

Title (150 characters including spaces):**The loss of neurons over the whole MS neocortex****Abstract text (2500 characters including spaces)****Background**

Neuro-axonal loss appears to be the major substrate of chronic and irreversible functional deterioration in people with multiple sclerosis (pwMS). Significant variation has been reported regarding the degree cortical neuronal loss. Accurate quantification of cell populations including neurons is of fundamental importance to better understand MS cortical pathophysiology and the significance of changes detected using non-invasive techniques, such as brain atrophy using MRI.

Objectives

To quantify in the total number of neocortical neurons across the entire cortical grey matter in MS and control brain.

Methods

Formalin fixed brain hemispheres of five people with MS (4 women, one man, aged 74 ± 7 years, disease duration = 30 ± 9 years) and three reference cases (men, age 83 ± 3 years) were studied. Hemispheres were dissected into 1.1 cm thick coronal slices. Neurons were identified on Giemsa-stained $40\mu\text{m}$ -thick sections (x60 oil immersion) using a microscope equipped with stage controlled by stereology software. The area of interest (AOI) was outlined to include the whole cortex on each section and disectors were placed using a random grid. The total number of neocortical neurons (TNNN) was calculated as $N_V \times V_{REF}$ in each slab, where N_V = neurons counted/volume of disectors quantified and V_{REF} = AOI x slab thickness (t), adjusted for tissue shrinkage, and multiplied by 2.

Results

All brains were quantified using the same mean frequency of disectors (MS= 466 ± 37 ; controls= 464 ± 24 , $p = 0.95$). TNNN was 14.1 ± 2.9 billion in MS and 23.6 ± 3.1 billion in controls brains ($p= 0.078$), suggesting an overall neuronal loss of 40%. Strong correlation was detected between TNNN and cortical volume ($r= 0.85$, $p= 0.0082$).

Conclusions

MS affects the entire CNS. To the best of our knowledge, this is the first study to accurately estimate neuronal loss throughout the MS cortex. The mean TNNN in our (male) controls was very similar to figures previously reported. The apparent 40% reduction of neocortical neurons in pwMS is biased by the fact that 4/5 pwMS were women whilst our control cases were all men. However, the previously reported TNNN in women is 21.4 billion, thus 34% higher than our TNNN in pwMS (including four women and one man). Though requiring confirmation in a larger and more balanced cohort, this data shows the loss of functionally important brain cells is massive and suggests strong correlation of TNNN with neocortical volume loss.