Cortical atrophy predicts neuronal loss in MS: a *post mortem* study using unbiased sampling

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INTRODUCTION

- The loss of cortical neurons is a likely contributor to the progressive physical and cognitive impairment of people with MS (pwMS).
- There is variation of the reported degree of neuronal loss in the cortex, and the topographic distribution of this loss over the entire cortex is unknown.
- Brain volume measures including cortical volume loss are used as MRI biomarkers of disease deterioration. However, the histological correlates of these indices are only partially known.

AIMS

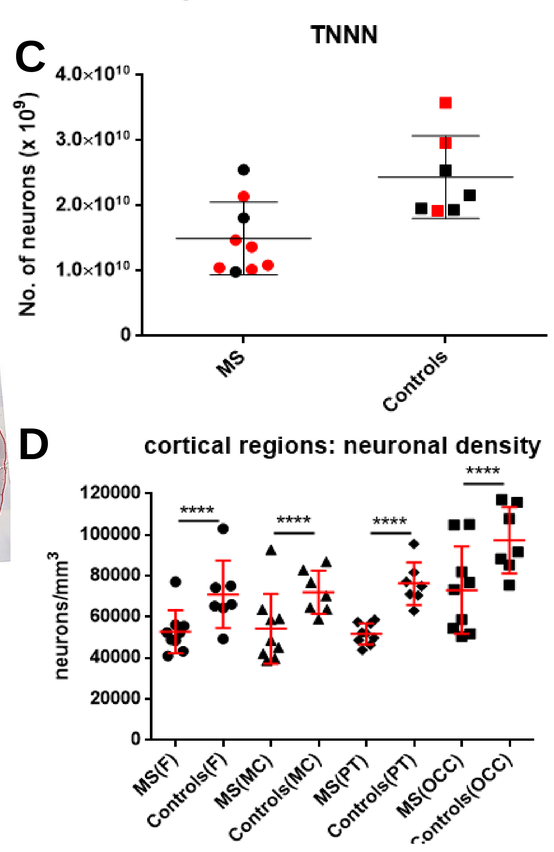
- To quantify the total number of cortical neurons in MS vs controls in *post mortem* brain.
- To explore the relationship between neuronal loss, cortical volume and demyelination.

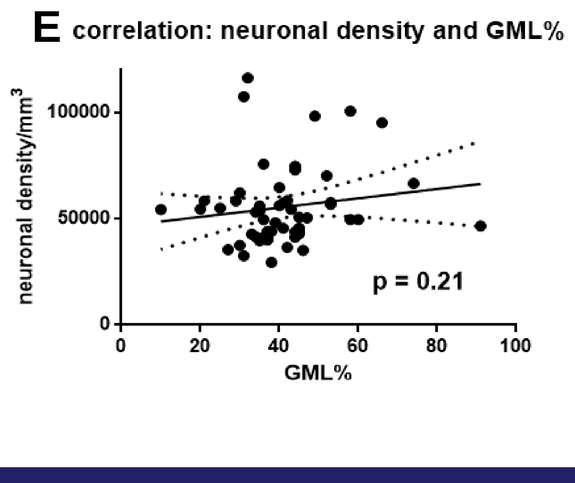
METHODS

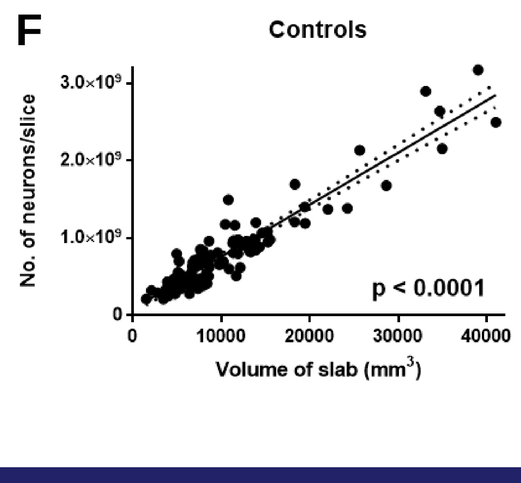
- Formalin fixed brain hemispheres of nine pwMS (6 F, 3 M, age = 68 ± 14, disease duration = 27 ± 5 years) and seven reference cases (4 M, 3 F, age= 75 ± 18 years) with no known neurological disease were studied.
- Lobar topography of motor and occipital corteces was marked before dissection into 1.1 cm-thick coronal slabs.
- Slabs were embedded for processing in paraffin and sections stained for Giemsa/40µm **(A)** and myelin basic protein (MBP)/10µm **(B)**.
- Neurons were identified on Giemsa-stained sections using a microscope equipped with a stage controlled by Stereo Investigator. Areas of interest (AOI) were outlined to include the entire cortex on each section **(A, blue)**; disectors were placed using systematic random sampling.
- The total number of neocortical neurons (**TNNN**) was calculated as the sum of $\mathbf{N_V \times V_{REF}}$ in each slab, where $\mathbf{N_V}$= neurons counted/volume of disectors quantified and $\mathbf{V_{REF}}$(cortical volume)= AOI x slab thickness (T), multiplied by 2.
- Demyelination extent in the cortex was manually outlined on MBP images **(B, red)**.

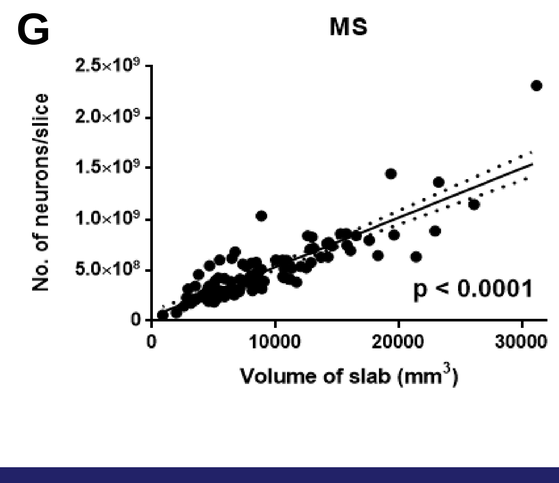
RESULTS

- The mean **TNNN** was 14.9 ± 1.9 billions in MS and 24.4 ± 2.4 billions in controls (p= 0.007) **(C)**.
- pwMS showed a 63% smaller number of neocortical neurons ($p < 0.0001$). This figure applied to all four cortical regions examined ($p = 0.719$).
- In pwMS cortical volume was reduced by 50% ($p < 0.0001$), which was very similar in all four cortical regions analyzed.
- The mean neuronal density [neurons/mm^3] in pwMS was 57468 ± 3636 and in controls 79787 ± 3869 indicating a similar reduction in all cortical regions of 28% ($p < 0.0001$) **(D)**.
- Neuronal density in MS was decreased by 20% in men ($p < 0.0001$) and by 26% in women ($p < 0.0001$) compared to controls. Neuronal density was significantly smaller in women compared to men, in MS by 19% ($p < 0.0001$) and in controls by 12% ($p < 0.0001$).
- The mean proportion of demyelinated cortex was 41 ± 14 %. There was no evidence that demyelination varied by gender ($p = 0.99$), age ($p = 0.794$) or disease duration ($p = 0.2$). Frontal cortex was significantly more affected by demyelination than all other cortical regions (motor, $p = 0.004$; parietal/temporal, $p = 0.017$; occipital, $p < 0.0001$).
- Across the entire MS neocortex the extent of demyelination and the decrease in neuronal density were not linked ($p = 0.21$) **(E)**. This lack of correlation was confirmed in a region-specific analysis ($p = 0.322$), including adjustment for gender ($p = 0.118$), age ($p = 0.56$) and disease duration ($p = 0.963$).
- The correlation between number of neurons and cortical volume was different in pwMS and controls ($p < 0.0001$). pwMS showed a smaller and shallower regression line (49787/mm^3) than controls (68848/mm^3) reflecting their lower neuronal density in the cortex **(F, G)**.
- All results are adjusted for cortical region, age at death, disease duration and gender.









DISCUSSION and FUTURE WORK

- To our knowledge, this was the first study estimating the extent of neuronal loss across the entire MS cortex using unbiased sampling techniques. The TNNN we obtained in control cases was similar to previously reported figures **[1]** indicating robust methodology.
- In our cohort of MS cases TNNN were more than halved while the overall neuronal density was reduced of about a third.
- This severe neuronal loss was not linked to the extent cortical demyelination. However, it was strongly correlated with volume as expected using stereology derived measuring methods. This observation is indicating volume reduction, which in our MS cohort was also halved, as the closest measure to predict neuronal loss over time.
- We are currently validating our measure of cortical volume with MRI measures on the same cohort of cases and assess whether cortical demyelination is spatially associated atrophy in isolated GM lesions.

[1] Pelvig, D.P., et al., Neocortical glial cell numbers in human brains. Neurobiol Aging, 2008

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Disclosures: None Relevant