The Hypothalamic Lateral Tuberal Nucleus in Alzheimer’s Disease

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The hypothalamic lateral tuberal nucleus was investigated in 5 young patients, aged 45 to 64 years, with Alzheimer’s disease (AD) and in 5 age-matched control subjects. Combining conventional histopathological and immunocytochemical staining with neuronal counts, a peculiar form of neuronal pathology was characterized. Although neurons and neurites in the lateral tuberal nucleus of AD specimens were heavily stained by Alz-50, silver and thioflavine-S stains disclosed few neurofibrillary tangles or neurites. The numbers of neurons in the lateral tuberal nucleus of patients with AD (67,450; SEM = 5,050) were no different from those of control subjects (58,900; SEM = 2,450). In the AD patients, few plaques were present and were almost exclusively of the amorphous variety. We conclude that neurons in the lateral tuberal nucleus show an early stage of AD-related cytoskeletal pathology (Alz-50 positivity), but without plaques or neuronal death.


The lateral tuberal nucleus (NTL) is a ganglion cell mass in the posterolateral floor of the hypothalamic tuber cinereum. Recently, we estimated that in adults without neurological disease it contained about 60,000 neurons [1]. Its connections, neurotransmitters, and function are unknown. The NTL is present in humans and higher primates [2]; homologies in other mammals are disputed [3]. Thus, the nucleus is considered to be a phylogenetically young structure.

Having been alerted by early descriptions [4–6], we recently quantified severe neuronal loss and glioblast changes in the NTL of patients with Huntington’s disease [1]. Braak and Braak [7] noted preferential changes in the NTL in a disease characterized by adult-onset dementia, intraneuronal argyrophilic grains, and silver-staining coiled bodies containing straight filaments. These observations suggested that structural changes in the NTL might be associated with dementing illnesses. In the present study we examined the NTL in patients with presenile Alzheimer’s disease (AD) and in control subjects.

Materials and Methods

The brains of 5 patients with presenile AD and of 5 age-matched control subjects were investigated. All patients had a history of gradual intellectual deterioration over a mean period of 7.8 years (standard error of mean [SEM], 2.2 years). A neurologist had made a clinical diagnosis of probable AD in every patient. Each patient had undergone a neuropsychological test on at least one occasion, and in all the results indicated organic dementia. Retrospective analysis of the clinical data according to accepted criteria [8] confirmed the clinical diagnosis. Postmortem examination followed the US National Institute on Aging guidelines [9] and showed cerebral atrophy with neuronal loss, plaques, and tangles in several neocortical areas and the hippocampus in all AD brains. The control subjects were free of neurological diseases. The Table gives the clinical details and the pathological findings in patients and control subjects.

Tissue Processing and Staining

Brains were weighed and fixed in 10% buffered formalin. The hypothalamus was dissected, dehydrated, embedded in paraffin, and serially sectioned at 6 μm in the coronal plane. Every 50th section was stained by cresyl violet and Luxol fast blue (Klüver-Barrera). Selected sections were stained by hematoxylin and eosin (H&E), Congo red B, thioflavine-S, silver methenamine according to Jones [10], and silver stains according to Palmgren and Holmes.

Immunohistochemical investigations were performed with the following primary antibodies: a monoclonal mouse antibody against glial fibrillary acid protein (GFAP, 1:10) (Sanbio/Monosan, Uden, The Netherlands); Alz-50, an antibody that recognizes a neuronal antigen in the brains of patients with AD [11] (IgM mouse monoclonal antibody, 1:100) (Abbott Laboratories); and SP28, a rabbit antibody to a synthetic

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Received Mar 30, 1990, and in revised form Jul 12. Accepted for publication Aug 26, 1990.

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### Clinical and Pathological Features of Patients with AD and Control Subjects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at Onset (yr)</th>
<th>Age at Death (yr)</th>
<th>Cause of Death</th>
<th>Brain Weight (gm)</th>
<th>Postmortem Time (hr)</th>
<th>Fixation Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>34</td>
<td>45</td>
<td>Cachexia</td>
<td>1,130</td>
<td>4</td>
<td>119</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>43</td>
<td>52</td>
<td>Meningitis, encephalitis</td>
<td>1,250</td>
<td>23</td>
<td>102</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>51</td>
<td>56</td>
<td>Bronchopneumonia, septic &lt;br&gt;shock</td>
<td>1,180</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>57</td>
<td>61</td>
<td>Pulmonary embolism</td>
<td>1,260</td>
<td>84</td>
<td>63</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>54</td>
<td>64</td>
<td>Pneumonia</td>
<td>800</td>
<td>3</td>
<td>35</td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td></td>
<td>47.8 (4.2)</td>
<td>55.6 (3.4)</td>
<td></td>
<td>1,124 (84.4)</td>
<td>27.6 (14.8)</td>
<td>73.4 (16.0)</td>
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<table>
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<tr>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>—</td>
<td>49</td>
<td>Suicide (barbiturates)</td>
<td>1,440</td>
<td>24</td>
<td>49</td>
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<tr>
<td>2</td>
<td>F</td>
<td>—</td>
<td>52</td>
<td>Carcinoma of the breast</td>
<td>1,370</td>
<td>5</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>—</td>
<td>53</td>
<td>Myeloid leukemia</td>
<td>1,410</td>
<td>+</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>—</td>
<td>61</td>
<td>Myocardial infarct</td>
<td>1,400</td>
<td>22</td>
<td>51</td>
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<tr>
<td>5</td>
<td>M</td>
<td>—</td>
<td>65</td>
<td>Myocardial infarct</td>
<td>1,420</td>
<td>32</td>
<td>35</td>
</tr>
<tr>
<td>Mean (SEM)</td>
<td></td>
<td>55.6 (2.7)</td>
<td></td>
<td></td>
<td>1,408 (11.6)</td>
<td>20.8 (5.7)</td>
<td>37.2 (6.0)</td>
</tr>
</tbody>
</table>

*p Value* 0.91 0.009 0.71 0.09

*Only differences in brain weight are statistically significant for patients with AD and control subjects (Mann-Whitney).* 
+ = time not known.

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**polypeptide, identical to the N-terminal 28–amino acid sequence of the A4/β amyloid-forming protein in plaques (1:300) [12]. For SP28 immunohistochemistry the sections were pretreated with 85% formic acid for 20 minutes [13]. Immunoreactivity was visualized by a second peroxidase-conjugated antibody (rabbit-anti-mouse or goat-anti-rabbit) with diaminobenzidin (DAB) as the end point.**

**Morphometrics**

The NTL could be unambiguously identified in patients as well as in control subjects (Fig 1). Estimates of the total number of neurons in the left NTL of each subject were made according to a systematic sampling procedure presenting about 0.4% of the total volume of the nucleus for counting. In each Klüver-Barrera–stained section, the outlines were marked in ink on the coverslip. At magnification of ×320, an ocular micrometer grid (microscope view, 187.5 × 187.5 μm²) was systematically moved over this area in an interlocking tessellation pattern. In every fifth position, the nucleolated NTL neurons within the confines of the grid were counted. The total count (i.e., the sum of the counts for the individual sections) was multiplied by 250 (the sampling periodicity). Nucleoli were considered to be hard particles which, during sectioning, were pushed either completely into or out of the section [14]. Thus no correction factor for split nucleoli was needed.

Counts might have been biased toward excessive numbers by the occurrence of extranucleolar chromatim lumps in the nuclei, mimicking nucleoli. Therefore, in each patient and each control, it was recorded what percentage of 100 randomly selected nucleolated neurons contained extra chromatim lumps.

Due to the small sample sizes involved, the quantitative data of the patients with AD and the control subjects were compared using the nonparametric two-tailed Mann-Whitney two-sample test, with the level of significance set at 0.05.

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**Results**

The neuronal and glial density in the NTL of the 5 patients with AD did not seem to differ from that of the control subjects in the Klüver-Barrera- and H&E-stained sections. GFAP immunostaining showed identical patterns in patients and control subjects.

Although plaques occurred in the NTL of all the patients, the densities were limited and nowhere approximated those observed in the hippocampus or the neocortex. Some plaques could be found in 2 control subjects as well. NTL plaques presented as whirly structures stained with SP28 and silver methenamine, less with thioflavine-S, and hardly with Congo red (Fig 2). Involvement of astrocytes (GFAP) or Alz-50–positive neurites in the plaques occurred infrequently. A few classic plaques with a central amyloid core were encountered in 2 patients with AD, but never in control subjects. Perivascular amyloid was sometimes observed in the patients.

Striking changes were found in the Alzheimer NTL neurons themselves. The cell bodies and the neuropil showed such a prominent Alz-50 reactivity that in most cases the NTL clearly stood out from the rest of the hypothalamus (Figs 3 and 4). Only the adjoining tuberomammillary nucleus was affected in an equally strong manner (see Fig 4). A large proportion of ganglion cells (although not the majority) exhibited this Alz-50 immunoreactivity (Fig 5). In such cells the perikaryal cytoplasm was completely stained, with the staining extending into the neurites. Staining of these neurites created a dense network of Alz-50–positive fibers throughout the whole of the NTL. In some places these were associated with plaques, but they occurred...
in equal densities in areas free of plaques. In the oldest 2 control subjects, aged 61 and 63 years, only a few Alz-50-positive neurons and neurites were recognized.

With conventional silver stains (Palmgren, Holmes) and thioflavine-S, neurofibrillary tangles were only rarely observed. Some cells displayed fine argyrophilic threads, but the network of affected cells and neurites, visible in the Alz-50-stained sections, was absent in these preparations.

Despite these changes in the NTL, the mean estimated number of ganglion cells in the patients (67,450; SEM = 5,050) did not differ significantly from that of the control subjects (58,900; SEM = 2,450; p = 0.13). Similarly, the mean percentage of neurons containing extra chromatin lumps in their karyoplasm did not differ between the two groups (patients: 2.8%, SEM = 1.0; controls: 3.6%, SEM = 1.7; p = 0.83). This implies that the counts in both groups were equally biased by this phenomenon.

As a corollary finding, our results suggest that neither postmortem autolysis time (up to 84 hours) nor duration of fixation (up to 119 days) is critically important for the proper staining with Alz-50.
Discussion
The hypothalamic NTL revealed peculiar changes in 5 patients with presenile AD. On the one hand, there were characteristics indicating that the NTL is relatively spared. In contrast to the neuronal loss found in Huntington's disease [1], in AD the number of neurons did not differ from that in control subjects and gliosis was absent. The number of plaques was low and they were almost exclusively of the amorphous variety [15]. Neurofibrillary tangles were rare in conventional silver-stained sections. Yet immunocytochemical staining with Alz-50 showed such an abundant reactivity of both perikarya and neurites that the NTL of the patients could be recognized with the naked eye.

Alz-50 is a monoclonal antibody that recognizes an epitope on an intraneuronal 68-kDa, microtubule-associated protein tau [11, 16, 17]. Tau is normally compartmentalized in axons [18]. Abnormally phosphorylated [19], primitive [20], or otherwise altered tau proteins are part of the paired helical filaments of the neurofibrillary tangles in AD. In AD brains, altered tau immunoreactivity is also found in neurites and perikarya lacking silver-staining tangles [21, 22], and the same has been reported for Alz-50 [11, 23]. Consequently, Alz-50 seems to be able to demonstrate early AD-related changes in the cytoskeletal distribution or composition of tau [11, 22, 23]. According to this view the NTL represents a brain area in which AD affects the neurons in a limited way, without progress to the classic changes of silver-staining tangles and neuronal loss. A similar evolution seems to occur in the hippocampal CA3 field, the deep layer underlying the lamina
principalis externa of the presubiculum (area 27), and perhaps area 20 of the inferior temporal association cortex [23].

In previous studies, Alz-50 reactivity has been associated with neuronal death, whether occurring from disease [11, 23, 24] or as a result of developmental rearrangements [25, 26]. According to our quantitative data, neurons in the NTL do not die in AD, and thus the view of Alz-50 as a marker of neuronal death does not seem to hold for this nucleus. Alternatively, Alz-50 labeling may not indicate that neurons are dying but rather that they become changed by a disease process, comparable to alterations taking place in the developing brain [25–27].

Abnormally staining neurites with a variety of abnormal shapes have been recognized as a feature of AD in silver stains [28], as well as in immunohistochemical preparations using anti-tau antibodies [21] and Alz-50 [29]. Although several names have been used, an appropriate one seems to be “dystrophic neurites.” They may be part of the AD plaques [29] or, alternatively, may be found unassociated with plaques in the cortex of patients [21, 23, 28]. Apparently, they are abundantly present in the NTL. We do not know whether these changes represent early cytoskeletal alterations in axons, redistribution or de novo expression of tau in the dendritic compartment, or neuritic sprouting and outgrowth [30, 31].

It has been suggested that plaques constitute a point source of trophic and/or toxic substances that affect surrounding neurites and glia in a centrifugal fashion [32]. In the NTL the dystrophic neurites were found to be evenly distributed over the entire area of the nucleus. The few plaques present were not associated with locally increased densities of neurites. Thus in this nucleus no evidence was found for the hypothesis that plaques induce local neuritic changes which spread through the neuropil to the ganglion cell bodies and beyond.

The brains were obtained from the Netherlands Brain Bank, Amsterdam. Postmortem neuropathological examinations were performed by Drs W. Kamphorst and D. Troost, neuropathologists at the Free University Hospital, Amsterdam, and the Academic Medical Centre of the University of Amsterdam, respectively. Technical expertise was demonstrated by G. Dingjan, Department of Neuropathology, Leiden State University. Alz-50 was donated by Abbott Laboratories. SP28 was kindly provided by Dr Frangione, New York.
References