Direct demonstration of a correspondence between the dopamine islands and acetylcholinesterase patches in the developing striatum
(tyrosine hydroxylase/brain development)

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ABSTRACT The distribution of dopamine-containing processes in the striatum of fetal and neonatal cats was studied by immunohistochemical and glyoxylic acid histofluorescence methods and compared to the distribution of acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7) observed by thiocholine histochemistry in the same or serially adjoining sections. Both methods for demonstrating the dopamine innervation revealed the characteristic patchwork of dopamine "islands" in the caudoputamen (8-10) and in the ventral accumbens (8-10) with thiocholine histochemistry. However, the distribution of acetylcholinesterase activity that appears to be evenly, dispersed in the fetal and neonatal striatum was observed to be localised in discrete patches or "islands" (11-14) and, though fluorescence is broadly, and apparently even, dispersed in the striatum of the normal adult (11, 12, 15-17), fluorescent islands have been observed by Fuxe and his colleagues (12, 18) in the striatum of rats pretreated with an inhibitor of tyrosine hydroxylase [tyrosine 3-monoxygenase; L-tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2]-like immunoreactivity was concentrated in 0.2- to 0.6-mm-wide patches. Both methods also demonstrated a high degree of patterning of the dopamine innervation in the ventral striatum, including the nucleus accumbens septi. A detailed and striking match was found between these configurations and the compartmental distribution of acetylcholinesterase observed in the caudoputamen and ventral striatum of the same brains. The correspondence between the dopamine and acetylcholinesterase figures was most obvious in the fetal brains, in which the background acetylcholinesterase staining was lightest, but matches between the dopamine islands and acetylcholinesterase patches could still be seen in the kittens. There was no clear alignment of striatal cell bodies stained for acetylcholinesterase with either the dopamine or the acetylcholinesterase-positive patches. Nor was there an obvious correspondence between dopamine and acetylcholinesterase in the striatal background matrix. We conclude that, at least during ontogenesis, it is the clustered arrangements of dopamine and acetylcholinesterase that are, in particular, tightly linked, and we suggest that information about the maturation of these clusters may be crucial in assessing the functions of striatal dopamine and acetylcholinesterase in the adult.

A surprising outcome of recent work on the caudate nucleus and putamen is that these telencephalic nuclei, although subcortical, are divided up into orderly sets of anatomical compartments with distinct histochemical properties and fiber connections. In the mature striatum, incoming afferents from the neocortex (1, 2) and thalamus (3-5), striatal projection cells (6), and certain neuropeptides (7) form three-dimensional patchworks, and we have shown that these heterogeneous distributions are for the most part aligned with a network of acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7)-poor zones called striosomes (8-10). The implications of these correspondences are of at least two sorts. First, though electrophysiological evidence is still lacking, it is clear from the anatomy that these striosomal units must be functional units because they represent subdivisions in which connections become established between particular sets of striatal inputs and outputs. Second, the findings suggest that there is a compartmentalization of different pharmacological interactions in the striatum and, accordingly, that this chemoarchitecture could be important in determining the effects of certain drug treatments on striatal function.

From the standpoint of the potential relevance of these findings to work on basal ganglia disease, it would clearly be of great importance to learn how these modular arrangements are related to the dopaminergic innervation of the striatum. At least some of the dopamine-containing fibers do appear to be organized into compartments. In the fetal and neonatal striatum catecholamine fluorescence is localised in discrete patches or "islands" (11-14) and, though fluorescence is broadly, and apparently evenly, dispersed in the striatum of the normal adult (11, 12, 15-17), fluorescent islands have been observed by Fuxe and his colleagues (12, 18) in the striatum of rats pretreated with an inhibitor of tyrosine hydroxylase [tyrosine 3-monoxygenase; L-tyrosine, tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2]. In neither the young animals nor the adults, however, has a direct correlation been established between the dopamine islands and any other set of striatal patches. To test for one such possible linkage, we have examined the relationship between the dopamine islands and the dense clumps of acetylcholinesterase activity that appear in the caudoputamen of the immature brain. We chose the acetylcholinesterase clumps for this initial study because a remarkable similarity between their clustering and the patterning of the dopamine islands has already been noted in laboratory animals (19) and in the human (20), and because the findings might have some bearing on cholinergic-dopaminergic interactions in the striatum despite the fact that striatal acetylcholinesterase has been implicated in functions other than the hydrolysis of acetylcholine (21-23).

MATERIALS AND METHODS

The striatum was studied in three kittens 1, 5, and 21 days old and in nine fetal cats extracted by cesarean operation from three pregnant females at gestational ages ranging between 49 and 57 days (E49 to E57). In five of the fetuses (E49 to E57) and in the kittens, perfusion-fixed 30-μm-thick frozen sections were allowed to react with antisera (24) to tyrosine hydroxylase by the peroxidase-antiperoxidase method (25) and the distribution of tyrosine hydroxylase-like immunoreactivity (TH) was compared to the pattern of acetylcholinesterase activity in serially adjoining sections prepared by the thiocholine method of Nieser-Jensen and Blackstad (26). Most sections were intensified with potassium ferricyanide/silver (refs. 27 and 28, acetylcholinesterase) or with cobalt (ref. 29; peroxidase-antiperoxidase).

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Abbreviation: TH, tyrosine hydroxylase-like immunoreactivity.

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and they were studied at the light microscope and macroprojector. In four fetuses, catecholamine fluorescence in the striatum was visualized in sections from brains cut at 32 μm on a cryostat and prepared by de la Torre’s modification of the glyoxylic acid method (30). Three of the brains were unfixed; the fourth was perfused with glyoxylic acid. Patterns observed by incident fluorescence microscopy were recorded photographically and compared with staining patterns in adjoining sections in which acetylcholinesterase reactions had been visualized. In several cases a sequential protocol was followed in which unfixed cryostat sections were first prepared by the histofluorescence method and photographed, and then stained for acetylcholinesterase activity. As controls to test whether the acetylcholinesterase staining in these sections was dependent on the presence of dopamine, catecholamines were eluted from a few of the sections by immersion in 2 M HCl (31) prior to exposure to glyoxylic acid. In other sections, the thiocholine incubation was carried out in the presence of the acetylcholinesterase inhibitor BW285c51 (Burroughs Wellcome, Research Triangle Park, NC) at 1 mM.

RESULTS

Figs. 1 and 2 illustrate the clumping patterns of TH and acetylcholinesterase activity in two pairs of serially adjoining cross...
sections through the caudate nucleus of an E57 fetus. In each section of the more rostral pair (Fig. 1) about a dozen darkened 0.2- to 0.6-mm-wide patches appear scattered at 0.5- to 0.8-mm intervals against a lighter background. The clumps of high acetylcholinesterase activity are somewhat larger and fuzzier than those stained for TH, and they lie in a more darkly stained matrix. It is clear at a glance, however, that the patches in the two sections have similar shapes and locations and thus are in topographic register with one another.

The patterns in the more caudal pair of sections (Fig. 2), despite their complexity, reinforce the impression of a detailed correspondence of the TH and acetylcholinesterase patches. Almost every patch in the tyrosine hydroxylase section has a counterpart in the acetylcholinesterase pattern, and the match extends both into the ventral striatum–nucleus accumbens region and into the anterior tip of the putamen (see arrows in Fig. 2). Characteristic gradients in the two staining patterns are also apparent in this pair. In the section stained for TH the patches in the dorsal part of the caudate nucleus tend to be more sharply delimited and more intensely immunoreactive than the ventral patches. Along the mediolateral dimension, figures are most common laterally, some sending streamers from a pericapsular origin toward the interior of the nucleus; the patches in the medial part of the nucleus tend to be more sparsely distributed and both lighter and simpler in shape. Similar gradients appear in the acetylcholinesterase section but, in addition, the matrix stain is organized so as to be darkest dorsally and laterally and weakest in the subependymal region and ventral district of the striatum. This background staining is punctuated by intensely reactive cell bodies whose locations do not seem to bear a clear relationship to the arrangement of macroscopically visible patches (see Inset in Fig. 1B).

The patterns just described for the E57 fetus are generally representative of the findings in the entire fetal series. By birth the background stain in the acetylcholinesterase sections had increased enough to nearly obscure some patches, and matches between the acetylcholinesterase and TH were correspondingly more difficult to see than in the fetal material. The pattern of TH in the newborns remained mainly one of dark patches on a light ground, but the TH background stain increased non-uniformly so as to produce also some light-on-dark figures. Such a marbling pattern is common in the acetylcholinesterase at these ages (7, 10, 32).

The dopamine islands were originally demonstrated by the catecholamine fluorescence method, and because this technique would add an independent means of identifying catecholamine-containing processes in the striatum, most of which are known to be dopaminergic (11, 12, 15–17), we carried out

![Image](image-url)
a second set of experiments comparing the distribution of acetylcholinesterase and histofluorescence induced by the glyoxylic acid method. Fluorescent islands appeared in the striatum of all fetal brains and were in obviously tight register with the densifications present in neighboring sections stained for acetylcholinesterase activity.

The fluorescence experiments were especially valuable in providing us the opportunity to demonstrate dopamine and acetylcholinesterase in the same sections by first carrying out the glyoxylic acid method and then, after fluorescence photography, staining the sections in the usual way for acetylcholinesterase activity. The photomontage of Fig. 3A illustrates the dopamine islands in one such cross section through the caudate nucleus, taken from an E54 fetus, and the distribution of acetylcholinesterase patches in the same cross section is shown in the macrophotograph of Fig. 3B. Histological detail was preserved through the successive procedures well enough so that it is possible to compare the two patterns patch for patch. Except in the most ventral part of the caudate nucleus, where the fluorescence appears murky, the correspondence between the two sets of patches is quite exact, certainly within the range of variation to be expected considering that the methods for demonstrating the patches were not systematically matched for relative sensitivity along any dimension of biochemical reactivity. Fig. 4 shows matched photographs of a second section treated according to the double staining protocol and makes the point that the dopamine–acetylcholinesterase patterns are aligned in the nucleus accumbens and adjoining regions of the ventral striatum as well. It should be added that, with brief exposures to 2 M HCl, the catecholamine fluorescence was abolished but typical acetylcholinesterase staining, sensitive to BW28c51, could still be obtained. This suggests that the acetylcholinesterase stain in these double protocol experiments did not depend on the presence of dopamine in the tissue, at least in a form that can be visualized by histofluorescence.

**DISCUSSION**

The present findings establish an exact and detailed correspondence between the islands of dopamine-containing fibers in the immature striatum and the system of acetylcholinesterase patches that is present during development. The correspondence in and of itself does not prove that acetylcholinesterase and dopamine coexist in the same fibers, but this hypothesis (19, 20) is certainly strengthened by the remarkably detailed similarity of the dopamine and acetylcholinesterase patches visualized in the same and in serially adjoining sections. A linkage between dopamine and acetylcholinesterase in the striatal background staining was not as obvious. There were clear mediolateral and anteroposterior gradients in the background acetylcholinesterase in brains in which comparable distributions of diffuse fluorescence or TH could not be detected. There were also acetylcholinesterase-positive cell bodies in local regions of the striatum where neither acetylcholinesterase background staining nor patches of acetylcholinesterase and dopamine were present. Although the failure of match in backgrounds could have a technical cause, these observations do suggest that there are at least three classes of acetylcholinesterase-containing elements in the developing striatum and that only one of these, the system of acetylcholinesterase patches, may be in rigid alignment with the incoming dopamine-containing fibers forming the islands.

Not only are there different acetylcholinesterase-containing elements in the striatum, potentially related to different functions (21–23), but also the islands themselves may be one of at least two classes of dopamine fibers. According to Fuxe and his colleagues (12) the fibers assembled into islands are characterized by a dotted fluorescence, in contrast to the diffuse fluorescence of the broadly distributed dopaminergic innervation, and also differ from the diffuse type by having lower rates of dopamine turnover, activating receptors linked to adenylate cyclase, and responding differently to certain drug treatments. One implication of the present study is that fibers of the island system may further be differentiated from the diffuse type by having high levels of acetylcholinesterase during ontogenesis or, at minimum, by developing in precise register with acetylcholinesterase-containing fibers.

Though dopamine islands can be made to appear in the adult by pretreatment with a tyrosine hydroxylase inhibitor (12), no pharmacological manipulation has been found to elicit a comparable reappearance of the acetylcholinesterase-rich patches in the mature striatum. Instead, the main adult pattern is one of acetylcholinesterase-poor patches on a darker background.

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**Fig. 4.** Matched photographs illustrating patterns of catecholamine fluorescence (A) and acetylcholinesterase (B) in the nucleus accumbens septi (NA) and adjoining regions of the ventral striatum (VS) from a section processed by the sequential glyoxylic acid–thiocholine method. From an E57 littermate of the fetus illustrated in Figs. 1 and 2. Note the correspondence of patterns in VS and heightened reactivity in medial and dorsal NA in both sections, but more delicate patterning in the NA visible in the fluorescence picture. Bar indicates 1 mm.
The apparent lack of parallelism could mean that the acetylcholinesterase in the patch fibers occurs only transiently during development. According to Butcher and his colleagues (18, 32), however, acetylcholinesterase can be found in most cell bodies of the pars compacta of the substantia nigra even in adulthood. The explanation for this discrepancy is not obvious, but it may be pertinent that Olson et al. (12) found the dopamine island fibers to resemble those of the limbic striatum. On these grounds a differential mesencephalic origin of the diffuse and dotted systems might be suggested.

To get to the crux of this problem it will be necessary to extend the present study to work on the adult. There is already a hint, however, that the acetylcholinesterase-rich patches of the young striatum might be the forerunners of at least some of the adult’s acetylcholinesterase-poor striosomes because there are patchworks of enkephalin-like immunoreactivity in the striatum that in the fetus (34) and neonate (7) match acetylcholinesterase-positive patches, but that in the adult (7) match zones of low acetylcholinesterase activity. This is a tantalizing possibility, because it would mean that the dopamine islands fit into a general architectural framework that governs also other afferents and efferents of the striatum. If so, the system of dopamine fibers making up the islands may well be found to have functional specificity by virtue of modulating a particular, identifiable subset of striatal connections.

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