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Diffuse cortical atrophy in a marmoset model of multiple sclerosis

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ABSTRACT

Marmoset experimental autoimmune encephalomyelitis (EAE) has previously been shown to replicate the essential features of both white matter and grey matter lesions of MS. This study set out to investigate whether cortical atrophy occurs in marmoset EAE and whether cortical thinning is related to the presence of focal, demyelinated cortical lesions. Seventeen leucocortical lesions and 13 subpial lesions were identified in 6 EAE cases. Cortical thickness surrounding these lesions was recorded and compared with matched cortical areas from five control animals. We found a diffuse 13–21% loss of cortical thickness in all areas of EAE cortex compared with control animals but there was no additional loss seen in demyelinated versus myelinated EAE cortex. These findings could not be accounted for by effects of age, sex and disease duration. We conclude that localised cortical demyelination is not responsible for the major part of the atrophy observed and that cortical thinning is largely due to more diffuse or more remote factors. Marmoset EAE is an invaluable tool which can be used to further investigate the cause and the substrate of cortical loss in demyelinating diseases.

MRI studies have demonstrated that cortical atrophy in multiple sclerosis (MS) can be substantial [13,2,9,6,5], progresses from the earliest stages of the disease and is correlated with clinical disability scores [6,5,16]. Despite this there is only a limited understanding of the underlying substrate of cortical thinning and the pathological mechanisms which lead to cortical loss. Atrophy could be caused by retrograde or trans-synaptic degeneration secondary to distant inflammatory lesions although previous studies have at best shown only a moderate correlation with white matter (WM) lesion load [2,9,6,5,13,3]. Alternatively, these changes could be due to a more diffuse neurodegenerative process or a consequence of localised inflammation in normal appearing grey matter (NAGM) or demyelinated cortical lesions.

Immunohistochemical techniques have demonstrated that cortical demyelinating lesions occupy 12.5–26.5% of the total cortical volume [1,10,19]. Cortical lesions may contribute to cortical thinning, as they are characterised by neuronal, glial and synaptic loss and show evidence for neuritic transection [19,14,21]. However, elucidation of the mechanisms contributing to cortical thinning in MS is difficult using post-mortem tissue, derived from patients with end-stage disease. An alternative strategy is to use an animal model which has a well-defined insult and time period for evolution of the pathology and which allows for optimal tissue handling. Cortical lesions which share many of the key features of cortical demyelination in MS have been described in marmoset experimental autoimmune encephalomyelitis (EAE). As in MS, cortical marmoset EAE lesions are predominately located in the leucocortical or subpial regions and subpial lesions are most frequent in the cingulate cortex [1,10,21,15].

This study sets out to establish whether significant cortical atrophy occurs in marmoset EAE and examines whether localised cortical thinning is associated with the presence of leucocortical or subpial cortical lesions.

Tissue was acquired from six animals with EAE and five control animals, all animals were kept according to the National Institute of Health Guide for the Care and Use of Laboratory Animals. Animals were induced with EAE through a subcutaneous injection of myelin oligodendrocyte glycoprotein (MOG) and underwent a progressive relapsing disease course with survival times of 26–70 days. Full clinical data and details of tissue selection and histological methods have been provided elsewhere [15]. All tissue blocks which were found to contain a leucocortical or subpial cortical lesion were used in this study. This included sections selected from along the entire length of the brain. Sections from each block were stained for myelin using anti-proteolipid protein (anti-PLP), Serotec

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UK to identify regions of interest (ROIs) containing cortical lesions or matched control areas. Cortical thickness in ROIs was measured in adjacent 10 μm thick sections stained with luxol fast blue and periodic acid Schiff stain (LFB/PAS). Sections were viewed using an Olympus BX41 microscope (Olympus UK) at a magnification level of 12.5× and digital images were captured using an AxioCam MRc5 camera (Carl Zeiss associates) at a resolution of 2584 × 1936 pixels. Images were viewed and analysed on a computer using AxioVision 4.4 software (Carl Zeiss associates).

Cortical thickness at sites containing leucocortical lesions was recorded by marking the centre of the lesion and measuring the shortest distance from the leucocortical border to the pial surface. This was repeated three times in each ROI and the mean thickness was recorded. Each ROI containing a leucocortical lesion was matched with an area of normal appearing grey matter taken from the homologous position in the contralateral hemisphere and with a matched area of control cortex from a non-EAE animal.

Cortical thickness adjacent to cingulate subpial lesions was assessed by recording the mean of five measures which were evenly spaced along the cingulate cortex. This measurement was repeated in homologous regions in control animals. In the EAE animals, as cingulate subpial lesions were always bilateral, a corresponding measure of NAGM was not possible and thickness of adjacent NAGM from the paracentral cortex was compared with the equivalent region from a control animal (Fig. 1).

Intra-rater reliability was assessed through a blinded count–recount analysis of eight randomly selected ROIs (Pearson's correlation coefficients $r = 0.89$ for cingulate cortex, $r = 0.92$ for paracentral cortex and $r = 0.98$ for leucocortical lesions, $p < 0.01$ in all cases). Differences in cortical thickness between lesions and matched control areas were assessed using a paired t-test. The influence of the potential confounding effects of age, sex and disease duration was assessed using linear regression. For age, this was performed by plotting the difference in cortical thickness for each pair of control and EAE ROIs against the difference in age between the animals. The constant in the regression equation ($y = a + bx$) was then analysed to look for a statistically significant difference in cortical thickness when age was theoretically equal. The same analysis was performed to examine the effect of sex by assigning male animals a nominal value of '1' and female animals a value of '0.'

Seventeen leucocortical lesions were analysed in nine tissue blocks from six EAE cases. Homologous regions of cortical NAGM from the contralateral hemisphere of the same case, and anatomically matched cortex from five control cases were also studied. The mean thickness of cortex containing leucocortical lesions was 1.48 mm (S.D. = 0.39 mm), compared with 1.51 mm (S.D. = 0.44 mm) for cortex surrounding NAGM and 1.73 mm (S.D. = 0.62 mm) for control cortex (Fig. 2A). This represents a 14.5% decrease in thickness of cortex containing leucocortical lesions and a 12.7% decrease in thickness in (non-lesional) NAGM in the same cases compared with control cortex. A paired t-test confirmed significant cortical thinning in lesional vs. control cortical regions ($\mu = -0.25$ mm, $p = 0.005$) and in NAGM vs. control regions ($\mu = -0.22$ mm, $p = 0.01$), but found no significant difference between lesional and NAGM regions in the same animals ($\mu = -0.03$ mm, $p = 0.433$).

Thirteen cingulate cortical regions with subpial lesions were analysed in six tissue blocks from five EAE cases, as well as anatomically matched regions in healthy controls. Nineteen regions of paracentral NAGM in 10 tissue blocks taken from 6 EAE cases were studied as an additional comparison (homologous cingulate NAGM was not found in the EAE animals). The mean cortical thickness of cingulate cortex in EAE animals was 1.08 mm (S.D. = 0.02 mm) compared with 1.29 mm (S.D. = 0.08 mm) in controls. Mean thickness of paracentral NAGM was 1.25 mm (S.D. = 0.15 mm) compared with 1.58 mm (S.D. = 0.11 mm) in controls (Fig. 2B). This represents a 16.3% decrease in cortical thickness in the lesion-containing cingulate cortex relative to controls ($\mu = -0.21$ mm, $p = 0.02$) and a 20.9% relative loss in the paracentral NAGM cortex ($\mu = -0.29$ mm, $p < 0.001$).

A potential confound is that the control animals were older than the EAE animals. There was a negative association between age and cortical thickness difference for cortex surrounding leucocortical lesions ($r^2 = 0.66$, $p < 0.001$), deep cortical NAGM ($r^2 = 0.38$, $p = 0.008$), cingulate subpial lesions ($r^2 = 0.66$, $p = 0.002$) and the paracentral NAGM ($r^2 = 0.61$, $p < 0.001$) (Fig. 3). Using linear regression, we corrected for age effects in the primary contrasts above and confirmed a highly significant loss of cortical thickness in areas containing leucocortical lesions ($a = -0.43$ mm, $p < 0.001$), deep cortical NAGM ($a = -0.36$ mm, $p < 0.001$), lesion-containing cingulate cortex ($a = -0.45$ mm, $p < 0.001$) and paracentral NAGM ($a = -0.46$ mm, $p < 0.001$) in EAE animals relative to controls (Fig. 3). No significant association was found between cortical thickness and sex ($r^2 = 0.001$–0.009, $p = 0.779$–0.970) or between cortical thickness and disease duration ($r^2 = 0.023$–0.220, $p = 0.146$–0.565).

Our results demonstrate significant loss of cortical thickness occurring in an animal model which reproduces many neuropatho-

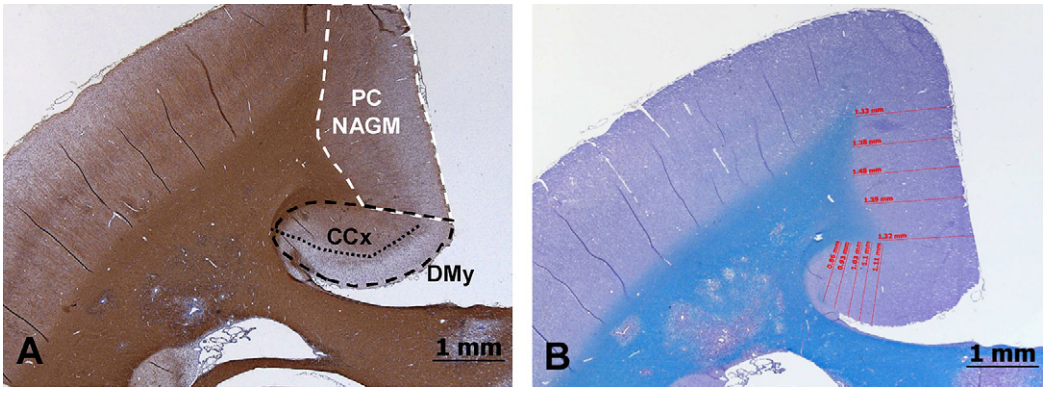

Fig. 1. Illustration of method for calculating cortical thickness. Low power photomicrographs illustrating the methods used for detecting cortical lesions and calculating cortical thickness for ROIs surrounding cingulate subpial lesions. (A) (anti-PLP, 12.5×) demonstrates the neuroanatomy of the paracentral NAGM (PC NAGM) and cingulate cortical (CCx) regions. Note the presence of a demyelinated subpial lesion (DMy) in the cingulate cortex. (B) (lfb/pas, 12.5×) illustrates the method used to calculate cortical thickness. Five lines were drawn at equally spaced intervals throughout the cortical areas and the mean length of the lines was calculated. Lines were orientated so as to cover the shortest distance between the leucocortical border and the pial surface.

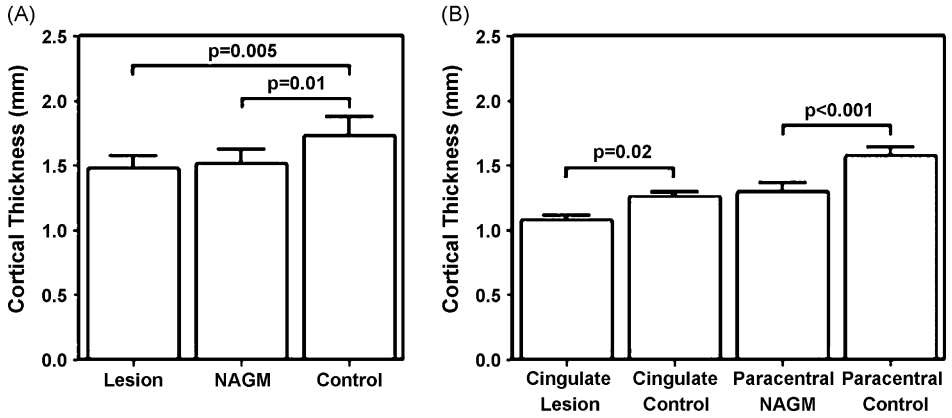

Fig. 2. Cortical thickness in ROIs surrounding lesions and control areas. Bar charts showing the distribution of cortical thickness in cases compared with controls in deep cortical layers (A) and superficial cortical layers (B). Bars represent mean cortical thickness difference and error bars represent standard error. All significant differences between matched ROIs are marked on the charts.

logical features of human MS [15,11,12]. Similar rates of atrophy were seen in demyelinated cortex and in normal appearing grey matter thereby suggesting that direct effects of inflammation and demyelination do not account for the major part of the cortical atrophy seen in this model.

The possible confounding factors of sex and disease duration showed no association with cortical thickness. However, we were able to confirm the previously well-described observation that cortical thickness decreases with age in the healthy animal [18]. Since the control cases in our study were older than the EAE cases, the 13–16% loss of cortical thickness seen in diseased animals may underestimate the true effect of disease, despite efforts to model this confound out. It is unlikely that the failure to identify greater cortical thinning in lesioned regions relative to NAGM in the EAE animals was a consequence of tissue expansion with oedema, because there is little inflammatory cell infiltration of these lesions relative to white matter lesions and the tissue was studied after fixation [15]. Three of the six EAE animals were fixed by perfusion fixation which could lead to higher concentrations of fixative around lesions due to disruption of the BBB. This confounding factor could cause artefactual shrinking around lesions but this would not explain the atrophy seen in areas of NAGM or the failure to find additional atrophy in demyelinated compared with myelinated cortex.

Previous studies in MS have found evidence of localised WM atrophy which correlates with regional WM lesion load [7] and diffuse damage occurring throughout the NAWM [1]. However, the observation in this study that cortical thinning is similar in both NAGM and in demyelinated cortical lesions suggests that cortical atrophy is predominately a consequence of more diffuse or more remote processes. Distant WM lesions could lead to remote atrophy through Wallerian, retrograde or trans-synaptic degeneration. Alternatively, a diffuse neurodegenerative process may be involved. Studies have suggested that calcium-mediated axonal loss can occur outside demyelinated areas due to altered expression of sodium channels and deficiencies in energy production within neurons [20]. In the context of this disease induced in otherwise healthy animals (i.e., animals without an additional cause for neurodegeneration), we speculate that a diffusible factor associated with inflammation may be involved as has been suggested in previous studies [10,14]. The pathological substrate of the cortical atrophy seen in this study also requires further elucidation. Early

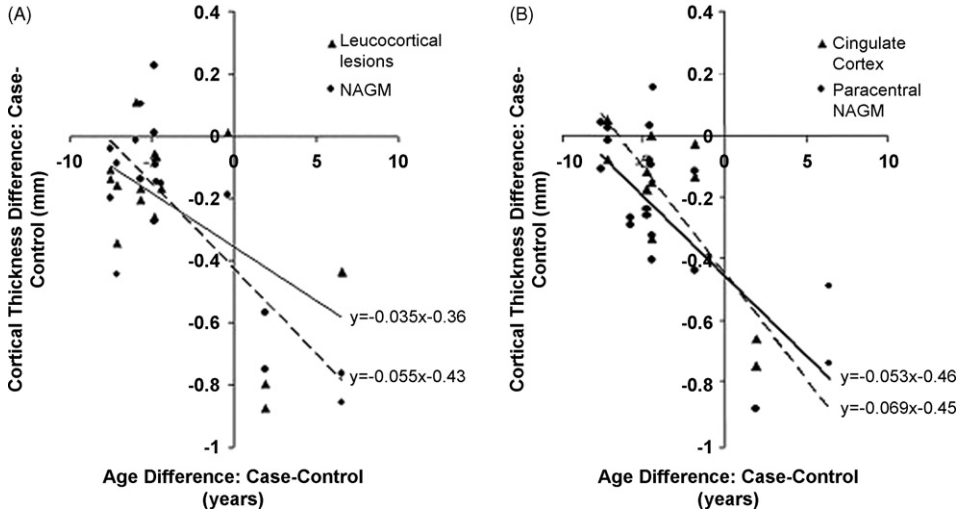

Fig. 3. Paired age differences vs. cortical thickness differences in EAE cases and matched control areas. Scatterplots display the difference in age between EAE cases and control animals plotted against the difference in cortical thickness. Graphs represent cortex associated with leucocortical lesions (A) and cingulate subpial lesions (B). Separate regression lines are displayed on each graph for areas representing lesions (triangles, dotted lines) and NAGM (circles, continuous lines). All four sets of data confirmed a strong negative correlation between age difference and cortical thickness difference. The constant in the regression equation was significantly less than zero in all cases thereby confirming cortical thickness loss in cases compared to controls.

cortical atrophy in Alzheimer's disease is associated primarily with loss of synapses and the extent and complexity of dendritic arborisation [8,17]. There is suggestive evidence that a similar process occurs in MS [14,21] but this requires further study.

Neuropathologically, marmoset EAE shares key features with MS [15,12]. The presence of cortical inflammatory lesions analogous to those of MS within weeks of induction suggests that cortical involvement in MS also is not only extensive [3] but early [5]. Our observations would be consistent with those in MS suggesting that cortical atrophy, while displaying some regional variation, is more generalised than focal [4]. Marmoset EAE provides a useful model which can be used to further characterise this phenomenon.

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