# Significant Brain Atrophy Precedes the Onset of Disability in a Murine Model of Multiple Sclerosis

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**Objective:** To determine the temporal relationship of central atrophy of the brain to disability in a murine model of multiple sclerosis (MS)

**Background:** Brain atrophy is a widely recognized component of MS(1). Both early cortical atrophy(2) as well as ongoing central atrophy(3) have been reported in MS. The pathogenesis of atrophy and its relationship to gray or white matter damage remains unclear. Animal models of brain atrophy would be critically important in determining the pathomechanism and clarifying the substrate of atrophy (gray or white matter, or both). Cerebellar cortical atrophy has been very elegantly demonstrated in a recent paper (4). However, the extensive central atrophy that is often seen in human MS has not yet been reported in animal models. Theiler's Murine Encephalitis Virus (TMEV) infection of mice is an accepted MS model(5). In SJL mice, the resulting disease is characterized by progressive demyelination and motor deficits. Our hypothesis was that in this model, progressive central atrophy accompanies the demyelinating condition. This hypothesis was based on our preliminary observations of ventricular enlargement in chronically TMEV infected and significantly disabled SJL mice. The goal of this pilot study was to determine whether a neurodegenerative component leading to brain atrophy is truly detectable in this model, and to clarify its temporal relationship to the onset of disability and to $^{1}$H MRS detectable markers.

**Design/Methods:** Eight TMEV infected SJL mice and 4 controls were scanned bimonthly for a year, using volume acquisition T1 weighted (FLASH sequence, TRl:15ms, TE:4.5ms, NEX:2, FOV: 3.2x1.92x1.92 cm, matrix: 256x128x128) and T2 weighted (RARE sequence, TR:1500ms, TE:70ms, RARE factor: 16, FOV: 3.2x1.92x1.92 cm, matrix: 256x128x128) sequences in a Bruker Biospec 7 Tesla horizontal bore small animal MRI system. Co-registration and slice extraction for presentation, total brain and ventricular volume measurements were performed using the coregistration, slice extraction, and the semiautomated volumetric 3D ROI and 3D Scan tools in Analyze 8.0(6). Short TE (20 ms) MRS of single 8mm$^3$ brainstem voxels were acquired (PRESS method, TR:2500ms, TE: 20ms, NEX:256, CHESS water suppression) and analyzed using LCModel(7). We monitored disability by monthly Rotamex rotarod assay (Columbus Instruments, Columbus, OH). This assay determines the time the animals are able to walk on a constantly accelerating rotating rod. Intergroup differences were analyzed statistically using Student's t-test in JMP (SAS Institute, Cary, NC).

**Results:** Brain atrophy was analyzed using BPF (brain parenchymal fraction) as well as average ventricular volumes; there was no difference between the two methods in capturing the extent of atrophy (p=0.91). Significant brain atrophy was found as early as 3 months with both methods (p=0.005), and reached its peak at 6 months post infection (Figure 1). Differences in disability became significant by 4 months (p=0.0005) but not at 3 months (p=0.45), and continued to progress until 9 months (Figure 2). MRS markers showed decrease in total NAA (p=0.025) at 3 months, progressing further until 12 months (p=0.00014) consistent with axonal/neuronal damage. Ins peak elevation was seen at 3 months (p=0.034) further increasing at 12 months (p=0.014) suggesting ongoing gliosis. The membrane turnover/cell infiltration marker Cho was significantly increased at 3 months (p=0.033) but later normalized (p=0.18), suggesting that inflammatory infiltrates are less prominent in the chronic, burnt out stage of the process.

**Conclusions/Relevance:**

In this first known MS model of progressive central atrophy of the brain we determined the rate of atrophy and its relationship to disability and MRS markers. Both the T1 and the T2 weighted sequences adequately captured the atrophy and lead to the same results; this is likely related to the fact that unlike in human MS, periventricular T2 hyperintense lesions don't interfere with volumetric measurements. In addition, there was no difference between the BPF method and ventricular volumetry. This is likely due to the insignificant variation of head and brain size among the genetically identical SJL mice. Our results demonstrated very significant brain atrophy in this model, and also showed that atrophy precedes the development of motor deficits. Axonal and neuronal processes likely contribute to this, as evidenced by the significant NAA decrease at 3 months and beyond. Axonal/neuronal dysfunction also appears to correlate with the progression of atrophy. This model will allow us to characterize the pathomechanisms for atrophy and may pave the way to novel therapeutic strategies aimed at neurodegeneration in MS.

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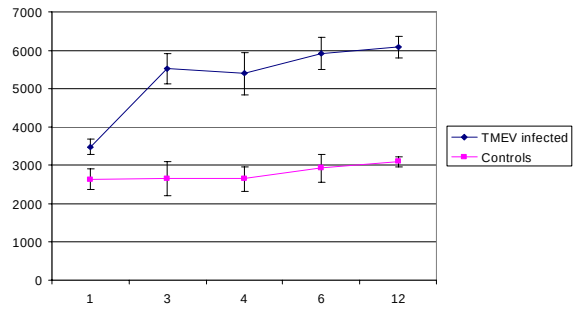


**Figure 1**.Brain atrophy.Y axis:ventricular volumes (0.01mm$^3$), X axis: months

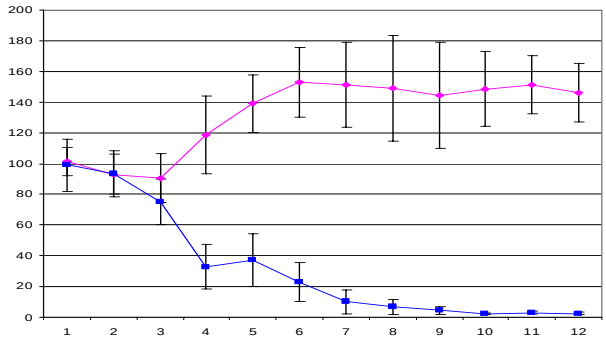


**Figure 2.** Disability. Y axis: Rotarod scores (sec), X axis: time (months).

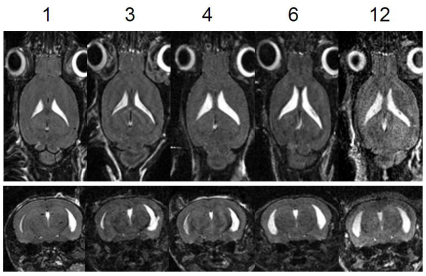


Figure 3. Representative axial (top row) and coronal (bottom row)slices showing progressive brain atrophy. Time (month) indicated above the figure.

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