the head of the caudate nucleus, one or more zones appeared that were char-

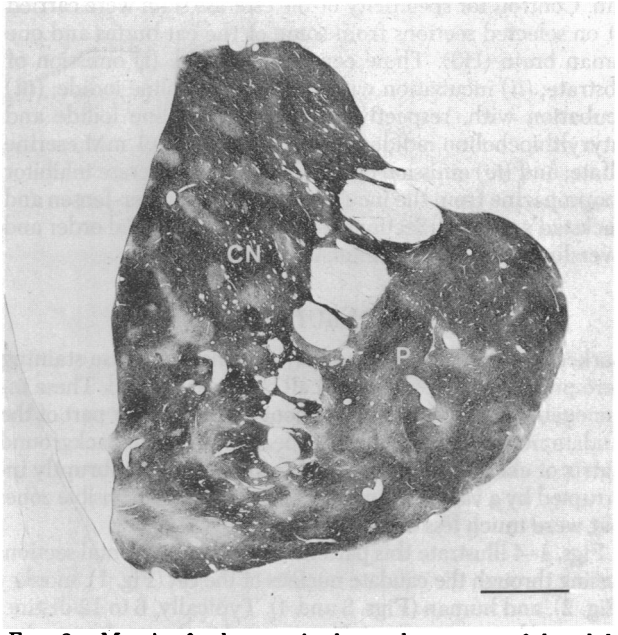

FIG. 2. Mosaic of pale zones in the caudoputamen of the adult rhesus monkey is illustrated in this 50-μm transverse section stained for acetylthiocholinesterase (4). Only the right side of the striatum is shown. Abbreviations as in Fig. 1; bar indicates 2 mm.

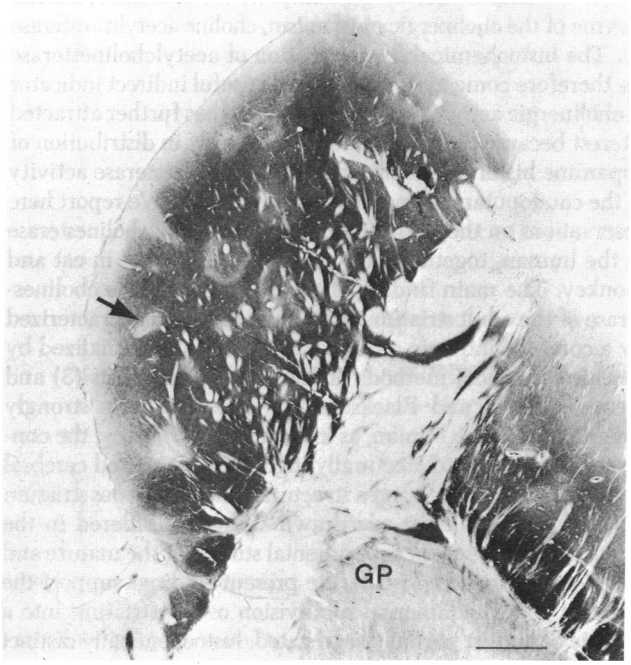

FIG. 3. Zones of weak acetylthiocholinesterase activity in caudate nucleus of the human (H3) shown in 75-μm-thick section stained for cholinesterase by the Geneser-Jensen and Blackstad method (4). Edge of caudate nucleus (to left) was probably overexposed to the fixative during immersion-fixation and looks bleached. Arrow points to one of the zones of low enzyme activity. Edge of the globus pallidus (GP) is at bottom. Bar indicates 2 mm.

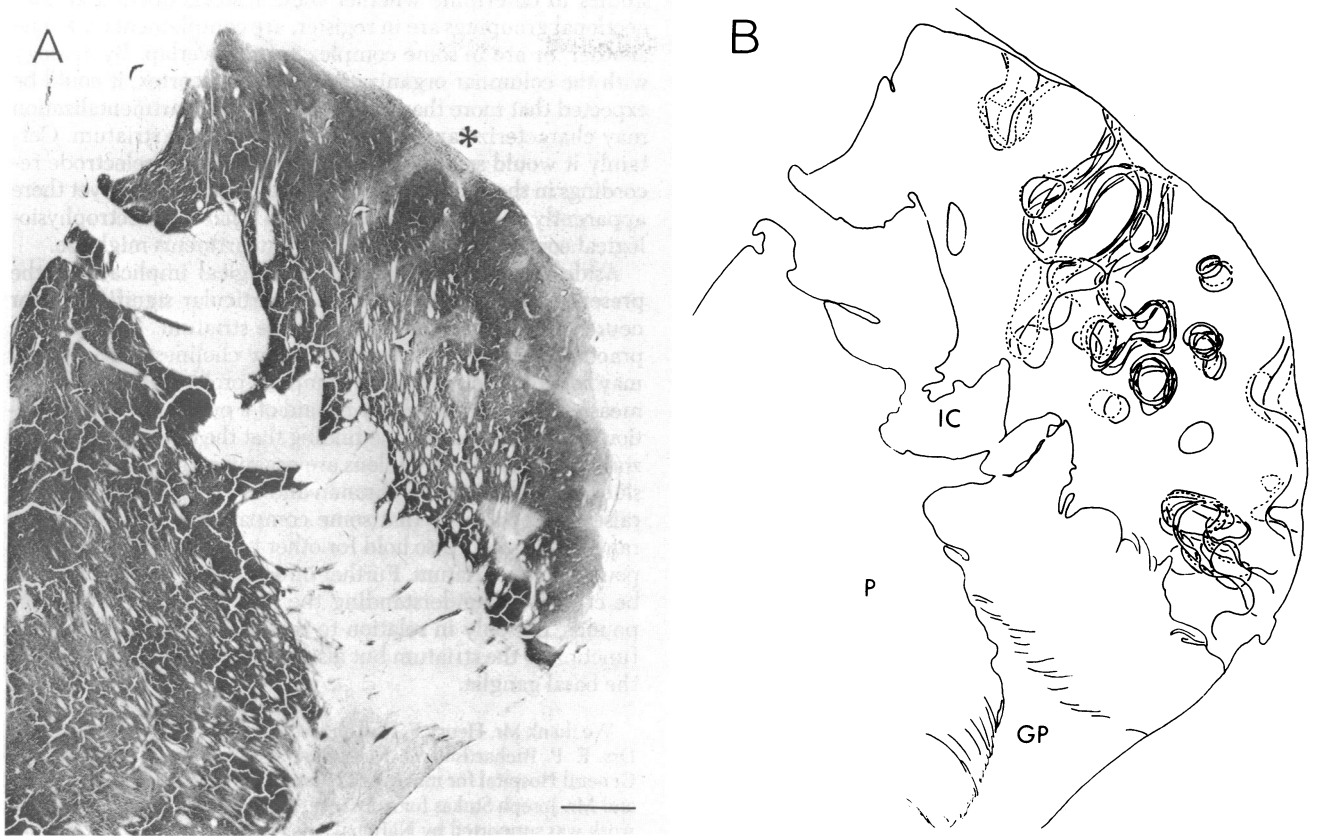

FIG. 4. Pattern of acetylthiocholinesterase staining in postmortem striatal tissue from human (H2). (*A*) Section (75 μm) through caudate nucleus and, to left of internal capsule, part of lentiform nucleus. Circumscript zones of low enzyme activity in caudate nucleus appear pale against dark background stain. Well-delineated cholinesterase-poor zones are not visible in putamen although some unevenness in staining is apparent. An example of characteristic association of pale zones with the ventricular aspect of the caudate nucleus is shown at asterisk. Bar indicates 2 mm. (*B*) Projection drawing of section in *A*, at same magnification. Outlines of pale zones in that section and in seven serially adjoining sections (three anterior and four posterior) have been superimposed to show continuation of pale zones. Solid outlines indicate clearly delineated pale zones; dotted outlines show zones that were less distinct. IC, internal capsule; GP, globus pallidus; P, putamen.

acterized by a cholinesterase content higher than that of the surrounding tissue. For the most part, however, the cholinesterase-rich background stain was fairly even, as in the sections illustrated.

The omission of the pseudocholinesterase inhibitor ethopropazine did not seem to change markedly the visibility of the zones, but the appearance of the zones was sensitive to other changes in protocol. First, prolonged fixation resulted in a general reduction of cholinesterase activity and hence a blurring and eventual disappearance of the zones. An example of such fading can be seen in Figs. 4 and especially 3, which show the effects of overfixation of the surface of the tissue blocks trimmed from the striatum in the human. Second, with the control treatments, staining in the striatum was slight (butyrylthiocholine) or not evident at all (eserine, omission of substrate). Finally, prolonging the incubation times led to increased density of striatal staining, and, after long enough times (e.g., 4 hr in the Geneser-Jensen and Blackstad method), to complete obscuring of the pale zones.

Although on first impression the enzyme-poor zones illustrated in Figs. 1–4 seem to be randomly placed, several observations suggest that this may not be the case. A general topography appeared to govern the locations of the pale zones, at least in the cat and monkey. In the cat, the regions of low enzyme activity were prominent in the head of the caudate nucleus but became indistinct and finally disappeared at more caudal levels. Within the rostral half of the caudate nucleus, such regions were most numerous medially and most sparse in the dorsolateral quadrant (Fig. 1). There were also marked variations of enzyme activity in the nucleus accumbens, but the regions of weak staining were less circumscript in this part of the striatum than in the caudate nucleus proper. The zones of low activity were least well-defined in the putamen of the cat. Where inhomogeneities did appear, their borders tended to be vague. Interestingly, as shown in Fig. 1, some of the weakly stained zones in the putamen appeared to be continuations of more clearly defined pale zones within the ventral part of the caudate nucleus. In the human striatum, the topography of the cholinesterase-poor compartments has not yet been studied but, as shown in Figs. 3 and 4, clearly outlined pale zones were rarely visible in the putamen. Curiously, the zones of low activity seemed better defined in the putamen of the monkey; this point needs further study.

In sections through the caudate nucleus of the human, it was common to find one or more zones of low cholinesterase activity stretching away from the ventricular face of the nucleus. A typical example of this pattern is shown in Fig. 4*A* at the asterisk. This arrangement also occurred in the cat and was particularly prominent in the rostral part of the *caput*. At these rostral levels of the cat's striatum, the arrangement of the zones seemed most regular; in some sections the zones were quite evenly spaced, about 1 mm from one another.

To determine whether the zones of low activity observed at a single transverse level were continued in neighboring sections, projection drawings were made of uninterrupted series of serial sections through the anterior part of the caudate nucleus of the cat and human. By lining up the drawings of successive sections, using blood vessels as fiducial marks, it became clear that in-

dividual pale zones did actually extend through several and sometimes many sections (see Fig. 4B). In addition, a surprisingly large number of the cholinesterase-poor zones in any particular section could be shown to be connected with one another at some point out of the plane of section. This arrangement will be described more fully in a separate account, but it is already clear that many and probably most of the pale zones seen in frontal sections are actually parts of complex labyrinths of low enzyme activity.

DISCUSSION

The finding of marked discontinuities in striatal acetylthiocholinesterase activity in the adult brain was unexpected. In the adult rat, Jacobowitz and Palkovits (15), Butcher and Hodge (7), and others (16) have described a uniform distribution of this enzyme in the caudoputamen. In a recent developmental study, however, Butcher and Hodge (7) made the interesting observation that in the neonatal rat, unlike the adult of that species, striatal cholinesterase is confined to a number of small patches. We have so far had the opportunity to study the neonatal striatum only in the kitten, but have found, in complete agreement with Butcher's observations in the rat, a patchwork of cholinesterase-positive clumps in the caudate nucleus in the newborn cat. It is not clear what developmental sequence might account for the nearly complete reversal of pattern (at least in the cat) from one of enzyme-rich patches in the immature striatum to one of enzyme-poor patches as here described in the adult. It is an open and obviously interesting question whether the zones of low enzyme activity in the adult represent the trails of a secondary compartmentalization of the striatum occurring after attainment of an even cholinesterase dispersal or are the effect of a primary process of cholinesterase distribution that failed to invade the pale zones.

Another important question raised by the findings described here is how the discontinuities in acetylthiocholinesterase activity relate to other recently described inhomogeneities of the striatum. Anatomical evidence indicates that striatal afferent fibers originating in the thalamus and neocortex terminate in the caudoputamen in a complex array of clusters (8–11). At least part of the dopaminergic nigrostriatal connection appears also to terminate in clusters, because islands of fluorescence have been found in the striatum of the neonatal rabbit (6) and in the caudoputamen of adult rats pretreated with a tyrosine hydroxylase inhibitor (5). Diprenorphine binding studies in the rat (17) have shown that one set of putative receptors in the striatum, the opiate receptors, occur in patches. Finally, some tendency toward clustering of striatal neurons has been noted with the Nissl method in the adult rat (12) and, very recently, with the [^{3}H]thymidine method in the fetal monkey (13). In axon transport studies still in progress in the cat, we have found (unpublished findings) that the cells of origin of at least one great efferent system of the striatum, the striopallidal connection, are clustered so as to form mosaics reminiscent of, and indeed partly overlapping with, the zones of low cholinesterase activity in the caudate nucleus.

The three-dimensional arrangement of these various clustering patterns in the striatum is still largely unknown. It will clearly require combined pharmacological and anatomical studies to determine whether these histochemical and connectional groupings are in register, are complementary to one another, or are in some complex partial overlap. By analogy with the columnar organization of the neocortex, it could be expected that more than one functional compartmentalization may characterize any particular region of the striatum. Certainly it would seem worthwhile to make microelectrode recordings in the caudate nucleus to study this point; as yet there apparently is little information as to what the electrophysiological correlates of such striatal compartments might be.

Aside from their possible physiological implications, the present observations may have a particular significance for neuropharmacological studies of the striatum. First, from a practical standpoint, the zones of low cholinesterase activity may be large enough to cause sampling problems when enzyme measurements are made by the aid of a punch or microdissection technique. Second, the finding that the cholinesterase-poor zones in the caudate nucleus are organized in a three-dimensional maze, both in the human and in experimental animals, raises the possibility that some comparable architectural arrangement might also hold for other transmitter-related compounds in the striatum. Further information on this point could be critical for understanding the interactions of these compounds, not only in relation to the development and normal function of the striatum but also in relation to disease states of the basal ganglia.

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