

MRI Detectable Spinal Cord Atrophy Correlates with Disability in a Murine Model Of Multiple Sclerosis

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Objective: To determine the association of MRI detectable spinal cord atrophy with disability in TMEV infected SJL/J mice.

Background: In SJL/J mice, TMEV infection triggers a progressive demyelinating disease leading to disability over several months^{1,2}. This serves as an accepted and relevant model for MS³. In this model, disability as detected by rotarod (see *methods* below) first becomes significant at 4 months after disease induction, and progresses to maximum disability by about 11-12 months. Recently, the development of early and significant brain atrophy was reported in this model, with over twofold enlargement of the lateral ventricles compared to controls by as early as 3 months post disease induction. Brain atrophy preceded and predicted the development of motor disability. Brain atrophy reached its peak by 6 months post disease induction and did not progress further, whereas the animals' disability continued to deteriorate. The aim of this study was to address whether MRI detectable spinal cord atrophy is present in this model, and to study its correlation with disability, especially at the late time points when brain atrophy has already reached its peak. Brain and cord atrophy has received increasing attention over the last several years as some of the most important non-lesional features of MS, with strong correlations to clinically relevant outcome measures⁴⁻⁷. Models of MS-related brain and cord atrophy are very limited at this time, and so is our understanding of the pathomechanism of these important MS features.

Design and Methods: The experiments were approved by the Institutional Animal Care and Use Committee. 8 TMEV infected and 6 age matched control SJL/J mice were studied. **MRI scans** were performed at 1, 4, 6 and 12 months using a Bruker Biospec 300 MHz horizontal bore system (Bruker Biospin, Billerica, MA) equipped with custom-built coils. A respiratory gated T2 weighted volume acquisition RARE sequence was used, with the FOV incorporating the spinal cord down to the T3/T4 area (TR: 1500ms, TE: 70ms, RARE factor: 16, FOV: 3. 20x1. 92x1. 92cm, matrix: 256x128x128).

Disability was monitored using the rotarod assay (Columbus Instruments, Columbus, OH): mice are trained to march on a constantly accelerating rotating rod. Eventually, the animals are no longer able to keep up with the rod and fall. The time spent on the rod serves as a quantitative disability measure. Two measurements are taken each time the animals are assessed, and the better score is used for analysis. **MRI post-processing:** 3D images were co-registered to a base image using the 3D Voxel Registration module in Analyze 8. 1^{8,9}, (Mayo Clinic BIR, Rochester, MN) with the registration voxel centered on the lower brainstem and the cervical spinal cord. From the co-registered scans, the oblique sectioning tool was used to extract a specific slice at the C5 level using strict anatomical landmarks. To measure cord atrophy, the surface area of these C5 axial cord sections was calculated using the smart edge detection tool in the ROI module of Analyze. Due to the semi-automated nature of this process, two trained investigators analyzed the datasets at least twice, with superb (>95%) intra- and inter rater reliability as determined by appropriate kappa statistics. **Statistical analysis:** Mixed effect models were used to assess associations of numerical measures to time and treatment, using a random effect (individual mice) to account for within subject correlation caused by repeated observations. *Post hoc* comparisons of means longitudinally between months in each group and cross sectionally between groups at each month were performed under the mixed effect model framework and adjusted for type 1 error using Tukey's method in SAS 9.1 (SAS, Cary, NC).

Results: To our surprise, the C5 cord section surface area (CSA) significantly ($p<0.05$) increased between 1 to 4 months by 8 % in cases vs 5% in controls, without significant intergroup differences at 1 or 4 months ($p>0.2$) (Figure 1 and 2). The likely reason for this was continued growth of the cord as opposed to inflammatory infiltration, which would have only affected the infected mice. Between 4-6 months, a less than 1% non-significant ($p=0.7$) increase was seen among the cases, whereas an additional 5% increase was detected among controls with a trend for significance ($p=0.07$). At the six month time point, the cases showed a 9.6% lower CSA compared to controls ($p=0.056$). By 12 months the CSA was 19.2% lower than in controls ($p=0.0019$). The overall 22% CSA decrease from 6 to 12 months among cases was highly significant ($p=0.0017$), and it was accompanied by a significant drop in rotarod scores. No significant age-related CSA decrease was detected in controls between 6-12 months (3.5% decrease, $p=0.16$). The correlation between CSA and rotarod scores for the 4-12 months period was strong in the cases ($r=0.63$, $p=0.001$); this represents some of the strongest known associations between MRI measures and disability. Interestingly, the association between rotarod scores and CSA was also very strong in controls ($r=0.94$, $p<0.001$) suggesting that this MRI measure has an important relationship with motor performance in general.

Conclusions/Relevance: Cord atrophy (expressed as CSA) correlated very well with disability at the late time points in our MS model, when brain atrophy has already reached its peak. Brain atrophy appears to be an early feature in this MS model, and it strongly predicts the development of disability, while cord atrophy appears later in the model, and correlates with the progression of disability. The model will allow for investigations of key components of CNS atrophy and its pathomechanism. It will also allow us to determine which potential components (demyelination, axonal damage, gray matter damage) are the key contributors to cord atrophy at various disease stages. Since atrophy has some of the strongest correlates with disability in MS, we believe that this model will allow us to understand the true substrate of disability in MS.

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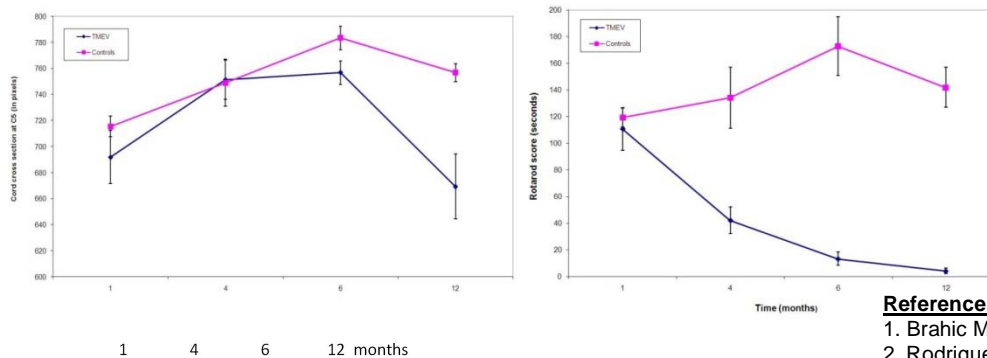


Figure 1. Left panel: changes in cord surface area at the C5 level, pink: controls, blue: cases. Note the initial increase (likely normal growth) followed by significant decrease in cases. **Right panel:** rotarod scores representing disability. Note motor learning in controls vs significant disability in cases. See results section for more details.

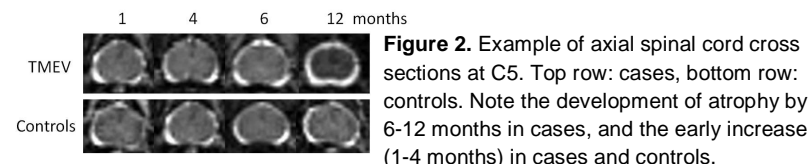


Figure 2. Example of axial spinal cord cross sections at C5. Top row: cases, bottom row: controls. Note the development of atrophy by 6-12 months in cases, and the early increase (1-4 months) in cases and controls.

References

1. Brahic M. 2002. Virology 301:1-5.
2. Rodriguez M et al. 1987. Crit Rev Immunol 7:325-65.
3. Nelson AL et al. 2004. Int MS J 11:95-9.
4. Zivadinov R et al. 2004. Front Biosci 9:647-64.
5. Simon JH. 2000. Neuroimaging Clin N Am 10:753-70 .ix.
6. Bermel RA et al. 2006. Lancet Neurol 5:158-70.
7. Bakshi R et al. 2005. J Neuroimaging 15:30S-45S.
8. Robb RA. 1999. Annu Rev Biomed Eng 1:377-99.
9. Robb RA. 2001. IEEE Trans Med Imaging 20:854-67.