Hypothalamic Lesions in Multiple Sclerosis

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Abstract. Demyelinating lesions of fiber bundles in and adjacent to the hypothalamus (i.e. the fornix, anterior commissure, internal capsule, and optic system) may be the basis for autonomic and endocrine alterations in multiple sclerosis (MS) patients. Therefore we investigated the presence and immunological activity of lesions in hypothalamic fiber bundles of 17 MS patients and 14 controls. In the MS group, 16 of 17 patients showed demyelinated lesions. The incidence of active lesions was high (60%) and outnumbered chronic inactive lesions in the internal capsule (p = 0.005). In 4 of 17 MS patients, axonal damage was observed and in 3 of 17 MS patients grey matter lesions were apparent. Duration of MS was inversely related to the active hypothalamic MS lesion score (r = -0.72, p = 0.001). Since comparison of hypothalamic lesions with MS lesions in other areas of the brain in the same patients (n = 7) showed a great similarity both as stage and appearance was concerned, this negative relation in all likelihood reflects the clinical consequences of high disease activity throughout the whole brain. In controls no demyelinating lesions were seen but in 11 control cases HLA expression was observed that was lower than that present in MS patients (p = 0.02). In the median eminence region that lacks a blood-brain barrier, all controls showed a strong HLA expression around the blood vessels. We conclude that systematic pathological investigation of the hypothalamus in MS patients reveals an unexpected high incidence of active lesions that may impact on hypothalamic functioning.

Key Words: Axonal damage; Demyelination; HPA-axis; Hypothalamus; Multiple sclerosis; Neuropathology.

INTRODUCTION

Various autonomic, endocrine, and behavioral disturbances have been described in MS patients. Disturbed autonomic functions include abnormalities in the regulation of the cardiovascular system and of sleep, eating, and temperature, the control of bowel and bladder activity, and sexual behavior (1–9). Alterations in endocrine function in MS include hyperactivity of corticotropin-releasing hormone (CRH)-producing neurons, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, and altered basal or stimulated cortisol concentrations (10–16). In addition, plasma levels of prolactin, luteinizing hormone, follicle stimulating hormone, growth hormone, thyroid-stimulation hormone, and testosterone have been reported to be altered during MS (17–20). In turn, the endocrine disturbances may influence the course of the disease, since both glucocorticoids and sex hormones have profound effects on the immune response (21, 22). Experimental animal studies show that activity and reactivity of the HPA system may be implicated in both susceptibility to the disease and recovery from a relapse of MS (23–25). Also, the increased risk of developing mood disorders and depression in MS may be the result of the chronic activation of the HPA-axis (26–31).

The pathogenic mechanisms underlying hypothalamic disturbances in MS patients are still poorly understood. Hardly any information is available on the incidence of MS lesions in and adjacent to the hypothalamus (32). It is conceivable that demyelinating MS lesions in myelinated fiber bundles in and adjacent to the hypothalamus, i.e. the fornix (FX), the anterior commissure (AC), part of the internal capsule (IC), and optic nerves, chiasm, and tract (optic system, OS, Fig. 1), may contain MS lesions that compromise hypothalamic functioning. Demyelination of these fiber bundles can cause a conduction block and thus may reduce input and output to hypothalamic neurons. Alternatively, inflammatory mediators that are produced in such lesions may diffuse to targets in the hypothalamus (33) to influence neuronal activity. Indeed, hypothalamic neurons respond to various cytokines and other mediators that are produced in active MS lesions, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α. These cytokines have been implicated in the regulation of body temperature, functioning of the HPA and HP-gonadal and HP-thyroid axes, and control of sleep and food intake (34–42).

The current study was designed to systematically investigate whether the hypothalamus of MS patients contains lesions and/or shows signs of immune activation. For this purpose we examined formalin-fixed hypothalami of 17 MS patients and 14 controls. In serial sections at the level of the paraventricular nucleus (PVN), we

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studied the degree of demyelination, activation of resident microglial cells, the presence of infiltrating macrophages and T cells, gliosis, and axonal damage.

MATERIALS AND METHODS

Brain Material

Hypothalami of 17 MS patients and 14 control subjects were obtained at autopsy under the management of the Netherlands Brain Bank (Dr. R. Ravid, Coordinator). All patients had given consent for a brain autopsy and the use of their brain tissue and clinical information for research purposes. The clinical correspondence of MS patients was evaluated by Prof. C.H. Polman (Vrije Universiteit Medical Centre, Amsterdam, The Netherlands) and the diagnosis of MS was determined according to the Poser criteria (43) and confirmed by neuropathological examination. The clinical and autopsy data are shown Table 1. The severity of MS at the time of death was determined using the expanded disability status scale (EDSS) (44). The mean disease duration ± SEM of MS was 21 ± 3 yr. Two of 17 MS patients had a primary progressive course (#91-072 and #92-033). The other 15 patients had a relapsing, remitting course that entered a chronic progressive phase of the disease before autopsy. The controls did not suffer from a primary neurological or psychiatric disease. Treatment with glucocorticoids or interferon-β within a period of 3 months prior to death was used as exclusion criterion. The control group did not differ significantly from the MS group in sex (p = 0.39), postmortem delay (p = 0.20), brain weight (p = 0.39), fixation period (p = 0.06), or pH of CSF (p = 0.34), which is a marker for agonal state (45) (Table 1).

Hypothalamus Tissue

The hypothalamus was dissected dorsally just above the third ventricle, laterally at approximately 2.5 cm from the third ventricle through the caudate-putamen complex and the internal capsule, rostrally at the caudal end of the diagonal band of Broca, and caudally through the corpus mamillare. Sometimes both the left and the right side, but usually only the left side, were fixed in 4% v/v formalin for 13 to 72 days at room temperature (RT) (Table 1), dehydrated in a graded ethanol dilution series, and embedded in paraffin (HistoWax, Histo-Lab Ltd., Göteborg, Sweden). Six-μm frontal sections were cut on a microtome. Sections were mounted on slides coated with 2% (v/v) 3-aminopropyl-triethoxysilane (Sigma, St. Louis, MO) dissolved in acetone (Merck, Darmstadt, Germany), and at 100-section intervals (i.e. 600 μm) sections were stained with 0.1% thionin in acetic acid to delineate the PVN. At 25%, 50%, and 75% rostro-caudal distance from the beginning of the PVN serial sections were taken for the immunohistopathological analysis (46) (Fig. 1).

Klüver Staining

Sections were deparaffinized in xylene and rinsed in 100% and 96% ethanol, stained with 0.1% Luxol Fast Blue (BDH Chemicals Ltd., Poole, UK), dissolved in 96% ethanol with 0.05% acidic acid for 3 hours (h) at 60°C, and rinsed in 96% ethanol and distilled water (DW). Subsequently the Luxol Fast Blue staining was differentiated in 0.05% lithium carbonate (Merck) in DW and rinsed in DW. Finally a standard cresyl violet (Merck) counterstaining was performed. Sections were dehydrated and enclosed in entellan (Merck).

Bodian Staining

Sections were deparaffinized in xylene and rehydrated in a graded ethanol dilution series and incubated overnight in 1% silver protein (Merck) at 37°C. The sections were rinsed with DW and incubated for 10 min with 1% (w/v) hydroquinone (Merck) in DW and 2% formol to reduce the silver staining. Subsequently the sections were rinsed with DW and incubated with 1% (v/v) gold chloride (Engelhard-Clal Drijfhout Edelmetall Bedrijven B.V., Amsterdam, The Netherlands) in DW for 3 to 5 min. After rinsing with DW the sections were stained for 5 min with 1.5% oxalic acid in DW and rinsed with DW. Finally the sections were fixed for 5 to 10 min with 5% sodium sulfate (Merck) in DW, dehydrated, and enclosed in entellan. Bodian-stained sections were evaluated microscopically at ×250 magnification. Alterations in axonal density were determined by comparison of the same structures and at the same level of the hypothalamus of an MS patient and a control case.

Primary Antibodies

Monoclonal mouse antibody CR3/43 (1:100, Dako, Glostrup, Denmark) is directed against the β-chain of all products of the major histocompatibility complex class II gene subregions of the human leukocyte antigen (HLA)-DR, -DQ and -DP. CR3/
### TABLE 1A

Clinicopathological Data

<table>
<thead>
<tr>
<th>NBB # MS</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>pmn (yr)</th>
<th>fxp (d)</th>
<th>brw (g)</th>
<th>pH CSF</th>
<th>Duration of MS (yr)</th>
<th>EDSS</th>
<th>Course of MS</th>
<th>Clinicopathological data and cause of death</th>
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<tr>
<td>91–012 m</td>
<td>33</td>
<td>52:30</td>
<td>28</td>
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<td>nd</td>
<td>11</td>
<td>8–9</td>
<td>sp</td>
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<tr>
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<td>36</td>
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<td>6.46</td>
<td>10</td>
<td>6</td>
<td>sp</td>
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<tr>
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<td>40</td>
<td>07:00</td>
<td>30</td>
<td>1,134</td>
<td>6.74</td>
<td>14</td>
<td>8–9</td>
<td>sp</td>
<td>dehydration</td>
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<td>95–065 f</td>
<td>41</td>
<td>03:40</td>
<td>199</td>
<td>1,294</td>
<td>6.52</td>
<td>7</td>
<td>8–9</td>
<td>sp</td>
<td>respiratory insufficiency</td>
<td></td>
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<tr>
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<td>30</td>
<td>1,080</td>
<td>6.39</td>
<td>8</td>
<td>6</td>
<td>sp</td>
<td>cachexia due to refusal of food</td>
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<td>30</td>
<td>1,010</td>
<td>6.10</td>
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<td>8–9</td>
<td>sp</td>
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<tr>
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<td>32</td>
<td>1,193</td>
<td>5.83</td>
<td>32</td>
<td>8–9</td>
<td>sp</td>
<td>respiratory insufficiency</td>
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<tr>
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<td>07:16</td>
<td>31</td>
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<td>18</td>
<td>8–9</td>
<td>sp</td>
<td>pneumonia</td>
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<tr>
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<td>55</td>
<td>09:10</td>
<td>29</td>
<td>1,378</td>
<td>6.52</td>
<td>20</td>
<td>8–9</td>
<td>sp</td>
<td>respiratory insufficiency; sepsis</td>
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</tr>
<tr>
<td>95–095 m</td>
<td>56</td>
<td>05:24</td>
<td>28</td>
<td>1,375</td>
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<td>13</td>
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<td>sp</td>
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<td>21</td>
<td>8–9</td>
<td>pp</td>
<td>sepsis</td>
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<tr>
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<td>26</td>
<td>1,240</td>
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<td>29</td>
<td>8–9</td>
<td>sp</td>
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<tr>
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<td>62</td>
<td>10:10</td>
<td>28</td>
<td>1,050</td>
<td>nd</td>
<td>32</td>
<td>8–9</td>
<td>sp</td>
<td>respiratory insufficiency; ileus; urinary tract infection; sepsis</td>
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<tr>
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<td>63</td>
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<td>30</td>
<td>1,071</td>
<td>6.43</td>
<td>40</td>
<td>8–9</td>
<td>pp</td>
<td>renal failure, cachexia, sepsis</td>
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<td>34</td>
<td>1,272</td>
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<td>19</td>
<td>8–9</td>
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<td>1,385</td>
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<td>96–076 f</td>
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<td>28</td>
<td>1,159</td>
<td>6.93</td>
<td>49</td>
<td>6</td>
<td>sp</td>
<td>cachexia; recurrent urinary tract infections</td>
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</tr>
<tr>
<td>NBB #</td>
<td>controls</td>
<td>Sex</td>
<td>Age (yr)</td>
<td>pmd (h)</td>
<td>fxp (d)</td>
<td>brw (g)</td>
<td>pH</td>
<td>CSF</td>
<td>Clinicopathological data and cause of death</td>
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<td>90-043</td>
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<td>31</td>
<td>1,325</td>
<td>nd</td>
<td>6.50</td>
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<td>arrhythmia; bacterial endocarditis; Fallot’s tetralogy</td>
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<tr>
<td>98-031</td>
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<td>33</td>
<td>&lt;46:25</td>
<td>72</td>
<td>1,588</td>
<td>nd</td>
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<td>hemothorax secondary to traumatic rupture of the left ventricle due to traffic accident</td>
<td></td>
</tr>
<tr>
<td>87-045</td>
<td>m</td>
<td>37</td>
<td>42:00</td>
<td>31</td>
<td>1,510</td>
<td>nd</td>
<td></td>
<td></td>
<td>alcohol and benzodiazepine intoxication</td>
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</tr>
<tr>
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<td>04:00</td>
<td>42</td>
<td>1,274</td>
<td>nd</td>
<td></td>
<td></td>
<td>Pickwickian syndrome; dystrophia myotonica and cardiac arrest</td>
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</tr>
<tr>
<td>86-039</td>
<td>f</td>
<td>53</td>
<td>&lt;27:00</td>
<td>17</td>
<td>1,410</td>
<td>nd</td>
<td></td>
<td></td>
<td>chronic myelomonocytic leukemia</td>
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<td>34</td>
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<td>bleeding from carotis communis due to squamous cell carcinoma of the mouth</td>
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<tr>
<td>92-046</td>
<td>f</td>
<td>54</td>
<td>12:45</td>
<td>31</td>
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<td>nd</td>
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<td></td>
<td>traffic accident</td>
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<td>96-010</td>
<td>m</td>
<td>63</td>
<td>10:20</td>
<td>32</td>
<td>1,250</td>
<td>nd</td>
<td>6.37</td>
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<td>pneumonia; diabetes mellitus type II; Korsakoff’s syndrome</td>
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<tr>
<td>91-206</td>
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<td>63</td>
<td>03:30</td>
<td>13</td>
<td>nd</td>
<td>nd</td>
<td></td>
<td></td>
<td>cardiac arrest; Crohn’s disease</td>
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<td>97-042</td>
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<td>12:50</td>
<td>28</td>
<td>1,030</td>
<td>6.94</td>
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<td>cardiac arrest; metabolic acidosis and dehydration</td>
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<tr>
<td>96-013</td>
<td>f</td>
<td>68</td>
<td>10:30</td>
<td>32</td>
<td>1,122</td>
<td>6.83</td>
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<td></td>
<td>hematemesis; melena; mammary carcinoma and metastases</td>
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<tr>
<td>82-005</td>
<td>m</td>
<td>68</td>
<td>05:45</td>
<td>30</td>
<td>1,300</td>
<td>nd</td>
<td></td>
<td></td>
<td>myocardial infraction</td>
<td></td>
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<tr>
<td>96-057</td>
<td>f</td>
<td>69</td>
<td>08:30</td>
<td>31</td>
<td>1,074</td>
<td>nd</td>
<td></td>
<td></td>
<td>myocardial infarction; pulmonary embolus</td>
<td></td>
</tr>
<tr>
<td>93-139</td>
<td>f</td>
<td>78</td>
<td>06:25</td>
<td>32</td>
<td>1,135</td>
<td>6.69</td>
<td></td>
<td></td>
<td>lung carcinoma; progressive dyspnea; hypertension</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 1B**
Clinicopathological Data

- **NBB # controls**: Netherlands Brain Bank identifier
- **Sex**: m = male, f = female
- **Age (yr)**: age in years
- **pmd (h)**: postmortem delay of obduction in hours
- **fxp (d)**: fixation period in days
- **brw (g)**: brain weight in grams
- **pH CSF**: cerebrospinal fluid pH

**Clinicopathological data and cause of death**:
- arrhythmia; bacterial endocarditis; Fallot’s tetralogy
- hemothorax secondary to traumatic rupture of the left ventricle due to traffic accident
- alcohol and benzodiazepine intoxication
- Pickwickian syndrome; dystrophia myotonica and cardiac arrest
- chronic myelomonocytic leukemia
- bleeding from carotis communis due to squamous cell carcinoma of the mouth
- pneumonia; diabetes mellitus type II; Korsakoff’s syndrome
- cardiac arrest; Crohn’s disease
- cardiac arrest; metabolic acidosis and dehydration
- hematemesis; melena; mammary carcinoma and metastases
- myocardial infraction
- myocardial infarction; pulmonary embolus
- lung carcinoma; progressive dyspnea; hypertension

**Notes**:
- a = Korsakoff’s syndrome without neuropathological changes
- brw = brain weight
- CSF = cerebrospinal fluid
- h = hours
- m = male
- NBB = Netherlands Brain Bank
- nd = not determined
- ms = multiple sclerosis
- pmd = postmortem delay of obduction
- yr = year

**Immunohistochemistry**

Prior to immunohistochemical staining, the sections were deparafﬁned in xylene, hydrated by a graded ethanol dilution series, and washed in TBS buffered saline (TBS: 0.05 M Tris). The sections were incubated with primary antibodies dissolved in TBS containing 0.05% w/v gelatin (Sigma, St. Louis, MO) and 0.015% v/v hydrogen peroxide (Merck) in 0.05 M Tris buffer (pH 7.6). Finally, the sections were rinsed in DW, heated in a microwave oven at 90°C for 10 min for antigen retrieval. After incubation with primary antibodies dissolved in TBS containing 0.05% w/v gelatin (Sigma, St. Louis, MO), the sections were washed with 0.1% Tween-20 (Sigma, St. Louis, MO) and 0.05% w/v amonium nitrate buffered saline (BBS, Sigma, St. Louis, MO) for 10 min. The sections were incubated with biotinylated antibodies directed against mouse or rabbit IgG (Vector Labs, CA) at RT for 1 h. After rinsing in TBS, the sections were incubated with an ABC complex (ABC, Vector Labs, CA) at RT for 30 min. To visualize the ABC-complex, the sections were incubated with 0.01% w/v 3,3-diaminobenzidine tetra hydrochloride (DAB; Sigma, Poole, UK) and 0.015% v/v hydrogen peroxide (Merck) in 0.05 M Tris buffer (pH 7.6) for 10 min before staining for reaction product visualization. The sections were then washed in distilled water, rinsed in DW, and mounted in entellan (Merck).

**Microscopy**

The sections were studied at a magnification of ×400 and ×1,000. The number of reactive and active lesions together gave the "active lesion score" as follows: 1, lesion almost completely surrounded by a hypercellular rim; 2, lesion partially surrounded by a hypercellular rim; 3, lesion almost completely surrounded by a hypercellular microglial/macrophage-containing rim. The incidence of reactive and active lesions was scored per myelinated structure (i.e., FX, IC, AC, and OS) using a scale ranging from 0 to 4: 0, no lesions; 1, one small lesion; 2, two small lesions; 3, lesion covering the bundle almost completely; 4, lesion covering the whole bundle.
score” and chronic inactive lesions produced the “chronic inactive lesion score” that were used for correlation studies. HLA-DP, -DQ, -DR immunoreactivity was also scored on a 4-point scale: 0, no immunoreactivity; 1, few cells expressing HLA-DP, -DQ, -DR (mostly ramified microglial cells) in a part of the bundle; 2, many cells expressing strong HLA-DP, -DQ, -DR immunoreactivity, some exhibiting a ramified and some exhibiting a round morphology; 3, many rounded cells (in many cases foamy macrophages) expressing intensive HLA-DP, -DQ, -DR covering the major part of the bundle; 4, the whole bundle contains foamy macrophages expressing HLA-DP, -DQ, -DR abundantly. Scores at the 3 levels of the fiber tract that were analyzed were averaged and the mean score per fiber tract per case was used for further analysis. If a structure was present at only 1 or 2 levels (as was often the case with the AC), only 1 score was used or 2 scores were averaged, respectively. When the left and the right side of the hypothalamus were present, both sides were analyzed and the mean was taken. The total lesion scores and the total HLA score per case are the cumulative scores of the 4 fiber bundles together (with a maximal score of 16).

**Statistical Analysis**

Differences between the MS and the control group in post-mortem delay, fixation period, brain weight, pH of CSF, and HLA scores were analyzed by Mann-Whitney U-test. Differences in incidence of either sex between the control and MS groups were analyzed by χ²-test. Correlation analyses were performed using the Spearman non-parametric correlation test. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL). Statistical significance was defined as p < 0.05.

**RESULTS**

**MS Patients**

Lesions were found in the hypothalamus in 16 of 17 MS patients. The lesion load varied from a single small lesion in one of the myelinated bundles to complete demyelination of the bundles analyzed. In 12 cases, active as well as chronic inactive lesions were found in the hypothalamus of the same patient. In 1 case (#96-076) only chronic inactive lesions were found; in 1 case (#91-072) only active lesions; and in 3 cases (#96-026, 96-104 and #96-121) only (p)reactive lesions were found. Comparison of the active lesion score (= [p]reactive + active lesions) with the chronic inactive lesion score (Fig. 2) showed that in the IC the active lesion score was significantly higher (p = 0.005), whereas both scores were not significantly different from each other in the AC (p = 0.44), the FX (p = 0.29) and the OS (p = 0.20). The incidence of active lesions was not significantly different from the incidence of chronic inactive lesions in either fiber bundle (Fig. 2).

Active lesions always contained myelin debris-containing foamy HLA-DP, -DQ, -DR and CD68-positive macrophages (Fig. 3a, b). In line with this, the HLA-DP, -DQ, -DR score correlated positively with the active lesion score (r = 0.67, p = 0.02), but not with the chronic inactive lesion score (r = 0.02, p = 0.94). In 2 MS cases foamy macrophages were seen in active lesions that contained Luxol Fast Blue-positive particles, indicative of recent uptake of myelin (Fig. 3b). In many cases hypertrophic GFAP-positive astrocytes were seen in and near such active lesions as a sign of gliosis (Fig. 3c). Three of 16 MS hypothalami with lesions contained only (p)reactive lesions. One of these patients (#96-121) had 3 small (p)reactive periventricular lesions in the IC, whereas the other 2 patients (#96-026 and #96-104) had large widespread expression of HLA by microglial-like cells and perivascular lymphocyte cuffing but no demyelination (Fig. 3d). Chronic inactive demyelinated lesions contained isomorphic gliosis, low numbers of HLA-DR, -DP, -DQ-positive microglial cells (Fig. 3e), and frequently low numbers of infiltrating CD3-positive T cells. In 13 of 16 MS patients with lesions, CD3-positive T cells were seen, mostly packed together in perivascular cuffs and sometimes infiltrating the surrounding tissue (Fig. 3f). In the patients with hypothalamic lesions, comparison with lesions in other areas of the brain that had been dissected by MRI-guidance (NBB# 96-025, 96-026, 96-074, 96-076, 96-104, 96-121 and 97-006) showed a great
Fig. 3. Microphotographs of MS lesions. A: CD68-positive foamy macrophages in an active lesion in the IC of MS patient #95-065. B: Klüver staining of an active lesion in the OS of MS patient #95-065. Arrows point at 2 foamy macrophages at the edge of the lesion. Arrowheads point at luxol fast blue-positive particles in the macrophages. C: Gliosis in a chronic active lesion in the IC of patient #95-065. Arrows point at GFAP-positive hypertrophic astrocytes. D: HLA-DR, -DP, -DQ-positive microglial-like cells (arrow) and HLA-positive leukocytes (arrowhead) in the Virchow-Robin space around a blood vessel (Bv), indicative of a (p)reactive MS lesion in the IC of MS patient #96-026. E: A chronic inactive lesion in the OS of patient #93-051. Arrows point at HLA-DR, -DP, -DQ-positive microglial-like cells and arrowheads point at isomorphic gliosis and widened extracellular spaces typical for gliotic tissue. F: Perivascular accumulation of CD3-positive T cells near an active lesion in the IC of patient #95-065. Scale bar: 15 μm.
similarity in the appearance and the activity of the lesions. For example, 5 of 6 lesions that were investigated in patients #96-026 and #96-104 in a study by De Groot et al (51) were scored (p)reactive. In the hypothalamus of these patients only (p)reactive lesions were scored in the current study.

Controls

In 9 of 14 controls, low HLA-DP, -DQ, -DR expression was seen in microglial-like cells with a ramified morphology in myelinated areas. The HLA score was lower in the controls than in the MS group (p = 0.02). In 4 of 14 controls some intravascular, weakly CD3-positive T cells were seen.

Axonal Damage

The presence of axonal damage was assessed in sections using Bodian and APP staining. Based on the Bodian staining in 1 MS patient (#93-051, Fig. 4a), loss of axonal density as compared to another MS patient (#96-026, Fig. 4b) was observed in a chronic inactive lesion in the OS. APP-immunoreactive fibers were spotted in 4 of 17 MS patients, in active lesions (3 of 4), or nearby (1 of 4) active MS lesions (Fig. 4c). The diameter of the APP-immunoreactive axons was enlarged (5–7 μm). In 3 of 4 MS patients, APP was also seen in infiltrating cells in active MS lesions. Neuronal cell bodies in the PVN and the supraoptic nucleus were APP-immunoreactive in almost all MS and control cases with varying intensities. No axonal damage was found in Bodian- or APP-stained sections in controls.

Grey Matter and Median Eminence

In 3 of 17 MS patients, hypercellular regions were observed in the hypothalamic grey matter, containing relatively high numbers of HLA-DP, -DQ, -DR immunoreactive microglial-like cells and some perivascular accumulations of CD3-positive T cells. HLA-DP, -DQ, -DR-positive microglial-like cells were seen in close proximity to neuronal cell bodies in the PVN and SON (Fig. 4d). In the grey matter of controls, only sporadically microglial-like cells expressed HLA-DP, -DQ, -DR, mainly along the third ventricle and in the PVN and the SON. Hypercellular areas were not observed in the grey matter of controls. In contrast, in the median eminence, HLA-DP, -DQ, -DR expression was found in all control cases in which the median eminence was present (n = 7), as well as in all MS patients that could be investigated (n = 10) (Fig. 4e, f). In the MS group, the HLA-DP, -DQ, -DR expression in the median eminence was somewhat more intense and in 1 case a small perivascular infiltrate was present (Fig. 4f).

Relation between MS lesions and Disease Duration and Severity

The active lesion score showed a strong negative correlation with the duration of MS (r = −0.72, p = 0.001), whereas there was no significant relationship between the chronic inactive lesion score and disease duration (r = −0.41, p = 0.102) (Fig. 5). The group of 12 MS patients that had reached the chronic progressive phase of the disease (EDSS 8–9) had similar numbers of active and inactive hypothalamic lesions as compared to the group of 4 MS patients with EDSS 6.

DISCUSSION

The results of this study show for the first time that the hypothalamus and adjacent structures are prone to demyelinating lesions in MS patients. The high incidence of lesions in this area of the brain may represent a key neuropathological finding underlying the autonomic and neuroendocrine disorders frequently found in MS patients. In most cases, both active and chronic inactive lesions were present, illustrating the long-term ongoing nature of demyelination in this disease.

The incidence of active MS lesions in autopsy brain tissue collected after a long disease history is generally considered to be low (52). However, 2 recent histopathological autopsy studies of MS lesions that were dissected by MRI guidance show the presence of many active lesions (50, 51). In fact, 83% and 48% of the lesions that were dissected in these respective studies consisted of (p)reactive and (chronic) active lesions. Thus, when tissue is sampled by MRI guidance (50, 51), or when an unbiased open study strategy is followed, as was the case in the current study, it appears that active lesions within and outside the hypothalamus are a common feature in MS. In addition, comparison of the immune and histopathological appearance of hypothalamic lesions with that of lesions outside the hypothalamus in the same patients showed a striking similarity between the stage and the appearance. This indicates homogeneity in the type of lesions within 1 MS patient.

The active lesion score in the hypothalamus correlated negatively with disease duration, whereas there was no relation between disease duration and the chronic inactive lesion score. As mentioned above, there is considerable homogeneity in the appearance of lesions within the brain of 1 patient. The relation between duration of disease and the active hypothalamic lesion score therefore in all likelihood reflects the clinical consequences of strong disease activity throughout the whole brain rather than the effect of active hypothalamic lesions on the disease course. In the patient material used in the present study, a possible relationship between the hypothalamic lesion score and clinical severity of MS could not be studied because there was too little differentiation in clinical severity in the MS.
Fig. 4. Photomicrograph of axonal damage and HLA expression in the supraoptic nucleus (SON) and the median eminence. A: Bodian staining of the OS of MS patient #93-051. Note the reduced density of axons as compared to the axonal density in Figure 5b. B: Bodian staining of the OS of MS patient #96-026. There is no sign of axonal damage in the OS of this MS patient. C: APP-immunoreactive axons in the IC of MS patient #91-070. Note the large diameter (5–7 μm) of the APP-immunoreactive axons. Adjacent to this area is an active MS lesion (not shown). D: HLA-DR, -DP, -DQ, -DR-positive microglial cells in the SON of MS patient #96-026. Arrow points at an HLA-positive microglial-like cell that seems to be in close contact with an SON neuron. E: HLA-DR, -DP, -DQ-immunoreactive microglial-like cells in the median eminence of control #93-085. Arrows point at HLA-reactive microglial-like cells in close vicinity of blood vessels (Bv). F: HLA-DR, -DP, -DQ-immunoreactive cells in the median eminence of MS patient #95-095. Arrow points at a small lesion of HLA-positive cells. Scale bar: 15 μm.
Importantly, we recently found that the presence of hypothalamic neuronal cell bodies due to diffusion (33, 34) in the myelinated bundles may have remote effects on the hypothalamus. Note that there is a significant inverse correlation between the active lesions score and the duration of MS, but not between the chronic inactive lesions score and the duration of MS.

Grey matter lesions are present in MS (54), but are less easily detectable as compared to white matter lesions, due to the absence of the contrast between demyelination and myelinated surroundings, as well as the absence of myelin-containing foamy macrophages. The current study revealed that in the grey matter of the hypothalamus of MS patients, hypercellular areas are present that exhibit abundant expression of HLA-DP, -DQ, -DR molecules in this region is due to the absence of the blood-brain barrier and whether the increase in HLA expression facilitates antigen presentation to leukocytes remains to be established.

Grey matter lesions are present in MS (54), but are less easily detectable as compared to white matter lesions, due to the absence of the contrast between demyelination and myelinated surroundings, as well as the absence of myelin-containing foamy macrophages. The current study revealed that in the grey matter of the hypothalamus of MS patients, hypercellular areas are present that exhibit abundant expression of HLA-DP, -DQ, -DR molecules. The degree of demyelination in these areas is difficult to assess due to the modest thickness of the myelin sheets. Such hypothalamic grey matter lesions may have substantial impact on conduction in axonal projections between the different hypothalamic nuclei and may therefore alter neuronal functioning in the hypothalamus. Inflammatory mediators generated within active lesions in the myelinated bundles may have remote effects on hypothalamic neuronal cell bodies due to diffusion (33, 39). Importantly, we recently found that the presence of active but not of chronic inactive lesions in the hypothalamus shows a highly significant negative relation with the degree of activation of CRH neurons in MS patients, both at the protein and the mRNA level. The nearer the structure containing the lesion was situated to the PVN, the stronger the relation. Thus, active hypothalamic lesions do seem to act on CRH neurons. Given the strong immune modulating effects of cortisol, the presence of active hypothalamic MS lesions can, via this route, have an effect on the immune system and may in this way influence the course of the disease. Activation of CRH neurons as well as increased plasma cortisol concentrations has been implicated in the etiology of depression. Therefore, the presence of active hypothalamic lesions may also be responsible for the increased risk of mood disorders in MS (26–31, 56, 57).

Since most of the projections in the investigated myelinated bundles do not project into the hypothalamus but cross it, the clinical consequences of conduction block in these bundles can be many. Lesions in the optic nerve will cause visual problems; lesions in the anterior commissure may be related to disturbances in temporal lobe functions, such as memory and olfaction; lesions in the fornix may lead to memory problems; and lesions in the internal capsule can cause disturbed motor functions.

We found APP-expressing axons in 4 MS cases. APP is a normal constituent of neurons and is conveyed by fast axonal transport; however, damage such as transection blocks such transport and causes APP to accumulate in the axons (58, 59). Animal models have taught us that APP is expressed 1 to 9 days after contusional injury in rats and becomes less abundant after 9 to 21 days; APP expression thus seems to be a marker of early axonal damage (60, 61). Indeed, we found APP expression only in axons in or near active lesions, which is also in accordance with the findings of Ferguson et al (48) and Bitsch et al (62) and explains why we did not see APP expression in chronic inactive MS lesions with axonal loss as visualized by Bodian staining.

In conclusion, the hypothalamus is frequently affected during MS and contains many active MS lesions. Reduced axonal conduction due to demyelination and the effect of diffusing inflammatory mediators may have impact on the hypothalamic control of autonomic and endocrine functioning and may thus be the basis for autonomic and endocrine signs and symptoms in MS patients.

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