Early Loss of Neostriatal Striosome Neurons in Huntington's Disease

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Abstract. During the first years of symptomatic Huntington's disease (HD), no readily apparent pathology is seen in the neostriatum at autopsy. To investigate the pathological correlates of chorea and other early clinical signs, we examined the evolution of neuronal loss and accompanying astrocytosis in neostriatal tissue from autopsy cases of early HD. We found scattered islands of astrocytosis and neuronal loss that were present before the previously described ventrally progressive wave of generalized neuronal loss. Histological demonstration of these islands, which are apparently specific to HD, is very helpful in the pathological differential diagnosis of this disease. Immunohistochemical stains for glial fibrillary acidic protein and for markers of the neostriatal striosome-matrix system showed that these islands correspond to the striosome compartment. Striosomal neuronal loss was present throughout the dorsoventral extent of the caudate nucleus and putamen during the early phase of symptomatic disease, and this loss extended to the most ventral region of the nucleus accumbens in later stages. Analysis of the functional circuitry of the basal ganglia suggests that early degeneration of striosomal neurons may produce hyperactivity of the nigrostriatal dopaminergic pathway, causing chorea and other early clinical manifestations of HD.

Key Words: Basal ganglia; Chorea; Dopamine; Huntington's disease; Neostriatum; Patch-matrix; Striosome.

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease that manifests as chorea, eye movement abnormalities, voluntary motor impairment, cognitive deterioration, and emotional and psychiatric disorders (1–4). The responsible gene is located near the telomere of chromosome 4p (5), and the defect has been identified as an expanded, unstable polymorphic trinucleotide repeat in a new gene, IT15 (6). The function of the gene product has not yet been characterized. The clinical diagnosis of HD is usually made at the time that chorea appears in the appropriate clinical and family history setting. The psychiatric, voluntary motor and eye movement abnormalities also appear early in the disease. Impairment of cognition and impairment of voluntary movements, characterized by clumsiness, rigidity, and dystonia, are progressive until the time of death, whereas chorea generally progresses early, then plateaus and wanes in later stages (3, 4, 7, 8).

The most striking pathological manifestation in HD is loss of γ-aminobutyric acid (GABA)-containing medium-spiny neurons (type I neurons of Braak and Braak [9]) in the neostriatum (10–12), accompanied by fibrillar astrocytosis. In a landmark study, Vonsattel and colleagues (13) categorized pathological severity in autopsy cases of HD into five grades, from grade 0 (no readily identifiable pathology despite definite premortem symptomatology and positive family history) to grade 4 (severe neostriatal atrophy, neuronal loss, and astrocytosis). These authors (13) described a generalized loss of medium-sized neurons that progresses in a dorsomedial-to-ventral direction during grades 1–4. This wave of major cell loss begins in the dorsomedial caudate nucleus and dorsal putamen in grade 1, but does not become prominent until grade 2. Thus, despite premortem chorea and other symptoms, no readily identifiable pathology in grade 0 and only minimal changes in grade 1 were described. These findings provided no pathological correlate for chorea and other early symptoms.

Reiner and colleagues (14, 15) suggested a mechanism for chorea in HD, based on their observation of a selective decrease in immunostaining for enkephalin in the external pallidal axonal arborizations of striopallidal enkephalin-containing GABAergic neurons. They inferred that these neurons are selectively vulnerable in HD striatum at least through grade 2 relative to substance P-containing striopallidal neurons that project to the internal pallidal segment. Decreased inhibition of external pallidal neurons by enkephalin-containing striopallidal neurons was suggested as a logical mechanism for the early appearance of chorea (14, 15). The decrease in enkephalin immunoreactivity in the external pallidum in early and presymptomatic HD, despite normal preproenkephalin mRNA levels in the parent cell bodies, suggested an early dysfunction of the striato-external pallidal axonal projection (16). Our results are consistent with a possible early involvement of enkephalin-containing neurons as a cause of chorea, but we suggest a possible alternative or additional explanation, namely synaptic inhibition producing decreased activity and lower enkephalin levels in these neurons.

MATERIALS AND METHODS

Two series of investigations were carried out. In the initial study (17, 18), immunostaining for glial fibrillary acidic protein
(GFAP) and cresyl violet counterstaining were performed on sections of neostriatum from 43 autopsy cases of HD (Vonsattel grades 0–4; two grade 0, three grade 1, 14 grade 2, 15 grade 3, and nine grade 4); five control cases without neurological disease; and 18 cases of non-HD neurological disease potentially involving the neostriatum (seven cases of Alzheimer's disease, one case of Pick's disease, one case of hippocampal sclerosis with cortical neuronal loss [19], one case of multiple system atrophy, five cases of primary dystonia, one case of Sydenham's chorea, and two cases of hypertensive striatal status spongiosis with perivascular neuronal loss). The term "neostriatum" is used to include the caudate nucleus, putamen, and nucleus accumbens. The two grade 0 cases had had chorea for 3 and 5 years, respectively, had been seen prior to death by members of the HD Clinic at The Johns Hopkins Hospital and judged to have symptomatic HD, and had positive family histories; neither case showed readily identifiable striatal pathology characteristic of HD on gross inspection of brain slices or on routine microscopic examination of hematoxylin & eosin-stained sections (13). Unfixed tissue for polymerase chain reaction analysis of the IT15 gene trinucleotide repeat length was not available from these two cases.

Brains were fixed for 2–4 weeks in 10% buffered formalin before blocks were taken for paraffin embedding. Ten µm paraffin sections of neostriatum at the accumbens or anterior pallidal levels were stained immunocytochemically for GFAP. Primary (polyclonal) and secondary antibodies from a GFAP kit (DAKO, Carpinteria, CA) were used, with or without a preliminary microwave step in distilled water (20), followed by diaminobenzidine as the chromogen and cresyl violet counterstaining. The microwave step intensified immunostaining for GFAP, but reduced staining by cresyl violet. Negative controls, in which rabbit serum was substituted for the primary antibody, and positive controls, consisting of sections from other cases known to show good GFAP staining, were always used. Hematoxylin & eosin-stained sections were available from each block.

Type I medium-spiny neurons and other neuronal types described by Braak and Braak (9) were identified in cresyl violet-stained sections. Type I neurons are the abundant medium-spiny GABAergic projection neurons. Type II neurons are slightly larger cells with more basophilic nuclei presumed to comprise GABAergic parvalbumin-containing interneurons as well as nitric oxide synthase-containing interneurons positive for neuropeptide Y and somatostatin. Type IV neurons are large neurons presumed to be cholinergic; type III is a rare, morphologically distinct type of large neuron. Type V are small neurons that stain darkly with hematoxylin or cresyl violet; their transmitter is uncertain, but some or all may contain vasoactive intestinal peptide (21). In neostriatal areas in which type I cells were depleted, other cell types (II, IV, V) appeared to be relatively preserved, as previously described by Kowall and colleagues (11). Type III cells were not identified.

This first series of studies, using GFAP immunocytochemistry and Nissl cytology, demonstrated islands of astrocytosis and neuronal loss in early HD neostriatum, as described below; the term "astrocytosis" is used herein to refer to increased GFAP expression in astrocytes. A second series of studies (22) was therefore directed at investigating the possible correspondence between the islands and the striosome-matrix compartments of the neostriatum (23, 24). To reveal the striosome-matrix pattern and the islands, immunostaining was performed on serial paraffin sections from formalin-fixed striatal blocks from nine HD autopsy cases (grades 0–2, selected from the first series) and three normal controls in the same age range, and in serial frozen sections of paraformaldehyde-fixed striatal tissue (one control and one grade 3 HD). Immunocytochemical staining was done using antibodies against calbindin (monoclonal, Sigma, St. Louis, MO), leucine-enkephalin (monoclonal, Seralab/Accurate Scientific, Westbury, NY; polyclonal, Incstar, Stillwater, MN), and GFAP (with cresyl violet counterstain), following microwaving in boiling distilled water for 1–10 minutes (20). Controls were as described above, using rabbit or mouse serum. Acetylcholinesterase histochemistry, historically the first method for defining striosomes (23), was not used because it was not feasible in the paraffin-embedded material.

Calbindin, a cytoplasmic calcium-binding protein found in cell bodies and axonal arborizations of type I neurons in the matrix but rarely in striosomes, is an excellent marker for striosome-matrix compartmentation (24, 25). Enkephalin is a peptide transmitter-modulator found in approximately half of type I GABAergic neurons (26, 27). Variations in the density of enkephalin-containing local axonal arborizations provide a good marker for the patch-matrix system (28–30) and show good correlation with calbindin-defined compartmentation. In the dorsal neostriatum, striosomes normally have a more enkephalin-negative neuropil than the matrix; central neostriatal striosomes are often defined by an enkephalin-positive ring; ventrally, striosomes are enkephalin-negative relative to the matrix (29, 30). Thus, in dorsal and middle neostriatum, enkephalin immunostaining provides positive identification of striosomes. The success of calbindin and enkephalin immunostaining in the neostriatum varied from case to case in both control and HD tissues, presumably because of differences in the degree of fixation and/or autolysis, so that only limited numbers of cases were successfully stained. Despite the fact that these two markers are contained in the type I neurons that are vulnerable in HD, they provided, in cases with successful staining, a good demonstration of the striosome-matrix compartmentation throughout most of the neostriatum in Vonsattel grades 0 and 1, and in the more ventral parts of the neostriatum in grade 2. Maps of GFAP, calbindin, and enkephalin immunostaining were made using a microscope with a drawing tube. Maps of adjacent sections of neostriatum immunostained for GFAP and for calbindin, respectively, were made in grades 1 and 2 cases in order to investigate the relationship between islands of gliosis with neuronal loss (GFAP and cresyl violet; neuronal loss quantified as described below) and apparent striosomes (calbindin). Maps of serial sections immunostained for enkephalin, GFAP (with cresyl violet), and calbindin were made in a grade 0 case to investigate the correspondence of islands to striosomes positively identified by enkephalin immunostaining, and to the center (low enkephalin, low calbindin) versus rim (high enkephalin, low calbindin) of the striosomes in the central neostriatum.

Neuron counts of type I neurons with nucleoli within the section were made using a 63× objective. Sections (10 µm) from the HD cases of grades 1 and 2 shown in Figure 6 (stained by GFAP immunocytochemistry and cresyl violet [without microwaving]), and adjacent sections (10 µm) of neostriatum from three control cases stained respectively by calbindin immun-
cytochemistry and cresyl violet were used. To count neurons in the GFAP-positive islands, the island was first centered in the field of a 4x objective, which was kept out of focus so that neurons were not discernible; the 63x objective was then put in place and type I neurons with nuclei in the field of the objective were counted. The non-island neostriatum, at the same dorsoventral level as the islands, was counted by systematic sampling in horizontal sweeps across the neostriatum, stopping every 1 mm; the horizontal sweeps were 1 mm apart in the dorsoventral axis. Non-island fields were rejected if they contained islands, lateral ventricle, internal capsule, external capsule or large vessels or white matter bundles. Counts for islands and for non-island neostriatum in the same section were compared by t-test.

RESULTS
First Series: Islands of Astrocytosis and Neuronal Loss

In control neostriatum, astrocytes with high levels of GFAP were present in and at the edges of white matter, in the subependymal zone, and around large and medium-sized blood vessels. Staining of normal astrocyte perikarya in the neostriatal gray matter after microwave treatment varied considerably; in some cases all or almost all of these were well stained, whereas in other cases these astrocytes remained faintly stained or virtually unstained. Whether this variability is due to variation in antigen preservation or to a generalized increased production of astrocytic GFAP in some cases is not known. The same variability was noted in the HD cases, being high in all areas in some cases, including pathologically uninvolved areas such as claustrum. Occasional small clumps of a few astrocytes with GFAP levels higher than the rest of the astrocytes in the neostriatal gray matter were noted in control cases. These groups of astrocytes appeared similar to those at the edges of blood vessels or along small bundles of white matter and may have been adjacent to such structures present in neighboring sections. These clumps of astrocytes were much smaller than the islands and striosomes described below.

In the two grade 0 HD cases, the caudate nucleus and putamen contained sharply circumscribed patches or islands of neostriatal tissue defined by a moderate-to-marked increase in GFAP staining compared to surrounding neostriatum (Fig. 1A–C). These islands were also visible in cases of higher grade (Fig. 1D). Perikarya and primary processes of astrocytes within the islands usually contained increased GFAP staining compared to most (but not all, see Fig. 1C) astrocytes in surrounding areas of neostriatum at the same dorsoventral level or in corresponding regions in controls. However, the defining feature of the islands was that the neuropil within them contained numerous very fine GFAP-positive processes (Fig. 1C), giving a brown shading to the islands. The islands were of variable shape and size (commonly 200–700 μm in lesser diameter). In the grade 0 cases, the islands were distributed throughout the dorsoventral extent of the caudate nucleus and putamen, whereas the ventral nucleus accumbens was free of them (Fig. 2A, B). None of the five neurologically normal control cases or 18 control cases with other neurological diseases showed a similar pattern of GFAP-positive neostriatal islands. In the grade 0 cases, GFAP staining was also increased compared to controls along the ventricular border of the caudate nucleus. In these cases, type I neurons were always notably decreased in density within the GFAP-positive islands compared to surrounding areas of neostriatum (Fig. 3A–D). Loss of type I neurons in the islands was also obvious in later grades (Fig. 3E–H). The two grade 0 cases appeared in every way to be involved with an earlier stage of the same pathological process affecting the higher grade cases. In the non-island regions of neostriatum, scattered astrocytes with increased GFAP content compared to controls were also present, especially in dorsal regions of neostriatum; these became more numerous in higher grade cases (Fig. 3F). Non-island regions were also characterized by a local variation in the degree of astrocytosis; some areas demonstrated a pristine appearance (e.g. Fig. 3D), whereas others demonstrated a mild to moderate degree of astrocytosis (e.g. Fig. 4A).

The three grade 1 cases also showed neuronal loss and fibrillary astrocytosis within sharply bounded islands as well as increased astrocytosis along the ventricular border. In addition, a diffuse fibrillary astrocytosis, easily visualized with hematoxylin & eosin staining, and an increase in astrocytic GFAP staining were present in the dorsomedial edge of the caudate nucleus and the dorsalmost border of the putamen. These changes were accompanied by a mild to moderate degree of type I neuronal loss in the same regions.

In the grade 2 cases, the dorsal one-fifth to three-fifths of the neostriatum at the level of the nucleus accumbens contained a diffuse increase in GFAP staining, although a focal, patchy variation in its density could often still be appreciated within this zone (Fig. 4B). In this gliotic dorsal region, a generalized, moderately severe loss of type I neurons was observed. This dorsal zone of generalized neuronal loss and astrocytosis constitutes the ventrally progressive wave of pathological change described by Vonsattel et al (13). Within this zone, pathological changes were most severe dorsally and less so ventrally. Below this zone, in every case, sharply bounded islands of moderate to intense GFAP staining in astrocytic perikarya and fine processes (Fig. 1D) were present as in grade 0 and 1 cases, but these islands now extended down into the ventralmost part of the nucleus accumbens. The islands showed increased fibrillary astrocytosis compared to grade 0 and 1 cases, and few type I neurons remained. In coronal sections at levels caudal to the nucleus accumbens, the zone of neostriatal generalized neuronal loss...
and astrocytosis had progressed at least as far ventralward as at the accumbens level.

The pattern of GFAP staining in grade 3 cases resembled that in grade 2, but the generalized astrocytosis and neuronal loss in the dorsal neostriatum were intensified and had spread further ventralward, and neostriatal atrophy had increased. In the dorsal zone of generalized astrocytosis, a patchy variation in GFAP staining density resembling the pattern of the islands was often present. Small regions of apparently well-preserved neostriatum partly surrounded by the astrocytic zone were sometimes seen at the lower border of this dorsal zone (Fig. 4C, D), reminiscent of those described by Vonsattel et al (31). Below the zone of generalized neuronal loss and astrocytosis, typical GFAP-positive islands were still visible in the ventralmost putamen (Fig. 5A) and in the nucleus accumbens.

In the grade 4 cases, a more uniform blanket of severe astrocytosis covered all of the putamen and caudate nucleus and extended into the nucleus accumbens. A severe depletion of type I neurons was present throughout the astrocytic zone, and striatal atrophy was severe. However, in some cases, islands of neuronal loss and gliosis were still visible in the ventral nucleus accumbens, surrounded by tissue with a relatively intact density of type I neurons.

In almost every case, islands were visible in the sections immunocytochemically stained for GFAP both with and without microwave pretreatment. However, with some exceptions, in most cases only a few of the islands stood out sharply on microscopic observation at low power; delineation of the others required inspection at medium to high power because of the varied GFAP expression in the non-island gray matter and the high levels of GFAP in astrocytes near blood vessels and especially...
immunostained for GFAP with Nissl or hematoxylin counterstain, constitute a highly specific distinguishing feature for the pathological diagnosis of early HD.

Second Series: Are the Islands Striosomes?

The islands of neuronal loss and astrocytosis described above were noted to resemble in size, shape, and spatial distribution the striosome or patch component of the neostriatum (23, 24, 32, 33). The alternate hypotheses (A) that the islands are the striosomes, or (B) that they are instead focal regions of neuronal degeneration in the matrix compartment (or are scattered in the neostriatum independent of striosome-matrix compartmentation) were considered. These hypotheses concerning the relationship between islands and striosomes were investigated in grade 0 to 2 cases by immunocytochemical staining of serial sections with antibodies against calbindin and enkephalin, as markers for striosome-matrix compartmentation, and GFAP, as a marker for the islands. The same or adjacent sections were stained with cresyl violet to reveal neurons.

Islands and apparent striosomes (calbindin-free patches of neostriatum) were compared in adjacent sections in cases of grade 0–2 HD where calbindin immunostaining was successful (n = 5). A microscopic survey of section pairs from these cases immunostained respectively for immunostaining.
calbindin and for GFAP with cresyl violet counterstain showed that an island always corresponded to a calbindin-negative apparent striosome in the adjacent section (Fig. 5A–D). This finding held true even when the islands were only partly depleted of type I neurons.

A rigorous comparison of islands and apparent stro- somes, addressing the alternative hypotheses mentioned above, was made in section pairs from a grade 1 case and from a grade 2 case by mapping all GFAP-positive islands and all calbindin-negative apparent striosomes. The maps were made in the region of the ventral neostriatum that was not yet involved by the ventrally progressive wave of generalized degeneration. The two hypotheses concerning the relationship of islands and striosomes were approached with the following considerations in mind: The set of calbindin-negative patches, termed apparent striosomes, must contain all of the stro- somes, as well as any holes in the matrix that might have been created secondarily by the degenerative process manifested as the islands. Thus, if only a few of the ap- parent striosomes contain islands, one would conclude that most of the genuine striosomes are not involved, but, if all or almost all of the apparent striosomes contain islands, one must conclude that the striosomes are the locus of the islands. This second conclusion would not rule out an additional component of pseudostriosomes produced by patchy degeneration of the matrix; however, any major contribution of this type would be manifested by an increased density of apparent striosomes compared with the density of striosomes in controls.

The relationship between islands and striosomes was looked at in both directions. First, do all GFAP-positive islands correspond to an apparent striosome? Confirming the qualitative results described above, this was indeed the case (Fig. 6A–D). Second, do all apparent striosomes correspond to GFAP-positive islands? As shown in Figure 6A–D, all apparent striosomes, except in the ventral most nucleus accumbens in the grade 1 case, contained islands demonstrated by GFAP staining (Fig. 6A–D). These findings strongly suggest that the calbindin-negative patches containing the islands must be genuine stro- somes, rather than secondary holes in the matrix. The distribution and number of striosomes in the same region in two control cases was similar to that in the HD cases (Fig. 6E, F). This similarity lends additional support to the idea that all or the great majority of the apparent striosomes in the HD cases shown in Figure 6A, C are genuine striosomes. In relation to the maps from a grade 0 case described below, it is important to point out that the islands defined by GFAP immunopositivity in these grade 1 and 2 cases appeared to correspond closely in size to the matching calbindin-defined striosomes.

Each of the islands in the section from the grade 2 case shown in Figure 6, and the great majority of those from the grade 1 case, gave, on microscopic inspection, a qualitative impression of readily detectable loss of type I neurons. This apparent neuronal dropout in the islands was more severe in the grade 2 case. A full scale quan- titative study of neuronal loss in striosomes and matrix is planned; to provide, for the present report, an indication of the degree of neuron loss in the striosomes, we counted type I neurons in the grade 1 and 2 cases shown in Figure 6. The counts were made in the ventral neostriatal region shown in the maps, and in the same region of three control cases. Neuron count means for multiple 63× objective fields from island/striosomes and from the matrix/non-island neostriatum are shown in Table 1. Loss of striosomal neurons in the grades 1 and 2 cases compared to non-island neostriatum in the same region was clear, and the striosomal counts were distinctly lower in HD.
### TABLE 1
Counts of Type I Neurons in 63× Objective Fields in Island/Striosome and Non-Island/Matrix Compartments of Neostriatum in HD and Controls

<table>
<thead>
<tr>
<th></th>
<th>Island/striosome</th>
<th>Non-island/matrix</th>
<th>Ratio of island to non-island</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>(r-test)</td>
</tr>
<tr>
<td></td>
<td>(n; range)</td>
<td>(n; range)</td>
<td>p value for island vs non-island</td>
</tr>
<tr>
<td>Control</td>
<td>11.0 (2.92)</td>
<td>12.9 (3.96)</td>
<td>0.853</td>
</tr>
<tr>
<td></td>
<td>(6; 8–15)</td>
<td>(18; 8–23)</td>
<td>(N.S.)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>4.00 (2.41)</td>
<td>9.65 (3.35)</td>
<td>0.415</td>
</tr>
<tr>
<td></td>
<td>(12; 1–8)</td>
<td>(57; 4–21)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>0.933 (0.854)</td>
<td>4.69 (2.29)</td>
<td>0.199</td>
</tr>
<tr>
<td></td>
<td>(15; 0–3)</td>
<td>(42; 0–11)</td>
<td>(&lt;0.001)</td>
</tr>
</tbody>
</table>

SD: standard deviation, N.S.: not significant.

than control cases. In addition, the counting results suggest a loss of matrix neurons compared to controls, more severe in grade 2, notwithstanding the difficulty in perceiving this deficit on qualitative microscopic inspection (Fig. 3).

Immunostaining of enkephalin-positive neuropil in the neostriatum in HD was difficult to achieve even in the early cases. In three grade 0 and 1 cases, enough enkephalin immunostaining was preserved in the dorsal and middle regions of neostriatum to allow positive identification of striosomes. In control cases, the intensity of enkephalin immunostaining varied from case to case, presumably a result of differences in fixation and autolysis. In those early HD cases where immunostaining for enkephalin was relatively preserved in the dorsal and middle neostriatum, staining intensity was never as strong as in the best control cases. For example, in the grade 0 case illustrated in Figure 7, enkephalin immunostaining was light and, in regions progressively more dorsal than those shown in the map, enkephalin immunostaining was increasingly difficult to appreciate, making identification of the dorsalmost striosomes with this marker impossible. Enkephalin immunostaining in the lateral segment of the globus pallidus was always lighter in HD cases than in controls, even in grade 0 and 1 cases, except in the ventralmost pallidum where levels resembling those in controls were present.

Maps were made, in the mid-caudate region of the brain of a grade 0 HD case, of GFAP-positive islands and of striosomes identified by both positive enkephalin staining and negative calbindin staining. The enkephalin- and calbindin-defined striosomes were always the locus of islands of astrocytosis and apparent neuronal loss (Fig. 7A–F). However, the GFAP-positive islands were located centrally within the enkephalin- and calbindin-defined striosomes in this grade 0 case and did not take up the full extent of the striosome, avoiding the rim of heavier enkephalin staining, although this was calbindin-negative. In contrast, as mentioned above, in the grade 1 and 2 cases (Fig. 6) the sizes of GFAP-defined islands and calbindin-defined striosomes corresponded much more closely.

DISCUSSION

Early Striosomal Neuronal Loss in HD Neostriatum

The findings presented above (Fig. 5–7; Table 1) show that, although a few islands of neuronal loss and astrocytosis could conceivably occupy regions of matrix secondarily made calbindin-negative by disease, at least the
The first involves the striosomes and was already present throughout the caudate nucleus and putamen in grade 0 in the two cases examined in this investigation; the second phase is a ventrally progressive wave of type I neuronal degeneration that involves the matrix, having its major effect in grades 2–4.

The prominent, and as yet unexplained, dorsal-to-ventral progression of severe neuronal loss in the matrix raises the question as to whether striosomal neuron degeneration might also be a dorsal-to-ventrally progressive process. Our finding that the ventrally most striosomes in the nucleus accumbens show little evidence of involvement in the grade 0 and 1 cases and have readily apparent neuronal loss and astrogliosis only in grades 2–4 is consistent with this idea.

In our cases the matrix showed variation in intensity of astrogliosis with some focal regions remaining free of this process; this variation may be a reflection of the “matrisome” geography described by Graybiel (32).

The demonstration of GFAP-positive “islands” in the neostriatum in autopsy cases of grades 0–2 HD greatly facilitates pathological differential diagnosis in such cases. Using this approach, we recently diagnosed HD at autopsy in two patients from the John Hopkins Autopsy Service, one with the clinical diagnosis of Alzheimer’s disease and the other with the clinical diagnosis of Kuf’s disease. The pathological diagnosis of HD was confirmed in both cases by polymerase chain reaction demonstrating the triplet repeat expansion characteristic of HD.

The pathogenetic mechanisms responsible for the temporally separate degeneration of striosome and matrix GABAergic neurons in the neostriatum in HD are unknown. One possibility is that calbindin content is initially relatively protective for neurons undergoing excitotoxic challenge (35–37), although the calbindin-containing matrix neurons certainly undergo almost total degeneration later in the course of the disease (25, 38).

It has been reported that, in HD cases of grades 3 and 4, striosomes defined by histochemical staining for acetylcholinesterase (39) or immunocytochemical staining for calbindin (25) maintain their volume to a greater degree than does the matrix. These results on striosomal volume preservation in late cases, which do not address neuronal loss, are not necessarily incompatible with the present findings, which demonstrate striosomal neuronal loss in early cases but do not address striosomal volume. Conceivably, preservation of striosomal volume may result from proliferation of astrocytic processes. It should be noted that a late relative preservation of striosomal volume has no meaningful implication for basal ganglia circuit function, as most type I GABAergic projection neurons in both compartments have degenerated by the grade 3–4 stage (13).

Immunocytochemical staining for calcineurin (40), demonstrating all medium-spiny (type I) neurons, and for...
syaptophysin (41), demonstrating synaptic terminals, has been reported to show patchy loss in the neostriatum in early HD. It is likely that these findings reflect the loss of neurons in striosomes demonstrated in the present report.

NADPH diaphorase histochemical activity, presumed to be located within axonal arborizations of NADPH diaphorase-containing neostriatal neurons, was recently reported to be decreased in striosomes in a case of grade 0 HD, despite the preservation of diaphorase-positive neuronal cell bodies (42). It is unclear what relationship there may be between this early patchy loss of diaphorase-positive neuropil and the loss of type I neurons in striosomes that is described in the present report.

Hypothesis: Relationship of Chorea to Early Striosomal Neuron Loss

The initial appearance of chorea is the principal criterion for defining the onset of symptomatic disease in HD (3). During the period following clinical onset, our findings show that striosomal neuronal loss is readily apparent in the neostriatum; thus it is reasonable to inquire whether striosomal neuronal loss might be the cause of chorea. If such is the case, one might predict that, analogous to the situation with dopaminergic neurons in Parkinson’s disease, striosomal neuronal loss may begin preclinically, and symptoms may appear only when a sufficient degree of cell loss has occurred to overcome adaptive mechanisms.

In recent years a consensus model of the operation of basal ganglia circuitry (Fig. 9A) has been constructed. This model, although clearly preliminary, has proved valuable in understanding hypokinetic and hyperkinetic disorders (15, 33, 43–48), and suggests that chorea in HD derives from an increase in firing rate of pallidoreceptive thalamocortical neurons of the ventrolateral thalamic complex, secondary to decreased inhibition by internal pallidal neurons (15, 43, 45, 47).

In Figure 9B we show a postulated mechanism whereby striosomal neuron loss in early HD might cause increased firing of ventrolateral thalamus neurons, leading to chorea. In this model of choreogenesis, the primary event is degeneration of striosomal GABAergic type I

Fig. 9. Diagrams of functional circuitry of the basal ganglia. A. Major basal ganglia neuronal connections, their transmitter and their inhibitory or excitatory effect. B. Predicted changes in early HD consequent to striosomal neuronal loss. Predicted hyperactivity is shown by thick lines, normal activity by thin lines, and hypoactivity by dashed lines. DA, dopamine; EXT GP, external segment of globus pallidus; GABA, γ-ami-nobutyric acid; GABA/SP and GABA/ENK, GABAergic striatal type I neurons containing substance P and enkephalin, respectively; GLU, glutamate; IL THAL, intralaminar thalamic complex; INT GP, internal segment of globus pallidus; PPN, pedunculopontine nucleus; SN COMP, pars compacta of substantia nigra; SN RETIC, pars reticulata of substantia nigra; SUBTHAL NUCLEUS, subthalamic nucleus; SUP COLLIC, nigroreceptive component of superior colliculus; UPPER CORTICO STR and LOWER CORTICO STR, corticostriatal neurons situated closer to the pia (upper) and closer the white matter (lower); VL THAL, ventrolateral thalamic complex.
neurons. A projection to the pars compacta of the substantia nigra from striosomal neurons is postulated based on findings in experimental animals (49–51). These striosomal-nigral axons are likely to inhibit the electrical activity of nigral dopamine neurons (52–54). Our hypothetical model (Fig. 9B) predicts that degeneration of striosomal-nigral neurons will disinhibit nigrostriatal dopaminergic neurons, producing a net increase in their activity. Increased dopaminergic activity in the neostriatum would then decrease the excitability of D2 dopamine receptor-bearing neostriatal type I neurons that contain enkephalin and project to the external pallidum (26), and the result would be decreased production by these neurons of preproenkephalin mRNA and enkephalin, and decreased synaptic release of GABA. The decreased net activity of enkephalin-containing GABAergic neurons would lead to increased activity in external pallidal neurons, decreased activity in subthalamic nucleus and internal pallidal neurons, and increased excitation of the thalamocortical neurons, producing chorea (Fig. 9B). Increased excitability of tectal neurons would also occur secondary to decreased activity of nigrosectal inhibitory neurons (Fig. 9B). Such a change may be responsible for the eye movement abnormalities characteristic of early HD (55, 56).

In later stages of the disease, both striosomal and matrix neurons of the neostriatum will have degenerated and many corticostratial neurons in cortical layer V are also likely to have disappeared (57, 58). These changes would be expected to lead to a marked decrease in the participation of the cortico-striato-pallido-thalamic pathway in motor activity, a situation that may be associated with the decreased motor activity and the rigidity that occur late in HD (3). As a part of these later changes, the postulated hyperdopaminergic state may disappear.

Evidence against the model of choreogenesis that we have presented includes the finding of a normal abundance of preproenkephalin mRNA in surviving neostriatal neurons in grades 2–4 HD (16), whereas the model suggests a likely decrease in message early in the course of disease secondary to inhibition of these enkephalin-containing neurons by excessive dopaminergic stimulation. The latter alternative is supported by the decreased immunostaining for enkephalin that we found in the neostriatum and that Reiner et al (14) found in the external pallidum.

The choreogenic model of Reiner, Albin, Penney, Young and coworkers was based on this finding of decreased immunocytochemical staining for enkephalin in the external pallidum (14, 15, 59, 60). Their model suggested that degeneration and/or decreased function of enkephalin-containing GABAergic neostriatal neurons accompanied by the relative preservation of substance P-containing GABAergic neurons was the cause of chorea in HD. Our findings are consistent with the model of Reiner and colleagues in several ways. The present finding of decreased staining for enkephalin-containing axonal arborizations in the neostriatum, as mentioned above, is consistent with their finding of decreased staining in axonal arborizations in the external pallidum, although we interpret these changes as likely to be caused principally by increased dopaminergic activity, whereas they postulate a primary defect in the enkephalin-containing neurons. In addition, loss of striosomal-nigral neurons leading to increased dopaminergic activity might also lead to increased activity in substance P-containing neostriatal neurons that project to the internal pallidum (15, 43, 61, 62), and might cause an increased production of substance P in type I neostriatal GABAergic neurons that project to the internal pallidum and pars reticulata of the substantia nigra (61). Such an increase in substance P content could help explain the unexpectedly strong staining for substance P in the internal pallidum in HD found by Reiner et al (14) and Albin et al (59, 60).

In Parkinson's disease decreased dopaminergic activity in the neostriatum, resulting in increased activity in subthalamic and pallidothalamic neurons, is thought to be responsible for bradykinesia (15, 33, 45, 46, 63). In our hypothetical model of chorea, the reverse occurs: increased dopaminergic activity is predicted to cause decreased activity in subthalamic and pallidothalamic neurons, producing increased excitation of thalamocortical neurons, which is presumed to underlie chorea. This view of the "opposite" nature of bradykinesia in Parkinson's disease and chorea in HD was once widely accepted (64–66). Later, this concept fell out of favor for a variety of reasons. Although one laboratory (67) found increased dopaminergic markers in the neostriatum in HD, others failed to find such an increase (68–71). In addition, one group failed to find evidence of dopaminergic hyperactivity in animals with excitotoxic striatal lesions (68).

However, most neurochemical studies of the brain in HD have examined autopsy tissue from patients with advanced disease, when both matrix and striosomal neurons have degenerated and the postulated dopaminergic hyperactivity may have subsided. A recent study of cerebrospinal fluid in living patients with HD (72) has presented evidence for a hyperdopaminergic state based on the unconventional idea that dihydroxyphenylacetic acid is the major dopamine metabolite in humans, although other studies, which measure instead homovanillic acid, have not found evidence for dopaminergic hyperactivity (e.g. ref. 73). The failure to find evidence for increased neostriatal dopamine release in animal models of HD (68) is probably not relevant because selective striosomal neuronal degeneration has not been obtained in these models (74–76). Instead, they reproduce the terminal state in HD, when both striosomal and matrix neurons have degenerated.

The present hypothesis on choreogenesis is consistent
with the amelioration of chorea by dopamine-depleting agents and dopamine receptor-blocking agents (3, 66, 77), both of which would counteract excessive dopaminergic activity in the neostriatum. The decreased efficacy of these agents in late-stage disease (3) supports our suggestion that the predicted hyperdopaminergic state abates and is replaced by a generalized functional failure of the basal ganglia.

Our discussion has thus far focused on chorea. We hypothesize that this motor manifestation arises secondary to dysfunction and loss of those striosomal neurons located within the motor area of the putamen (78). Early degeneration of striosomal neurons in other regions of the putamen and in the caudate nucleus may be responsible for other early clinical manifestations. For example, a cognitive deficit of the frontal lobe type may derive from striosomal degeneration in the caudate nucleus, which receives corticostriatal input from the prefrontal association cortex, and mood disorders might conceivably be related to the predominantly limbic telencephalic input to many of the striosomes in the ventral anterior neostriatum (79–81). Clinical signs resulting from striosomal neuronal loss outside of the motor area of the putamen would also be predicted to be ameliorated by dopamine-receptor-blocking agents, whereas signs arising from changes in other neuronal systems (e.g. cerebral cortex) would be less likely to be affected by these agents.

In conclusion, the present studies have demonstrated an early loss of striosomal neurons in the neostriatum. A distinctive histopathological pattern is thereby created, so far apparently specific to HD, that is very useful in pathological differential diagnosis of postmortem cases of early HD and cases with other premortem clinical diagnoses. Furthermore, our results suggest a model of basal ganglia function in early HD that provides a mechanism for the appearance of chorea and other early clinical signs, is consistent with the beneficial effect of dopamine-depleting agents and dopamine receptor blockers on chorea, and provides a framework for searching for improvements in pharmacological therapy. This finding also suggests experiments involving the relative expression of message and gene product of the newly identified IT15 gene in striosomal and matrix type I neurons or their afferents and provides a challenge to purported animal models of HD, in which a differential involvement of matrix and striosomal compartments has not been described.

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