Morphometric Demonstration of Atrophic Changes in the Cerebral Cortex, White Matter, and Neostriatum in Huntington’s Disease

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Abstract. We performed morphometric analysis of five standardized coronal brain slices at anterior frontal (AF), caudate-putamen-accumbens (CAP), globus pallidus (GP), lateral geniculate nucleus (LGN), and parieto-occipital fissure (OCP) levels in 30 patients with Huntington’s disease (HD) and 13 controls. Associated with the 30% mean reduction in brain weight in HD patients ($p < 0.001$) were significantly smaller overall cross-sectional areas of brain at all five levels studied, with striking losses in cerebral cortex (21–29%), white matter (29–34%), caudate (57%), putamen (64%), and thalamus (28%) ($all p < 0.005$). In addition, the ventricular system was dilated up to 2.5 times normal at CAP, GP, and LGN levels, 9.5 times normal at the OCP level, and 13 times normal at the AF level. Higher grades of severity of HD had greater reductions in the cross-sectional area of the caudate, putamen, thalamus, and cerebral cortex ($p < 0.005$–0.001), and larger ventricles ($p = 0.08$) compared to lower (less severe) grades of HD. The findings confirm and quantitate the severe atrophy of the neostriatum, in addition to demonstrating a severe loss of cerebral cortex and subcortical white matter in HD. The global atrophy of cerebral cortex and white matter observed in all degrees of HD may account for the cognitive and neuropsychiatric impairments which often precede the onset of chorea.

Key Words: Atrophy, cerebral; Dementia; Huntington’s disease; Hydrocephalus; Morphometry.

INTRODUCTION

Huntington’s disease (HD) is an autosomal dominant genetically transmitted neurodegenerative disorder characterized by progressive chorea, neuropsychiatric manifestations, and dementia (1). The neuropathologic hallmark of advanced HD consists of gross atrophy of the neostriatum with loss of both large and small neurons (2–6), most strikingly in the mediodorsal portions (7, 8). Conspicuous degenerative changes in neuronal cell bodies of both the neostriatum and cerebral cortex are detectable by histopathologic, ultrastructural, and histochemical examination early in the course of disease and before the onset of dementia (9–12). The neurochemical abnormalities associated with neuronal degeneration and atrophy of the neostriatum in HD include quantitative reductions in substance P, enkephalins, gamma aminobutyric acid (GABA), and cholecystokinin (13–16). Also characteristic of this neurodegenerative process are loss of spiny neurons (17), and selective sparing of a subset of aspiny neurons that show immunocytochemical reactivity to somatostatin, neuropeptide-Y, and nicotinamide adenine dinucleotide phosphate diaphorase (16, 18–20), and large acetylcholinesterase-bearing neurons (18, 21, 22) in the neostriatum.

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While the abnormal movements in HD have been ascribed to the anatomic and neurochemical abnormalities in the neostriatum (14, 23, 24), the underlying basis for neuropsychiatric symptoms and dementia is unknown. The gross reduction in brain weight, and the fact that the pattern of neuropsychological impairment is not distinct from other neurodegenerative diseases with known abnormalities in the cerebral cortex (25) imply a cortical basis of dementia, a theory corroborated by the ultrastructural demonstration of neuronal degeneration in the frontal cortex of patients with HD (10). However, these observations are not incontrovertible as others have been unable to document significant reductions in neuronal density in the cerebral cortex of patients with HD (26). The failure to find consistent abnormalities in the cerebral cortex has led to the hypothesis that dementia in HD is subcortical in origin and may thus be due to lesions in the neostriatum (25). In this study, we performed morphometric analysis on coronal slices of the cerebral hemispheres to determine whether significant reductions in the cross-sectional area of cerebral cortex, white matter, and subcortical nuclei occur in HD, and whether dementia is positively correlated with atrophy of particular regions in the brain.

METHODS

Sources of Tissue

Brains from patients with Huntington’s disease (HD) were obtained from the Brain Tissue Resource Center at the McLean Hospital. As part of HD research protocol, the brains were weighed in the unfixed state and then divided in the mid-sagittal plane. One half was frozen at −70°C for biochemical studies, and the other half was fixed in 10% neutral buffered formaldehyde solution. The ventricular volume of the fixed half was measured by determining the quantity of water required to fill the ventricular system to capacity. The fixed specimens were sent to the Charles S. Kubik Laboratory of Neuropathology at the Massachusetts General Hospital for neuropathologic confirmation of the diagnosis. Brains from patients with no underlying neurologic disease (controls) were obtained through the autopsy service at the Massachusetts General Hospital. These specimens were fixed whole in 10% formaldehyde solution. The ventricular volumes of these brains were not measured because the control cases were identified retrospectively.

Clinicopathological Correlation

The clinical records of all patients were reviewed to obtain demographic data and information regarding the presence and nature of neuropsychiatric disturbances, clinical course, cause of death, and underlying non-neurological diseases. These variables were used in correlation matrices to determine whether and how they were associated with atrophic changes in the brain.

Methods Used to Examine Formaldehyde-Fixed Brains

All brains were subjected to routine gross and microscopic examination, although for the HD cases, a broader histopathologic survey was made for research purposes (8). The brains were cut in the coronal plane at 0.5–1.0 cm intervals, always including the following five standardized levels which were used for morphometric analysis: anterior frontal region, approximately 1 cm anterior to the temporal poles (AF); head of caudate nucleus with the putamen and nucleus accumbens (CAP); globus pallidus with the putamen, body of caudate nucleus, and amygdala (GP); hippocampus with the lateral geniculate nucleus and centromedian nucleus of the thalamus (LGN); and the parieto-occipital fissure (OCP). The five slices were photographed before further sectioning for histologic examination; a ruler was included in the view to determine magnification.

In addition to the routine examination, the brains of patients with HD were assigned grades for severity of HD using a scale from 0 to 4 as described previously (8). Briefly, Grade 0 refers

to the presence of clinical HD, with absence of the gross and microscopic changes that typify HD; in Grade 1 the head of the caudate nucleus is grossly normal, but shows microscopic evidence of neuronal loss and gliosis; Grade 2 shows moderate atrophy of the head of the caudate with preservation of its convexity; Grade 3 refers to marked atrophy with flattening of the head of the caudate; and Grade 4 represents marked atrophy of the head of the caudate with concavity of the nucleus. Paraffin-embedded histologic sections stained with luxol fast blue–hematoxylin and eosin were used for neuropathological diagnostic assessment in all cases, both HD and controls.

Morphometric Studies

The cross-sectional areas respectively of the entire coronal slice, the cerebral cortex, the white matter, the subcortical nuclei, and the ventricular system were measured or computed from high contrast, 11" × 14" black and white mat photographic prints. This was done by digitizing the perimeters of the entire slice (CTX-OUT), the innermost portion of cerebral cortex at the cortical–white matter junction (CTX-IN), the boundaries of each subcortical nuclear structure, and the walls of the ventricles. The cross-sectional areas of the various structures used in the data analysis were measured directly or computed as follows:

\[
\begin{align*}
\text{Entire cerebral slice} & = \text{CTX-OUT}; \\
\text{Cerebral cortex} & = (\text{CTX-OUT}) - (\text{CTX-IN}); \\
\text{Total nuclear mass} & = (\text{TNUC}) \text{ Sum of areas of each nuclear structure}; \\
\text{Ventricular System} & = (\text{VENT}) \text{ Sum of areas of all components of ventricles at a given level}; \\
\text{Cerebral white matter} & = (\text{CTX-IN}) - (\text{TNUC} + \text{VENT}).
\end{align*}
\]

In addition, the thickness of the cortical ribbon was measured (digitized) along the dorsal, ventral, medial, and lateral aspects of each slice using only regions where the cortex had been cut squarely across the long axis, rather than tangentially.

The digitizing was performed by tracing structures with a hand-held cursor and digitizing pad with data transmitted directly to a minicomputer. Computations were carried out using the Bioquant System IV morphometry software package (R&M Biometrics, Inc., Nashville, TN) interfaced with an IBM AT computer. Each structure was digitized twice, and the averaged values were used for data analysis. Data are expressed as proportions (percentages) or mean ± standard error. The data were analyzed statistically with Student t-tests, analysis of variance (ANOVA), correlation matrices, and linear regression using the SYSTAT programs (1986; Evanston, IL) interfaced with an IBM PC computer. The data analyses were focused on characterizing and quantitating regional losses of cerebral tissue in HD, and correlating these changes with clinical aspects of the cases.

RESULTS

Population Profile

Among the 43 patients included in this study, 30 (70%) had clinical and pathological evidence of HD, and 13 (30%) were controls. The group with HD ranged in age from 21 to 81 years, with a mean age of 54.8 ± 2.7 years (±SEM). Sixteen of the group were men, and 14 were women. The ages in the control group ranged from 36 to 75 years, with a mean age 52.9 ± 3.9 years. Six of the group were men, and seven were women.

Clinical Aspects

Among the patients with HD, all had choreiform movements, 21 (60%) were demented, 6 (19%) had a history of psychiatric depression (3 with suicide attempts),

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and 1 (3%) had a history of personality change. In 3 (10%), no history as to the presence or absence of dementia was available. None of the control patients had histories of dementia, psychiatric depression, or personality change.

The recorded causes of death among patients with HD were: pneumonia 6, myocardial infarction 5, acute respiratory failure 2, gastrointestinal hemorrhage 1, pulmonary edema 1, pulmonary embolism 1, shock 1, suicide 1, and unknown, i.e. not determined 10. The causes of death among the controls included: bronchopneumonia 3, myocardial infarction 2, hepatic failure 2, renal failure 1, malignancy 1, and thermal burns 1. Underlying chronic diseases among patients with HD included: coronary atherosclerosis 3, endocrine abnormalities, e.g. hypothyroidism 3, diabetes mellitus 2, and pituitary adenoma 1, recurrent bronchopneumonia 2, pulmonary emphysema 1, renal disease 2, systemic hypertension 2, extensive decubitus ulcers 1, systemic amyloidosis 1, epilepsy 1, and anemia 1. Chronic diseases in the control group included: cardiomyopathy 1, progressive spinal muscular atrophy 1, malignancy 1, systemic hypertension 1, and endocrine abnormalities 2 (pancreatitis—1; diabetes mellitus—1). Twelve patients with HD and four controls had no recognized chronic systemic illnesses.

Morphometric Differences in Cerebral Structures Between HD and Control Groups

The mean brain weight in patients with HD (1,041.1 ± 33.0 grams) was significantly lower than that of the controls (1,304.2 ± 30.8 grams) (p < 0.001). In addition, in the HD brains, the ventricular system was enlarged so that the mean volume in one hemisphere was 21.3 ± 2.8 cc (5.5–67.0 cc). This average represents a two-fold increase in ventricular size relative to the normal adult brain which has a mean hemispheric ventricular volume of approximately 12 cc (27–29).

At all five levels, brains from patients with HD had mean reductions of 22–31% in overall cross-sectional areas of cerebrum, 21–29% reductions in cross-sectional area of cerebral cortex, and 29–34% reductions in cross-sectional area of cerebral white matter (all p < 0.001) (Fig. 1). In general, the loss of cerebral cortex was proportionate to the loss of cerebral white matter, except at the AF and LGN levels where the loss of white matter was disproportionately greater (AF: 21% in cortex, 31% in white matter, p < 0.007; LGN: 26% in cortex, 34% in white matter, p < 0.005). The mean thickness of the cortical ribbon in HD brains was consistently smaller than in controls (overall: 3.03 ± 0.08 mm in HD vs 3.45 ± 0.07 mm in controls, p < 0.001). The cortical ribbon was maximally reduced (16%) at the CAP level (3.18 ± 0.11 mm in HD vs 3.82 ± 0.16 mm, p < 0.001); at the other levels, the differences ranged from 9% to 12%.

Among subcortical structures, marked, disproportionate atrophy with 60–62% reductions in cross-sectional area were observed in the caudate nucleus, putamen, and globus pallidus (all p < 0.001). In contrast, 19–31% reductions in cross-sectional area, comparable to tissue loss in cerebral cortex and white matter, were observed in the amygdala (p < 0.001), hippocampus (p < 0.05), thalamus (p < 0.05), and nucleus accumbens (p < 0.05). In brains with HD, the ventricular system was significantly enlarged to 13 times control at the AF level (p < 0.001), 2.1–2.5 times normal at CAP (p < 0.01), GP (p < 0.005), and LGN (p < 0.001) levels, and 9.5 times normal at the OCP level (p < 0.005). At all levels, the enlargement of the ventricular system was disproportionate and not simply ex vacuo, i.e. the relative dilation of the ventricles was significantly greater than the relative reduction in cross-sectional area of subcortical nuclei, cerebral cortex, and cerebral white matter (p <
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Fig. 1. Morphometric analysis of cross-sectional areas of cerebrum, cerebral cortex, and cerebral white matter in HD and control patients. Measurements were made from photographs of the cerebrum sectioned in the coronal plane at the following standardized levels: anterior frontal (AF), caudate, nucleus accumbens, and putamen (CAP), globus pallidus with amygdala (GP), lateral geniculate nucleus and hippocampus (LGN), and parieto-occipital fissure (OCP). Data points represent mean ± SE. Grades 1 and 2 HD are represented as mild HD, and Grades 3 and 4 HD are represented as severe HD. HD grades were assessed by gross and microscopic survey of the neostriatum (8). Patients with HD have marked generalized cerebral atrophy with 20–30% reductions in cross-sectional area of cerebral cortex and white matter. The degree of atrophy is more severe in brains with higher grades of HD.

0.005–0.001). Hydrocephalus was considered *ex vacuo* if the degree of ventricular enlargement was equal to the degree of atrophy in the basal ganglia. This was assessed by comparing HD and control brains using the fraction: (TNUCL + TVENT)/CTX – OUT, under the assumption that this ratio ought to remain constant since we observed comparable degrees of cortical and white matter atrophy in HD brains.
MORPHOMETRIC DIFFERENCES IN CEREBRAL STRUCTURES AMONG PATIENTS WITH DIFFERENT GRADES OF HD

Profile of Patients with HD (Table 1)

The distribution of HD by grade was as follows: Grade 1: 3 (10%); Grade 2: 9 (30%); Grade 3: 10 (33%); and Grade 4: 8 (27%). With increasing severity (grade) of HD, there was a gradual decline in the mean age at death from 61.1 to 49.4 years, a lengthening of the duration of HD from 10.4 to 17.3 years, a reduction in mean brain weight from 1,083.3 to 1,008.0 grams, and an increase in the hemispheric ventricular volume from 13.9 to 28.7 ml. There was no trend among the groups with respect to sex distribution.

Comparison of Morphometric Analysis of Cerebral Structures among the Different Grades of HD (Figs. 1, 2)

At all five levels, the mean cross-sectional areas of the cerebrum, cerebral cortex, and cerebral white matter decreased significantly with increasing severity (higher grades) of HD (ANOVA; p < 0.005–0.001). In addition, a significant progressive reduction in the mean thickness of the cortical ribbon with increasing grade of HD was observed at the CAP level (p < 0.001); at the other four levels, the differences were not statistically significant by four-way analysis of variance. However, the overall (averaged for all five levels) mean thickness of cerebral cortex did differ significantly among the groups, with a trend toward higher grades of HD having thinner cortical ribbons (p < 0.005). The degrees of atrophy in cerebral cortex and white matter relative to total cross-sectional area of cerebrum were consistently proportionate among the different grades of HD.

There were significant differences among the HD grades with respect to the sizes of the caudate nucleus (CAP and GP levels), putamen (CAP and GP levels), globus pallidus, and amygdala, so that higher grades of HD had smaller mean cross-sectional areas of these structures (all p < 0.001). In contrast, significant trends in cross-sectional area of the thalamus, hippocampus, or nucleus accumbens septi with respect to HD grade were not observed. That is to say, although these latter structures were atrophic relative to control brains, the degree of atrophy was similar across different grades of HD.

At all five levels, the cross-sectional area of the ventricular system was significantly larger with higher grades of HD (p < 0.05–0.001). In addition, the relative size of the ventricles compared to total cross-sectional area of the brain slice increased with grade of HD, i.e. the ventricles were disproportionately more dilated in brains with higher grades of HD (p < 0.05–0.001; all levels).

Clinicopathological Correlations

Attempts were made to correlate the clinical aspects of the patients with the pathologic changes in the brains. Higher grades of HD were positively correlated with younger ages (r = 0.36; p = 0.06), longer duration of disease (r = 0.37; p = 0.06), larger ventricular volumes (r = 0.36; p = 0.08), and dementia (r = 0.33; p < 0.05). Depression (N = 6) was correlated with more severe global atrophy of the cerebral cortex and white matter by multiple correlation analysis (r = 0.93; p < 0.001). In particular, patients with a history of depression had more striking atrophy of the cerebral cortex at the AF (r = 0.41; p < 0.05), CAP (r = 0.39; p < 0.05), and OCP (r = 0.57; p < 0.01) levels, white matter at CAP (r = 0.48; p < 0.01), LGN (r = 0.49; p < 0.01), and OCP (r = 0.38; p = 0.09) levels, and basal ganglia at the
TABLE I
Profile of Patients with Huntington's Disease

<table>
<thead>
<tr>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
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<tbody>
<tr>
<td>Number</td>
<td>3</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Age</td>
<td>66.0 ± 1.2</td>
<td>61.1 ± 4.6</td>
<td>52.3 ± 4.8</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>2:1</td>
<td>4:5</td>
<td>4:6</td>
</tr>
<tr>
<td>Duration of HD</td>
<td>8.0*</td>
<td>10.4 ± 2.9</td>
<td>14.2 ± 2.6</td>
</tr>
<tr>
<td>Brain weight (grams)</td>
<td>1,083 ± 46</td>
<td>1,067 ± 43</td>
<td>1,031 ± 80</td>
</tr>
<tr>
<td>Ventricular volume (cc)</td>
<td>23.0*</td>
<td>13.9 ± 2.2</td>
<td>22.8 ± 4.1</td>
</tr>
</tbody>
</table>

* Information available for only one of the three patients. This group was excluded from the ANOVA test for these variables.

CAP level (r = 0.45; p < 0.01). Dementia (N = 21) was correlated with longer durations of HD (r = 0.40; p < 0.05), higher grades of HD (based upon degree of atrophy of the caudate nucleus) (r = 0.33; p < 0.05), and global atrophy of cerebral cortex and white matter (all levels using multiple correlation analysis: r = 0.93; p < 0.001). In contrast, dementia was not correlated with atrophy of other structures such as the hippocampus, amygdala, or thalamus. Suicidal behavior (N = 3) was positively correlated with duration of HD (r = 0.46; p < 0.01) and marked dilatation of the ventricular system (r = 0.72; p < 0.005).

DISCUSSION

This study demonstrates a 30% reduction in brain weight in HD, which is associated with 20–30% areal reductions in cerebral cortex, white matter, hippocampus, amygdala, and thalamus, and a 60% reduction in the cross-sectional area of the caudate, putamen, and globus pallidus. Although atrophy of cerebral cortex and white matter would be expected from the reduction in brain weight, the difference in thickness of the cortical ribbon was relatively slight (10–15%), and not perceptible by visual inspection. In addition, sulcal widening often is not prominent in HD brains so that atrophic changes may be inapparent; this is best explained by the proportionate shrinkage of cortex, white matter, and thalamus in HD. The result is that the brains are small (by weight), but they do not appear atrophic. Dementia in HD has been correlated with regional reductions in blood flow in the frontotemporal regions (30). Other findings which suggest a functional or anatomic abnormality in the cerebral cortex are the abnormal cortical somatosensory evoked potentials in HD (31), and the gross reduction in brain weight generally observed in postmortem specimens from patients with HD. Despite the gross reduction in brain weight, it has not been possible to demonstrate significant reductions in the density of neurons in the cerebral cortex (26, 32). In contrast, the disproportionate atrophy of the caudate, putamen, and globus pallidus is readily discernible without quantitation.

The observation that higher grades of HD were found in patients with earlier onset and longer duration of disease suggests that the atrophic changes in the caudate and putamen increase progressively with duration of disease, and that with later onset of HD, the atrophic changes in the neostriatum occur more slowly than in younger patients with HD. This finding has been reported by others (33, 34). The fact that the degree of atrophy in the cerebral cortex, white matter, thalamus, hippocampus, amygdala, and nucleus accumbens was similar for all grades of HD suggests that the shrinkage of these structures occurs early in the disease process and is not progressive. In contrast, the degree of atrophy of the caudate, putamen, and globus pallidus increases with higher grades of HD, indicating that these structures progressively

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Fig. 2. Morphometric analysis of subcortical nuclei and the ventricular system in HD and control brains. Striking atrophy of the caudate (CAUD), putamen (PUT), and globus pallidus (GP) was observed in brains with HD. Less severe atrophy was present in the amygdala (AMYG), thalamus (THAL), and hippocampus (HIPPO). In contrast, the nucleus accumbens septi (ACCUM) was relatively intact in HD brains. Marked enlargement of the ventricular system was observed in HD. The degree of enlargement increased with more severe grades of HD. The ventricular enlargement was disproportionate to the degree of cerebral atrophy, suggesting primary hydrocephalus in HD.

degenerate with prolonged survival. The atrophy of cerebral cortex, white matter, thalamus, hippocampus, and amygdala must reflect loss of cells, principally neurons as well as fibers. The curious aspect about this atrophy is that gliosis is not readily apparent in these structures, and the density of neurons has been assessed to be normal (26, 35). The explanation for this discrepancy is that along with the loss of neurons, proportionate shrinkage of the tissue occurs. The absence of gliosis is not readily explained; possibly the cell loss occurs so slowly and over so long a period of time that the expected gliosis does not occur.

To some extent, the enlargement of the ventricular system is explained by the loss
of cerebral tissue. However, the 200 to 1,300% increases in cross-sectional area of the ventricles (the extent varying with the plane of section), and overall 200% increase in ventricular volume (compared to normal adult brains) are disproportionate and cannot be accounted for on the basis of 30–60% reductions in the cross-sectional area of various cerebral structures. This observation would suggest that ventricular dilation in HD is primary, or independent of tissue loss, i.e. predominantly not ex vacuo, although there is no overt evidence of an obstructive process. The etiology of hydrocephalus in HD was not discernible in this study. However, the degree of ventricular enlargement in HD is far greater and more disproportionate than in other neurodegenerative diseases associated with cerebral atrophy, including Alzheimer’s disease (36) and chronic ethanol abuse (37).

The clinical correlates of the morphometrically determined atrophic changes in cerebral tissue are that both dementia and depression were significantly associated with more severe global atrophy of cerebral cortex and white matter. In addition, dementia and depression were correlated with higher grades of HD or more severe atrophy of the neostriatum. The positive correlation between dementia and depression, and degree of global atrophy of the cerebral cortex, white matter, and striatum (grade of HD) suggests that the underlying basis of dementia and depression in HD resides in degeneration of the neostriatum and projections between neostriatum and overlying cortex. Although personality changes occur commonly in HD (38), pertinent information along these lines had not been documented in most cases. Nonetheless, the substantial reductions in cross-sectional area of cerebral cortex, white matter, amygdala, hippocampus, and thalamus demonstrated for all grades of HD, may account for the neuropsychiatric derangements that occur and that frequently precede the onset of choreiform movements.

REFERENCES


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