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Thalamic Neurodegeneration in Multiple Sclerosis

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Multiple sclerosis is still regarded primarily as a disease of the white matter. However, recent evidence suggests that there may be significant involvement of gray matter. Here, we have used magnetic resonance imaging and magnetic resonance spectroscopy in vivo and histopathology postmortem to estimate thalamic neuronal loss in patients with multiple sclerosis. Our results show that neuronal loss in multiple sclerosis can be substantial (30–35% reduction). We conclude that a neurodegenerative pathology may make a major contribution to the genesis of symptoms in multiple sclerosis.

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With an increasing focus on axonal injury and transection as the main cause of chronic disability with multiple sclerosis (MS),^{1,2} attention also has turned to un-

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derstanding neuronal pathology in gray matter (GM) of patients with MS, particularly to explain cognitive symptoms.³ Axotomy is associated with retrograde neuronal degeneration and apoptosis.⁴ Apoptosis of retinal ganglion cells is associated with optic neuritis in a rodent experimental allergic encephalitis model.⁵ GM also may be a common focus for inflammatory damage.⁶

The aim of our study was to assess the extent of neuronal loss in thalamic GM of patients with MS using imaging methods and then to validate the measurements with parallel histopathological studies performed on (different) postmortem material. The thalamic medial dorsal (MD) nucleus specifically was chosen for the histopathological study because of its relatively large size, clearly defined boundaries, and potential importance for understanding cognitive manifestations of MS.⁷

Subjects and Methods

Magnetic Resonance Spectroscopy and Imaging

Magnetic resonance studies were conducted for 14 secondary progressive MS patients randomly selected from our clinic database (Table 1). Healthy controls were age- and gender-matched. Subjects had a structural magnetic resonance imaging for thalamic volume measurements with a pulse sequence optimized for contrast between the thalamus and surrounding white matter and magnetic resonance spectroscopy of the thalamus. Informed consent was obtained.

All studies were performed using a 3T Varian-Inova scanner (Varian NMR Instruments, Inc., Palo Alto, CA). For volume measurements, a three-dimensional magnetization-prepared fast gradient echo sequence (inversion recovery time TI, 500 milliseconds; TE, 5 milliseconds; TR, 30 milliseconds; 64 × 3 mm) was used. Thalamic and intracranial volumes were outlined manually in the coronal plane (Fig. A and B). Ratios of thalamic volume to intracranial volume were calculated in every patient (multiplied by 10⁶ for a normalized thalamic volume [NTV]). Third ventricle width was measured from the coronal slice showing maximum width.

Proton magnetic resonance spectra were acquired using a localized PRESS sequence⁸ (echo time TE, 26 milliseconds; repetition time TR, 5 seconds) and a WET⁹ water suppression scheme. The use of a short echo time and a TR long enough to allow full relaxation minimized the effects of modest changes in relaxation times (eg, from factors such as increased iron content) on measurements of metabolite concentrations. Application of a specially designed 90 degree RF pulse in PRESS allowed simultaneous acquisition of the signal from two cubical approximately 1cm³ volumes of interest placed in the thalami. Metabolite concentrations were estimated (VARPRO method and MRUI software) using brain tissue water as a concentration reference.^{10,11} We confirmed that similar results were obtained using LCMoDel.¹² The relative volumes of water in cerebrospinal fluid and tissue compartments within the voxel were estimated with biexponential fitting of the water signal T2 decay (TR, 15 seconds; TE,

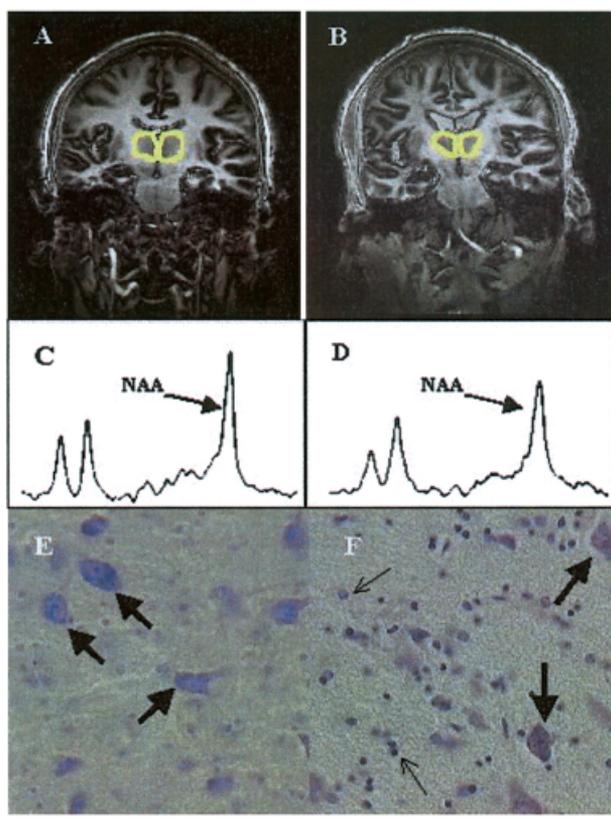
Table 1. Description of Multiple Sclerosis Patients and Healthy Controls Studied Using MRI and Localized MRS

Characteristic	Secondary Progressive MS		Controls	
	MRS	MRI	MRS	MRI
Number of subjects	13	14	13	14
Median age (yr) (range)	50 (38–58)	51.5 (38–60)	47 (34–64)	48.5 (34–64)
M:F	9:4	9:5	7:6	7:7
Median EDSS (range)	6.5 (4–8)	6.5 (4–8)	—	—
Median duration of disease (years) (range)	18.5 (7–24)	18.5 (7–24)	—	—

MS = multiple sclerosis; MRS = magnetic resonance spectroscopy; MRI = magnetic resonance imaging; EDSS = extended Disability Status Scale score.

25, 42, 72, 92, 132, 282, 512, 612, 812, 1,000, 1,200, 2,000 milliseconds). The relative water content of GM was assumed to be 0.745.¹³

Fig. Coronal structural magnetic resonance images of a healthy control (A) and a multiple sclerosis (MS) patient (B). The acquisition parameters were optimized to enhance the subcortical gray–white matter contrast (see Subjects and Methods). Proton magnetic resonance spectra from a normal brain (C) and an MS patient (D). The N-acetylaspartate (NAA) resonance is indicated by arrows. Microscopic field ($\times 400$) from the mediodorsal nucleus of the thalamus of a nonneurological control (E) and an MS case (F). Heavy arrows identify neuron (the density of which is reduced in the MS case), and light arrows point to inflammatory or glial cells, which cannot be distinguished reliably with this staining.



Case Selection for the Pathological Study

Brains from patients with MS were collected from three centers in the United Kingdom (The UK Multiple Sclerosis Tissue Bank, Charing Cross Campus, London; Radcliffe Infirmary, Oxford; Kettering General Hospital), weighed, and preserved in 10% formalin. Consent to the use of autopsy tissue for research had been obtained from the next of kin. Specimens from 10 MS patients (four men and six women; six secondary progressive, three primary progressive, one relapsing-remitting; median disease duration, 23.5 [range, 8–40] years; median age, 62 [range, 32–83] years; median postmortem interval, 75 [range, 34–120] hours; median brain weight, 1,212 [range, 1,042–1,375] g) and from 10 age- and gender-matched controls without a history of neurological or psychiatric disease (median age, 65 [range, 40–85] years; median postmortem interval, 38 [range, 24–104] hours; median brain weight, 1,260 [range, 1,015–1,465] g).

Brain Dissection and Preparation of the Sections

The brains were cut in 5mm coronal slices starting from a plane orthogonal to the mamillary bodies. From each brain slice, the thalamus was dissected and embedded in paraffin. One section from the front of each block was taken for measurement of gross thalamic volumes. Two adjacent 25 μ m sections were taken from the front and middle of each block at levels including the MD nucleus for definition of specific histopathological changes. One section from each pair was stained with cresyl violet for the neuronal counts, whereas in the contiguous section Weil's myelin technique was used to delineate the MD nucleus. The area of the whole thalamus and of the MD nucleus were calculated using a point counting method. The images were acquired with a digital camera (JVC TK-1280E) mounted on the microscope (Olympus BX40) and interfaced with a workstation (Indy; Silicon Graphics). An observer (A.C.) blind to patient/control status examined the images on the video display unit of the workstation. The neuronal number estimates were corrected with the Abercrombie formula.¹⁴

Statistics

Significance levels for comparisons between MS and control subjects were calculated with a two-tailed (*N*-acetylaspartate [NAA] concentration, NTV and MD neuronal density) or a

one-tailed *t* test (whole thalamic and MD volume) or a two-tailed Mann–Whitney *U* test (total MD neuronal numbers).

Results

MS patients showed a mean 17% decrease of the NTV ($p = 0.01$; see Fig. A and B) and a twofold increase in mean third ventricle width ($p < 0.002$; Table 2). There was a moderate correlation between this measure of ventricular enlargement and the thalamic atrophy ($r = -0.59$, $p < 0.05$). Magnetic resonance spectroscopy demonstrated a mean reduction in NAA (a measure of apparent thalamic neuronal density) of 19% in the MS group relative to the controls ($p = 0.004$; see Fig. C and D; see Table 2). No significant changes in either creatine or choline were found. From the product of mean NTV and NAA, an index of effective neuronal volume loss was estimated to be a mean 30% lower in the MS patients than in the healthy controls.

A reduction in effective neuronal volume could occur with either neuronal atrophy or loss. To assess the potential contribution of neuronal loss to thalamic pathology with MS, the MD volume and neuronal density were measured in postmortem specimens (see Fig. E and F). The mean neuronal density for the MD in the MS autopsy cases was reduced by 22% ($p = 0.003$; see Table 2). The mean MD volume also was decreased in the MS group relative to the controls (21%; $p = 0.024$). The relative decrease in the mean whole thalamic volume was similar (22%; $p = 0.03$). From the product of the combined measures of mean MD volume and neuronal density, we estimate a mean 35% reduction in total neuronal numbers in patients relative to controls. These changes occurred outside of any focal lesions, which were rare. More than a single,

small inflammatory lesion in the MD nucleus was identified in only three cases.

Discussion

These observations demonstrate that there is substantial thalamic neuronal loss with MS. Although interpretation is complicated by potential partial volume effects (mixing GM with cerebrospinal fluid or white matter), a similar loss may occur in the neocortical GM, for which a 22% decrease in relative NAA has been reported in secondary progressive MS patients¹⁵ and a smaller decrease has been reported for mildly affected relapsing-remitting patients.¹⁶ The extent of neuronal loss in the thalamus in this study is remarkably close to the previously reported loss of axons in “normal appearing” white matter of the corpus callosum,¹⁷ suggesting that damage to axons in the normal appearing white matter may be related directly to GM pathology. Neuronal injury or loss could be a consequence of local inflammatory activity in the GM, which may be common.⁶ Alternatively, it could result from changes secondary to lesions in white matter associated with axonal transection. A recent report, for example, has suggested that cortical GM atrophy increases with white matter lesion load.¹⁸

We chose the thalamus as the focus of our investigations of GM because it has reasonably well-defined boundaries, and there is only a minimal potential for partial volume effects. The thalamus also has extensive reciprocal connections with the cortex and subcortical structures and thus is likely to be sensitive to effects from pathology in widespread areas.

Recent work has further validated the use of NAA as a quantitative marker of neuronal or axonal density in

Table 2. Summary of Results from In Vivo MRI and MRS and Postmortem Histopathological Characterization of Thalamic Volume and Neuronal Density

Measure	MS, Mean (SD)	Controls, Mean (SD)	Difference (%)
In vivo study			
NAA (mmol/kg wet weight)	9.22 (1.82)	11.4 (1.62)	-19%
Creatine (mmol/kg wet weight)	7.60 (1.19)	8.02 (1.11)	ns
Choline (mmol/kg wet weight)	1.74 (0.56)	1.94 (0.38)	ns
Third ventricle width (mm)	7.6 (3.8)	3.9 (0.8)	105%
Thalamic volume (NTV)	2,547 (669)	3,089 (196)	-17%
Effective neuronal density, mean (SD) $\times 10^{-4}$	2.47 (0.82)	3.53 (0.49)	-30%
Postmortem study			
Neuronal density (cells/mm ³)	2293 (471)	2,929 (315)	-22%
Thalamic volume (au)	3,787 (1228)	4,798 (1018)	-21%
MD volume (mm ³)	637 (213)	806 (127)	-21%
Total MD neurone numbers, mean (SD) $\times 10^{-6}$	1.53 (0.72)	2.34 (0.36)	-35%

Studies in vivo measured total thalamic volume NTV and the concentration of NAA. Postmortem results describe volume changes in both the whole thalamus and the MD nucleus, for which direct neuronal density measurements were made.

MRI = magnetic resonance imaging; MRS = magnetic resonance spectrometry; MS = multiple sclerosis; NAA = *N*-acetylaspartate; SD = standard deviation; au = arbitrary units; ns = not significant; NTV = normalized to intracranial volume; MD = medial dorsal.

the adult brain.¹⁷ However, the imaging data alone only allowed us to speculate on the substrate of the NAA changes, which could be related to a decrease of neuronal numbers, individual neuronal atrophy, metabolic changes, or a combination of these factors. Our parallel histopathological study demonstrated that neuronal loss alone potentially could account for the NAA decrease measured. Although the patient populations were different, disease duration was not substantially greater in the postmortem relative to the in vivo groups. Although later life or terminal changes could have contributed to neuronal loss in the postmortem group, the similarity between measures of thalamic volume in vivo and postmortem gives us confidence in the comparison.

The mechanisms for neuronal loss remain to be established. Plausible candidate mechanisms for neuronal loss include retrograde degeneration, transsynaptic effects, or immune cytotoxicity (direct, from inflammatory cells, or indirect, from associated diffusible factors).²⁰ Potentially, such phenomena could originate with changes either in the white matter or in the GM itself.

In conclusion, we have supplied evidence of neuronal damage in the GM with MS. The substantial loss of neurons is a novel finding that complements axonal theories¹ regarding the genesis of symptom progression in MS. Our findings further support the notion that there is a substantial neurodegenerative component to MS. The identification of substantial neuronal loss has particular clinical relevance for understanding neuropsychological changes with MS, which need no longer be interpreted simply in terms of functionally impaired or disrupted brain connectivities.

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