Atrophy of the Hypothalamic Lateral Tuberal Nucleus in Huntington's Disease

H. P. H. Kremer, M.D., R. A. C. Roos, M.D., G. Dingjan, E. Marani, Ph.D., and G. Th. A. M. Bots, M.D.

Abstract. The hypothalamic lateral tuberal nucleus (NTL) was studied in the formalin-fixed brains of five patients with Huntington's disease (HD) and in five age- and sex-matched controls. With the Klüver-Barrera (luxol fast blue/cresyl violet) and hematoxylin and eosin stains the NTL was defined by its cytoarchitectonic characteristics. The nucleus was composed of one type of neuron and had about 60,000 cells. In HD, up to 90% neuronal loss was found in the NTL. The remaining neurons showed features of degeneration and there was astrocytosis. The estimated total number of glial cells in the NTL was reduced to 80% of the control values, which was exclusively accounted for by a reduction of 40% in the number of oligodendrocytes. The total number of astrocytes was unchanged. Grouping of astrocytes and the changes observed in glial fibrillary acidic protein immunocytochemistry suggested that astrocytic proliferation occurred.

Key Words: Huntington's disease; Hypothalamus; Lateral tuberal nucleus; Morphometry.

INTRODUCTION

Although chorea, personality changes, and dementia are the most striking features of Huntington's disease (HD), few patients fail to develop severe emaciation during the final stages, despite a normal or increased appetite (1). Retarded menarche (2), disturbances of central blood pressure regulation (3, 4), and various neuro-endocrinologic abnormalities (5) have been noted in HD. These findings suggest hypothalamic dysfunction. Whether this dysfunction is related to structural changes in the hypothalamus is unknown. Neuronal degeneration and depletion have been described in the supraoptic (6–8), paraventricular (6, 7), ventromedial (8) and tuberomamillary (6) nuclei, but systematic morphological studies have not been reported.

Neuronal depletion in the hypothalamic lateral tuberal nucleus (NTL) was described by Vogt and Vogt in 1951 (7) and Wahren in 1952 (9), but subsequent authors have neglected their findings. The possibility that this neglect might be related to the paucity of data concerning the normal anatomy and function of the NTL led us to study the normal localization and cytoarchitecture of the NTL before attempting to characterize the pathological changes in HD.

MATERIALS AND METHODS

Formalin-fixed brains from five randomly chosen Huntington's disease (HD) patients and from five age- and sex-matched controls were evaluated. The diagnosis of HD was based on the typical clinical signs, a positive family history, and the postmortem findings. The brains of all of the HD patients showed weight loss, the lightest (850 grams) belonged to a severely emaciated patient with typical features of HD. All cases showed neoistriatal atrophy with

From the Departments of Neurology (HPH, RACR) and Pathology (GD, GTAMB), of the Leiden University Hospital, and the Neuroregulation Group of the Department of Physiology (EM), the University of Leiden, The Netherlands.

Correspondence to: H. P. H. Kremer, M.D., Department of Neurology, University Hospital, Rijnburgerweg 10, Leiden, P.O. Box 9600, 2300 RC Leiden, The Netherlands.
neuronal loss and astrocytosis. The severity of these changes was graded according to a system devised by Vonsattel et al (10). Cortical atrophy was present in all five cases. The controls had no clinical or pathological changes associated with neurological disease. Clinical and postmortem data are given in Table 1.

Tissue Processing and Staining

The hypothalamus was dissected from coronal brain sections by placing a sagittal section lateral to the optic tract and a horizontal section through the thalamus. The hypothalamus, thus removed, included the optic chiasm and lamina terminalis rostrally, and the mamillary bodies caudally.

The blocks were dehydrated, embedded in paraffin, and serially sectioned at 15 µm in the coronal plane. Two consecutive sections out of every twenty were stained with cresyl violet/ luxol fast blue (Klüver-Barrera) and hematoxylin and eosin (H&E), respectively. Additional stains used to study selected features included silver impregnations, periodic acid/Schiff (PAS), Sudan Black B, and Schmorl's stain for lipofuscin (11).

In one control brain the serial Klüver-Barrera-stained sections were photographed and the NTL was outlined. A computer reconstruction of the nuclear mass was made with a MOP Videoplan (Kontron, Germany) computer.

Cell Counts

Estimates of the total number of neurons in the left NTL in HD patients and controls were made according to a systematic sampling procedure presenting about 1% of the total volume of the NTL for counting. In each Klüver-Barrera-stained section the area of the NTL was delineated. At magnification ×320, we moved an ocular micrometer grid (microscope view 187.5 × 187.5 µm²) over this area systematically in adjoining steps, and in every fifth position counted the nucleolated NTL neurons within the confines of the grid. Degenerated neurons were only counted if they were recognizable of NTL origin. The total count, i.e. the sum of the counts for the individual sections, was multiplied by 100 (the sampling periodicity). Nucleoli were considered to be indivisible particles which, during sectioning, were pushed either completely into or completely out of the section. On this basis no further corrections are needed for split nucleoli whose parts occur in two adjoining sections (12). Each neuron was assumed to contain one, and only one, nucleolus.

The number of glial cells was estimated in a similar manner. The glial nuclear profiles within the confines of the grid were counted. The sampling periodicity chosen for these estimates was 320. Again, no corrections were made. Commonly accepted morphological criteria were used to distinguish astrocytes from oligodendrocytes (13).

Results were expressed as means ± SD. Due to the small number of observations, differences were analysed statistically with the Wilcoxon summed rank test.

Immunocytochemistry

Gliarial fibrillary acid protein (GFAP) staining was performed by an indirect method in which a monoclonal mouse antibody against human GFAP (Sanbio/Monosan, Uden, The Netherlands; clone GF2) was used in a 1:10 dilution, followed by a second peroxidase-conjugated rabbit anti-mouse serum (Miles Laboratories; Mijdrecht, The Netherlands). The results were visualized with amino-ethylcarbazole (AEC).

RESULTS

Normal Controls

The single most important feature characterizing the NTL was the morphological appearance of its constituent neurons. These cells were easily recognized and were readily distinguished from the neurons of the surrounding nuclear formations. Lateral tuberal nucleus (NTL) neurons were triangular, polygonal, or rounded, with a maximal diameter of about 25 µm (Fig. 1a). One or two apical dendrites may be present.
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age at death (years)</th>
<th>Sex</th>
<th>Duration of illness (years)</th>
<th>Post-mortem interval (hours)</th>
<th>Brain weight (grams)</th>
<th>Grade (*)</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>F</td>
<td>7</td>
<td>24</td>
<td>1,125</td>
<td>4</td>
<td>pneumonia</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>M</td>
<td>18</td>
<td>12</td>
<td>850</td>
<td>4</td>
<td>pneumonia</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>F</td>
<td>10</td>
<td>72</td>
<td>1,200</td>
<td>3</td>
<td>pulmonary embolism</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>M</td>
<td>12</td>
<td>6</td>
<td>1,040</td>
<td>4</td>
<td>septic shock</td>
</tr>
<tr>
<td>5</td>
<td>74</td>
<td>F</td>
<td>15</td>
<td>4</td>
<td>1,060</td>
<td>3</td>
<td>pneumonia; septic shock</td>
</tr>
</tbody>
</table>

Mean age: 53.4 ± 18.1 year

| Controls |                      |     |                             |                             |                      |           |                |
|----------|----------------------|-----|-----------------------------|-----------------------------|----------------------|-----------|                |
| 1        | 33                   | F   | —                           | 8                           | 1,350                | —         | vulva carcinoma; septic shock |
| 2        | 63                   | M   | —                           | 7                           | 1,440                | —         | bronchial carcinoma; pneumonia |
| 3        | 42                   | F   | —                           | 6                           | 1,500                | —         | gastric carcinoma; pulmonary embolism |
| 4        | 53                   | M   | —                           | 13                          | 1,510                | —         | myocardial infarct |
| 5        | 77                   | F   | —                           | 6                           | 1,300                | —         | disseminated carcinoma of the breast |

Mean age: 53.6 ± 17.3 year

(*) According to Vonsattel et al (Ref. 10); Grade 4 shows the most severe pathological changes. F—female, M—male.
The nucleus, which was situated eccentrically, close to one of the apices, and often showed an indented nuclear membrane, accounted for one-third to one-half of the total neuronal surface on section. Its nucleolus measured 1–2 μm in diameter. The most conspicuous feature of the NTL cells was the perikaryon. It was smoothly limited and completely filled with a fine-grained pigment which was stained by lipophilic (Klüver-Barrera and Sudan Black B) and PAS dyes, but not by a lipofuscin stain (Schmorl's). Nissl substance was evenly dispersed over the cytoplasm. No other types of neuron were found within the confines of the NTL.

The neuropil around these cells had a relatively homogeneous structure with sparse myelinated fibers, some vessels, and few glial cells (Fig. 2a).

If these cytoarchitectonic criteria were used to establish the extent of the NTL, the nuclear mass was aggregated in two or more discrete subdivisions in the basal lateral tuberal hypothalamic region. The bulk of the nucleus was caudal and was contained within a space bordered laterally by the optic tract, dorsolaterally by the internal capsule and ansa lenticularis, mediadorsally by the descending fornix, mediocaudally by the mamillary body, medially by the arcuate nucleus, and ventrally by the pia mater of the tuber cinereum. Smaller aggregates extended rostrally toward the optic chiasm. To the unaided eye the NTL was revealed by what is called the lateral eminence on the ventral surface of the tuber cinereum (Fig. 3a). Figure 4 shows a computer reconstruction of the spatial arrangement of the NTL in one of the controls.

The NTL is partially surrounded by magnocellular neurons of the tuberomamillary nucleus (NTM). Tuberomamillary nucleus neurons can be easily distinguished from NTL neurons: they are larger (up to 40 μm), have an irregularly limited perikaryon, coarse patches of Nissl substance, and a centrally placed nucleus. In the posterior tuberal region especially, NTM neurons are closely apposed to the main mass of the NTL, demarcating it from the rest of the lateral hypothalamic area. The smaller rostral NTL extensions are usually not bordered by these large characteristic ganglion cells.

The NTL in Huntington's Disease

The typical NTL neurons disappear in HD. This depletion varied from a limited amount (case HD 5 in our series) to an almost complete loss of neurons (cases HD 1–4). The remaining ganglion cells showed marked degenerative changes. Spindle-shaped or shrunken forms were seen (Fig. 1b). Characteristic features of the perikaryon were lost: the cytoplasm lost volume and its texture became coarser, with lumps of basophilic Nissl-like substance. Satellitosis occurred rarely.

Gliosis was noted in what was recognized as remnants of the atrophied NTL. The density of astrocytes and oligodendrocytes was increased. Astrocytes were encountered in groups of three or four.

The neuropil lost its homogeneous structure and acquired a spongy appearance (Fig. 2b). The density of capillaries was increased.

Characteristically, the large NTM neurons surrounding the NTL were well preserved, although a limited increase in glial density was often suggested. These large ganglion cells indicated the position of the atrophied NTL by lining a space devoid


Fig. 2. Cytoarchitecture of the lateral tuberal nucleus (NTL). a. Control: a tuberomamillary neuron can be seen on the right side (arrow). H&E. ×80. b. Huntington's disease. Typical NTL neurons have disappeared; tuberomamillary neurons are preserved (arrows). The number of small cells is greatly increased; the neuropil has a spongy appearance. H&E. ×80.
of neurons but with conspicuous gliosis (Fig. 3b). This space may collapse to such an extent that the NTM neurons may approach the ventral pial surface, from which they are separated only by a rather narrow band of dense glial tissue.

The total volume of the NTL appeared to be diminished, as suggested by the replacement of the bulging lateral eminence on the ventral tuberal surface by a concavely curved floor of the lateral tuber in some cases.
Fig. 4. Computer reconstruction of the left lateral tuberal nucleus of a control. The complex is viewed along an antero-posterior, slightly left-to-right, slightly upward line. Thin lines indicate the contours of the NTL in coronal serial sections, 300 µm apart. Bold lines schematically outline the fornix (F), the optic tract (O), and the bottom of the tuber cinereum (T). Right (R) medial side and left (L) lateral side are indicated. Note the large caudal part of the nucleus and the small rostral aggregates.

Cell Counts

The normal NTL contains about 60,000 neurons (sample mean: 59,980; 95% confidence interval of the mean: 50,260 to 69,700). In HD this number may be as low as 3,000 (Table 2). Cases HD 1 to 4 showed such a severe reduction. Case HD 5 preserved about 40% of the normal cell count. Clinically this case was characterized by a mild form of chorea and dementia, which became evident only after the 60th year.

The mean glial estimate in normal NTL was 234,000 ± 30,600 cells (Table 3), made up of 114,500 ± 26,300 astrocytes and 119,500 ± 26,600 oligodendrocytes. In HD the mean glia cell estimate was decreased by 21%, amounting to 182,900 ±

<table>
<thead>
<tr>
<th>HD patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58,500</td>
</tr>
<tr>
<td>2</td>
<td>45,800</td>
</tr>
<tr>
<td>3</td>
<td>64,900</td>
</tr>
<tr>
<td>4</td>
<td>66,000</td>
</tr>
<tr>
<td>5</td>
<td>64,700</td>
</tr>
</tbody>
</table>

Mean ± SD: 59,980 ± 8,460

<table>
<thead>
<tr>
<th>Number</th>
<th>Total glial cells</th>
<th>Astrocytes</th>
<th>Oligodendrocytes</th>
<th>Total glial cells</th>
<th>Astrocytes</th>
<th>Oligodendrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>221,440</td>
<td>127,360</td>
<td>94,080</td>
<td>276,800</td>
<td>118,720</td>
<td>158,080</td>
</tr>
<tr>
<td>2</td>
<td>192,960</td>
<td>133,760</td>
<td>59,200</td>
<td>198,080</td>
<td>88,960</td>
<td>109,120</td>
</tr>
<tr>
<td>3</td>
<td>118,080</td>
<td>62,080</td>
<td>56,000</td>
<td>250,240</td>
<td>155,520</td>
<td>94,720</td>
</tr>
<tr>
<td>4</td>
<td>188,480</td>
<td>132,480</td>
<td>56,000</td>
<td>229,440</td>
<td>94,080</td>
<td>135,360</td>
</tr>
<tr>
<td>5</td>
<td>193,600</td>
<td>101,760</td>
<td>91,840</td>
<td>215,680</td>
<td>115,520</td>
<td>100,160</td>
</tr>
<tr>
<td>Mean</td>
<td>182,900*</td>
<td>111,500</td>
<td>71,400†</td>
<td>234,000</td>
<td>114,600</td>
<td>119,500</td>
</tr>
<tr>
<td>± SD</td>
<td>±38,500</td>
<td>±30,500</td>
<td>±19,700</td>
<td>±30,600</td>
<td>±26,300</td>
<td>±26,600</td>
</tr>
<tr>
<td>% of control</td>
<td>79</td>
<td>97</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05.
† p < 0.01.
38,500 (p < 0.05; Table 3). The mean astrocyte estimate did not differ from that of controls (97%; 111,500 ± 30,500; NS). The number of oligodendrocytes was reduced to 60% of the control values, i.e. 71,400 ± 19,700 (p < 0.01).

In HD it was sometimes difficult to locate the atrophied NTL, especially in the rostral sections, where the adjoining NTM cells are lacking. Only areas belonging unambiguously to the depleted NTL were counted.

Immunocytochemistry

All controls and HD brains showed dense GFAP staining of pial and ependymal surfaces. In the controls the basal tuberal region showed feeble to moderate immunoreactivity diffusely located from the optic tract to the median eminence. There was no predilection for any area.

In HD cases 1 to 4 the density of immunoreactive astrocytes was greatly increased in the area of the atrophied NTL, as well as in some directly adjoining areas occupied by NTM neurons. The rest of the basal tuber showed only weak reactivity. Case HD 5 showed no signs of immunoreactivity except pial and ependymal staining.

The GFAP-reactive astrocytes in the NTL of HD patients had a coarser shape, with more tortuous protrusions, than their counterparts in controls.

DISCUSSION

Descriptions of the normal anatomy of the lateral tuberal nuclei are conspicuously sparse. Nauta and Haymaker (14) describe them as “two or three spherical or oval bodies which bulge somewhat from the inferior surface of the hypothalamus.” Daniel and Prichard (15) did not define the limits of the NTL, while Strenge (16), although recognizing the space it occupies in the basal lateral hypothalamus, did not describe the extension of the nucleus to the caudal tuber cinereum and the rostral mamillary area in man. Earlier reports mentioned distinct nuclei (17), separate cell columns (18–20), or an irregular mass of typical cells in the basal parts of the lateral tuber cinereum (21). These differences can be explained by interindividual variations (21), a variable appearance in neighbouring sections leading to sampling errors, and the emphasis given to a demarcating “capsule” around the nucleus.

We used cytological criteria to define the extent of the NTL, as did Wahren (17). The intimate relationship with the tuberomamillary nucleus is stressed by all authors and can be used for closer delineation of the NTL (17–21). The fibrous capsule mentioned by several authors (14, 22) is, according to our observations, not a distinct structure.

The lateral tuberal nucleus is present in man and in higher primates, as well as in some lower primates such as Callithricidae (Simiae) and Lemuriformes (Prosimiae), but not in other Prosimiae such as Lorisiformes, Tarsiformes, and Tupariiformes (22). There is considerable controversy as to its presence in other mammals (16, 20). Neither fiber connections, nor neurotransmitter(s) of the NTL neurons are known. In Cynomolgus monkeys receptors for corticotropin-releasing factor are abundant in the NTL (23). Greving (24) observed connections with the basal ganglia. Modern work on this problem is not available, and studies on the functional significance of these nuclei have not been reported.

Huntington’s disease is neuropathologically characterized by neuronal loss and astrocytosis in the neostriatum (1, 10). Although changes have been described in several other regions of the brain (1, 25), they are far less pronounced than those in the caudate nucleus and the putamen. This has led some authors to doubt the occurrence of consistent changes outside the neostriatum (26).
With systematic sampling and an associated point counting method (12), we found severe neuronal loss in the NTL of our HD cases. The population may be reduced to less than 10% of that of normal controls. However, severely degenerated neurons may have been neglected in the counts.

In all five patients we found gliosis, i.e. an increased density of glia cells. Whether this increase is due to collapse of the neuropil or represents a real increase in the total number of glial cells is uncertain. Our estimates in our controls and patients showed a 21% decrease in the total glia cell number caused by a 40% decrease in the number of oligodendrocytes. The number of astrocytes had not changed. Several points, however, should be kept in mind. The difficulty encountered in delineating the rostral parts of the nucleus in HD may mean that we counted too few cells. If so, the actual total number of glia cells and the number of oligodendrocytes must have been higher than our estimates show and the number of astrocytes would also be higher. This implies astrocyte proliferation, which is supported by the observation of grouping of astrocytes and altered GFAP immunoreactivity. Since we did not correct our estimates for cut spheres, lost caps, or possibly increased nuclear volume, they indicate relative changes rather than absolute numbers.

The number of cases we investigated is too small to permit detection of correlations between neuronal loss in the NTL and neostriatal cell loss, as described by the classification according to Vonsattel et al (Table 1). Case HD 5 is interesting, because the late-onset clinical course was associated with a relatively small ganglion cell loss. This raises questions requiring further study of clinico-pathological correlates.

Qualitatively, the pathological changes in the NTL resemble those in the neostriatum (10). It is not clear whether this represents a direct effect of the defective HD gene on NTL neurons or is caused secondarily by affected NTL afferents.

Pathological changes in the NTL have been described in other diseases. In Parkinson’s disease, Lewy bodies were found in all parts of the hypothalamus, including the NTL (27). Recently, selective pyknosis and disintegration of neurons were found in the NTL of a patient with severe depressive illness who died of a malignant neuroleptic syndrome (28). In a patient with Kallmann’s syndrome (hypogonadotropic hypogonadism with anosmia) the lateral tuberal nuclei were “underdeveloped“ (29). The question of the specificity of the NTL degeneration in HD should be resolved by systematic neuropathological studies in HD and other neurodegenerative diseases.

ACKNOWLEDGMENTS

One hypothalamus (control 3) was obtained from the brain bank of the Netherlands Institute for Brain Research, Amsterdam. Prof. G. W. Bruyn and Prof. D. Swaab made critical comments; Mr. G. v. d. Giessen and Dr. H. Feirabend assisted in preparing the illustrations, and Mr. H. Choufoer made the computer reconstruction.

REFERENCES


(Received June 21, 1989/Accepted October 30, 1989)
MS 89-39