Decreased Neuronal and Increased Oligodendroglial Densities In Huntington's Disease Caudate Nucleus


Abstract. Decreased density of neurons was found throughout the head of the caudate nucleus in Huntington's disease (HD), with the most severe neuronal loss early in the disease in the medial region. The density of reactive astrocytes is inversely proportional to the neuronal loss. In cases of mild Huntington's disease which had no identifiable abnormality on conventional neuropathologic evaluation (grade 0), there is a reduction in neuron density without an accompanying reactive astrocytosis. The pattern for decrease in neurons and accompanying astrocytosis suggests that the earliest changes occur in the most medial portion of the head of the caudate nucleus and subsequently sweep laterally across the caudate nucleus to the internal capsule. An increased density of oligodendrocytes is observed in the head of the caudate nucleus for the lower grades (0, 1 and 2). The decreased neuronal and increased oligodendroglial densities may be of significance in understanding the pathogenesis of HD. These altered densities, observed in the absence of reactive astrocytosis, suggest that these changes may not represent recent effects of disease, but rather that HD gene expression may influence brain cell densities from early in the life of the gene carrier.

Key Words: Astrocyte; Caudate nucleus; Huntington's disease; Neuron; Oligodendrocyte.

INTRODUCTION

Huntington's disease (HD) is an inherited midlife onset neurodegenerative disease transmitted as autosomal dominant (1). The gene responsible for HD has been localized to the p16.3 region of chromosome 4 (2, 3). The primary neuroanatomical abnormalities are found in the striatum, although other regions of the central nervous system have been implicated as well (4–6). In the frontal cortex, a striking loss of volume has been noted (6, 7) with a concomitant decrease in pyramidal neurons (8). These cortical changes do not appear to be strongly related to the extent of striatal degeneration (7). Variation in the distribution of neurons in the putamen has been noted in HD with relative sparing of neurons in the ventral anterior putamen when

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compared to dorsal anterior and posterior parts of the putamen (9). Subregional loss of neurons has been reported in the substantia nigra (10). Variation in the distribution of caudate nucleus neurons in HD has been reported (11), but the subregional pattern of variation and its relation to the extent of pathological involvement is not known.

As previously reported (4), postmortem examination of a series of 163 HD brain specimens revealed marked variation in the severity of neuropathological involvement, and this led to the formation of a scale for classifying involvement based on macroscopic and microscopic criteria (4). In the present study, we investigate the subregional density of neurons, astrocytes, oligodendrocytes, and microglia in the head of the caudate nucleus among HD brains with varying degrees of neuropathological severity. We postulate that HD may produce regionally specific cell loss and that an understanding of these subregional changes may be of significance in understanding the early development and the progression of the disease.

MATERIAL AND METHODS

Brain specimens of 24 clinically diagnosed cases of HD and seven similarly aged control patients collected by the Brain Tissue Resource Center of McLean Hospital, Boston, MA, were examined. The brains were divided in the midsagittal plane at the time of the autopsy. One randomly selected half-brain was frozen and the other was fixed. The weight of each fixed half-brain was recorded at the time of neuropathologic examination. Age at death, duration of disease and the sex of the individual were recorded from medical record review and interview with family members.

The HD brains were randomly selected to represent five grades of neuropathological involvement in ascending order of severity. The grading system has been previously described (4). Briefly, a grade 0 case represents an individual with the clinical diagnosis of HD and a positive family history of the illness (12). However, on blind routine neuropathological examination these cases were considered to be normal on both gross and microscopic evaluation. In short, the caudate nucleus appeared to be of normal proportion macroscopically and to be unremarkable microscopically. A grade 1 appears normal macroscopically but has evidence of neuronal cell loss and astrocytic gliosis microscopically. Grade 2 has macroscopic evidence of atrophy in the head of the caudate nucleus but retains a convex outline at the lateral ventricle. Microscopically, mild loss of neurons and reactive astrocytic gliosis are present. In grade 3, the macroscopic appearance of the ventricular outline of the head of the caudate nucleus is a straight line. Neuronal cell loss and gliosis are moderate and diffuse. In grade 4, the caudate nucleus presents a medially concave, severely shrunken appearance with extensive nerve cell depletion and fibrillary astrocytosis.

Five grade 4, six grade 3, five grade 2, five grade 1, and three grade 0 cases were included in the analysis. No additional grade 0 cases were available for study. The 24 HD specimens were derived from the series of 163 previously described (4, 12).

For cell counting, cresyl violet (CV)-stained sections of seven μm thickness at the level of the caudoputaminal junction containing the caudate nucleus, nucleus accumbens septi and putamen were prepared. A strip the width of a single microscopic field was counted for each specimen. The length of the strip was from the lateral border of the subependymal glial layer to the medial border of the internal capsule (IC). To insure consistency in sampling between cases, a standard protocol was developed as follows (see Fig. 1): A line was drawn along the lateral border of the IC (line A), with a dorsal limit at the junction of the IC and external capsule and a ventral limit at the junction of the IC and basal white matter. A point was defined on this line at one-third its length from the superolateral end, and a perpendicular line (line B) was drawn medially which traversed the caudate nucleus. The counts were made upon this line, the lateral limit of which was the medial border of the IC (line C) and the medial limit the ependymal surface of the lateral ventricle.

A Zeiss microscope with a magnification of ×312 (×25 objective, 1.25 optivar, ×10 eye-piece) and a Zeiss camera lucida were utilized to produce a series of drawings of successive
Fig. 1. Cell counts were made in the region of the head of the caudate nucleus which is outlined by the dotted line.

microscopic fields, each field encompassing 0.196 mm² of the sampled region. Each field was defined by means of an eyepiece grid, and the first 0.12 mm from the ependymal surface were skipped. Locations of neurons, astrocytes, oligodendrocytes, and microglia were recorded on the drawings. Determination of the number and location of each of these cell types was made by a computer-aided digitizing grid. All “dead” areas (e.g. vessels) larger than 0.0025 mm² on the slide were deleted.

All cell type identifications were made by visual microscopic examination of CV-stained sections by an investigator (JVP) blind to the clinical diagnosis of the case. Morphologic features, and not merely cell sizes, were used for the definition of cell types (Fig. 2) (13, 14).
Fig. 2. A. A representative photomicrograph of normal medial caudate nucleus with neuron (n), astrocyte (a), and oligodendrocyte (o) indicated (×312). B. A representative photomicrograph of the medial caudate nucleus in HD grade 0 caudate nucleus with cell types indicated as in 2a (×312). The increased density of oligodendroglia and decreased density of neurons is representative of findings in this grade for this subregion of the caudate.
**CELL DENSITIES IN HUNTINGTON’S DISEASE**

*Neurons* were identified as cells with granular cytoplasm and at least one of the following: discrete nucleolus or nucleus with granular chromatin. Because the sections were only seven μm thick, it was not possible to differentiate small neurons from those large neurons sectioned at a region of small diameter. Therefore, we report neuronal counts as combined counts for both small and large neurons. *Astrocytes* were characterized by oval or bean-shaped nuclei, about ten μm in diameter, with pale, finely textured chromatin and neither nucleoli nor visible cytoplasm. These are distinguished from small neurons by readily recognizable morphologic features. Specifically, the neurons have an optically empty rim or halo between the cell body and neuropil. Furthermore, the dark, fine granules seen in some reactive astrocytes assist in characterizing this cell type. *Oligodendrocytes* were easily identifiable in CV staining as rounded, dark nuclei sized five to seven μm without visible cytoplasm and which, with CV, morphologically resemble lymphocytes. *Microglia* cells were defined as rod-shaped cells with darkly stained nuclei, having a process at each pole, and were distinguished from endothelial cells by greater chromatin density and absence of association with vessels. Any cell which could not be unequivocally categorized was eliminated (fewer than 5% of cells).

As an additional control for reliable cell identification, twelve of the 24 HD cases were stained by glial fibrillary acidic protein (GFAP) in adjacent sections to assess that astrocytes were properly identified. Counts of astrocytes for these cases closely paralleled those of the CV-stained sections.

The position and distance of each cell from the ependymal surface was recorded. The distance from the ependymal surface to the IC was divided into fifths or quintiles; 1 = region closest to ependymal surface and 5 = region closest to the IC. The average number of cells of each cell type was determined for each quintile with specimens grouped by grade of neuropathological involvement. In addition, the average number of each cell type per microscopic field was determined for fields within each quintile. The first method (mean cells per quintile) produced the total number of cells within a standardized proportion (%) of the caudate nucleus. The second method (mean cells per field) produced the density of cells within a fixed area of the caudate nucleus.

Regional comparisons of numbers of cells were made by analysis of variance between grades (nested for specimens within a grade) and for region of caudate nucleus by quintile position. An interaction between quintile and grade evaluated whether the pattern of cellular distribution across the quintile regions of the caudate nucleus was different for the different grades. The *t*-test was used to compare the total distance from the ependymal surface to the IC for grade 0 versus control cases and grade 1 versus control cases.

**RESULTS**

The grade of neuropathologic involvement is related to both the age at death and the duration of the disease (Table 1). Persons with shorter duration of illness and those with older onset ages (as represented by older death age preceded by shorter duration) have milder neuropathologic involvement. This pattern has been noted previously (12). In addition, the half-brain weight reflects the grade of neuropathologic involvement (Table 1).

The volume of the caudate nucleus decreases dramatically across the grades of neuropathological involvement. At the level of the caudate-accumbens-putamen at which the counts were performed, the area of the caudate nucleus is normal in grade 0 but in grade 4 is only 40% of that seen in grade 0 or controls. A comparison of the mean width of the head of the caudate nucleus for control (mean = 10.1 mm) and HD grade 0 (mean = 9.1 mm) found no difference (*t* = 0.69, *p* = 0.37), but a decrease in size for grade 1 (mean = 7.5 mm; *t* = 10.0, *p* = 0.01) was found. The area of the caudate nucleus from which the cell counts are performed also changes across the grades. The area from which cell counts are derived in grade 4 is 40% of the area counted for grade 0 or controls. However, it was calculated that 2% of the area of the caudate nucleus was counted at each grade, and that the proportion of...
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* Number of cases with information available in parentheses.
± standard deviation.
NEURONS

LOCATION

Fig. 3. Mean cell counts and standard error for neurons are presented. The area of the caudate nucleus from which cell counts were derived was divided into five equally sized segments or quintiles. The quintile closest to the lateral ventricle is at the leftmost position, and the quintile closest to the internal capsule is at the rightmost position of the figure. The counts represent the total number of neurons within that quintile. Progressive loss of neurons is seen across grades. C = control, 0 = grade 0, 1 = grade 1, 2 = grade 2, 3 = grade 3, 4 = grade 4.

the existing caudate nucleus which is counted is nearly constant across grades. Because the size of the caudate nucleus is different in different grades of pathological involvement, we report mean cell number for the entire quintile as well as mean densities per microscopic field within each quintile for specimens grouped by grade of neuropathologic involvement.

Analysis per Quintile

Neurons: Figure 3 presents the average numbers of neurons by grade according to quintile region. A clear loss of neurons was seen across grades (F = 66.63, p < 0.0001). In grade 4, the number of neurons per quintile region varied from 0 to 9, while the normal control number varied from 62 to 118. In grade 4, 94% to 98% of neurons were lost. The most severe early depletion of neurons occurred in the quintile region closest to the ependymal surface. Even the grade 0 cases, considered normal on conventional microscopic examination, had lost 40% of the normal number of neurons in the first quintile (near ventricle) but had lost only 26% of the neurons from the fifth quintile (near IC). The photomicrograph in Figure 2b is representative of the medial region of the caudate in grade 0. The neuronal cell loss in the region close to the ependymal surface was significantly greater—but only marginally—than that in the region near the IC (F = 2.19, p = 0.06).

Fig. 4. Mean cell counts and standard error for astrocytes are presented by quintile region as defined in Figure 3. The counts represent the total number of astrocytes within that quintile.

Astrocytes: Evidence of reactive astrogliosis was absent in grade 0 (Fig. 4) despite the finding of neuronal cell loss noted above. Glial reactive astrogliosis was apparent in grades 1 and 2, particularly in the region close to the ependymal surface, but no increase was seen near the IC. In grade 4, severe astrogliosis was prominent in the area close to the IC. The numbers of astrocytes were significantly increased across the grade of neuropathological involvement (F = 5.01, p = 0.0003). Furthermore, the difference in number of astrocytes was significantly related to the region of the caudate nucleus in the different grades (the lines are not parallel; F = 3.28, p = 0.01).

Oligodendrocytes: A remarkable increase in the number of oligodendroglia was seen (Fig. 5) in grades 0, 1 and 2 (F = 4.60, p = 0.0007). The difference in number of oligodendrocytes was not significantly related to the region of the caudate nucleus (the lines are parallel; F = 0.70, p = 0.6).

Microglia: The numbers of microglial cells were increased in HD (mean = 10.97 to 21.4) when compared to controls (mean = 12.14) (F = 6.3, p = 0.0001), and the increase was related to quintile (F = 2.9, p = 0.02).

Analysis per Field

Neurons: The density of neurons by field (Fig. 6) closely resembled the analysis of total number per quintile described above. A significant loss of neurons was seen across grades (F = 37.2, p = 0.0001) but the regional differences were less apparent. The lines are parallel (F = 1.64, p = 0.15).

Astrocytes: The per field density of astrocytes shows an absence of reactive astrogliosis in grade 0 (Fig. 7). A modest increase in density was seen in grades 1 and 2.
near the ependymal surface. In grades 3 and 4 the extent of reactive astrocytosis became more evident in the second, third and fourth quintiles. The increase in astrocytes was significantly related to the grade (F = 40.33, p < 0.0001) and, because of the pattern in grade 4, to the region of the caudate nucleus (the lines are not parallel; F = 4.6, p = 0.0004).

Oligodendrocytes: The density of oligodendrocytes was strikingly increased in all HD grades (Fig. 8) over controls (F = 9.48, p < 0.0001). There was little difference between the grades except in the region closest to the IC. The difference in number of oligodendrocytes was significantly related to the region of the caudate nucleus (the lines are not parallel; F = 5.58, p < 0.0001).

Microglia: The density of microglial cells was modestly increased in grades 0, 1, 2 and 3 (range 2.8 to 7.8 cells per field) over controls (range 2.2 to 3.2 cells). Grade 4 cases had significantly increased numbers of microglia (range 7.8 to 18.2 cells), particularly in the quintiles closest to the lateral ventricle (the lines are not parallel; F = 33.55, p < 0.0001).

DISCUSSION

Variation in the number of neurons was observed between subregions of the head of the caudate nucleus among different grades of neuropathologic involvement in HD. The extent of loss was related to the grade of neuropathological severity. The initial neuronal depletion occurred in the region nearest the ependymal surface with a 40% decrease observed even in grade 0, the mildest grade of neuropathological
involvement. This is of particular significance since the grade 0 cases had been considered normal on routine blind microscopic examination and, thus, abnormal cell density was only identifiable by quantitative analysis.

Reactive astrocytosis was also related to the subregion of the head of the caudate nucleus. However, no significant reactive astrocytosis was observed in the grade 0 cases. The grade 1 and grade 2 cases exhibited similar patterns of reactive astrocytosis, primarily in the quintile regions closest to the ependymal surface. The maximal amount of astrocytosis in grade 3 was in the second quintile while the maximum in grade 4 was in the third and fourth quintiles. The pattern of involvement of neuronal cell loss with the reactive astrocytosis in the head of the caudate nucleus suggests that the earliest degenerative effect was primarily in the region closest to the ependymal surface in grade 0, 1 or 2 and subsequently swept laterally across the caudate in grades 3 and 4.

The absolute numbers of oligodendrocytes in the caudate nucleus were increased in HD grades 0, 1 and 2 (mild to moderate neuropathologic involvement) to nearly double that found in control cases (Fig. 5). Similarly, the density of oligodendrocytes in HD was twice that of controls regardless of grade (Fig. 8). While the increase in oligodendrocyte density may be the result of collapse of the neuropil with relative sparing of these cells, the absence of appreciable decrease in size of the caudate in grade 0 and only moderate atrophy in grade 1 suggests that selective sparing is not the only mechanism involved. In addition, the middle of the caudate nucleus (second, third and fourth quintiles) was the region with the most dramatic increase in oli-
godendrocyte density. Thus, the region of greatest increase in oligodendrocytes appears to be different from the region of decrease in neurons with reactive astrocytosis. Relative preservation of oligodendrocytes may account for this pattern since most of these cells were in the mid and lateral regions of the caudate nucleus; however, the magnitude of increase appears to be disproportionate to the extent of tissue shrinkage.

The analysis of grade 0 cases is of considerable significance in understanding the early lesion in HD since these cases represent the mildest pathological involvement. Only five of the series of 163 brains (4, 12) were categorized as grade 0. Therefore, it is important to assess whether these cases actually represent HD. The clinical features of these cases have been previously presented (12). All had a clinical diagnosis of HD and all had a positive family history of the illness. Further support for their consideration as HD-afflicted is that these quantitative studies demonstrated that they did not have normal densities of neurons and oligodendroglia in the caudate nucleus. The pattern of abnormal cell density also argues for the HD diagnosis. The neuronal densities for the grade 0 cases resembled those for grades 1 and 2, with a pattern of successively more severe neostriatal involvement across grades. This trend supports the contention that the grade 0 specimens are indeed very mildly afflicted HD cases.

It has been suggested that oligodendrocytes “may multiply in degenerative conditions” with the precaution that “this is uncertain since six or more cells may be seen around a neuron under what appear to be normal conditions” (13). One must consider that the increase in oligodendrocytes observed here may be a consequence
Fig. 8. Mean cell counts and standard error for oligodendrocytes for a single microscope field from each quintile are presented.

of proliferation occurring with neuronal degeneration. However, the observation that the region of maximal increase in oligodendroglial density is different from the region of maximal neuronal depletion and the observation that the oligodendroglial density does not increase with increasing neuronal depletion suggest that the increased oligodendroglial density is not a consequence of proliferation in response to neuronal degeneration.

The finding of an absence of reactive astrocystosis in spite of a reduction in neuronal cell density in grade 0 is significant because it suggests that the neuronal cell loss does not represent a recent event. The observation of decreased neuronal density together with the increased oligodendroglial density suggests the hypothesis that the caudate nucleus of the HD gene carrier may have had less than the normal complement of neurons and perhaps more than the normal complement of oligodendrocytes from early in the development of the central nervous system (CNS). If the cellular distribution of neurons is compromised in the neostriatum of the HD gene carrier from early in life, onset of the disease may be related to two primary factors: first, the degree of compromise in the development of the CNS, and second, the rate at which additional neurons are lost as a consequence of mid-life effects of the HD gene. This model may be of significance in understanding the onset age and the course of the illness.

The pattern of neuronal cell loss in HD may support the hypothesis that the HD striatum is compromised from early in development. We found the periventricular region of the caudate nucleus to be the region of most pronounced neuronal cell death early in the disease. Earlier studies have suggested that the tail of the caudate

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nucleus is affected prior to the head (4). Thus, the course of the degeneration appears to progress in a caudo-rostral gradient and in a medio-lateral gradient. Studies of the embryologic development of the caudate nucleus in rat (15), rabbit (16), and monkey (17) show a clear caudo-rostral gradient with neurons in the tail forming before those in the body and head. Medio-lateral gradients are also reported (18, 19) but are not found in all studies (17). The pattern of decreased neuronal density in HD may be related to the pattern of development of the caudate nucleus such that the first-formed neurons may be those first affected in HD or those neurons may not have been generated in adequate numbers originally.

Studies in the age at onset of HD are also consistent with a model implicating dysfunction in the early development of the striatum in the pathogenesis of HD. The age at onset in HD is influenced by the sex of the affected parent, with offspring of affected fathers having younger onset than offspring of affected mothers (20, 21). Either an intrauterine maternal effect (22) or a genomic imprinting by DNA methylation paternal effect (23) has been postulated to account for the parental sex effect. Both intrauterine and genomic imprinting effects may be expected to have their most direct influence upon the developing fetus. For example, gene carrying mothers may confer a protection to their gene carrying offspring during fetal development which non-gene carrying mothers (i.e. the wives of gene carrying fathers) do not confer on their offspring. Thus, the sex of parent effect may be related to a modification of the extent of compromise in the development of the striation.

Substantial white matter atrophy has been reported in HD and this may exceed the relative loss in gray matter (4, 6, 7). An abnormality in oligodendroglial function may contribute to the white matter atrophy. These findings further support the involvement of oligodendroglial cells in HD and suggest that further study is warranted. Oligodendroglial cells have received little attention in past HD research (24).

With the advent of genetic linkage testing, it is now possible to establish HD gene carrier state for the occasional rare collection of postmortem tissues from clinically normal, asymptomatic, at risk persons. These tissues may assist in resolving whether asymptomatic HD gene carriers have a normal complement of neurons and oligodendrocytes prior to onset.

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