# MRI Detection of Spinal Lesions in a Rat MS Model

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## Introduction

Multiple Sclerosis (MS) is a neurodegenerative disease with a wide spectrum of clinical symptoms, which often makes it difficult to diagnose. Currently, there are no drugs or other forms of treatment that can cure patients with MS. MRI of the brain of MS patients has been the gold standard of diagnosis for some time. These images reveal hyperintense lesions in the white matter of T2-weigheted brain MRI, which represent areas of inflammation and demyelination that are associated with MS [1]. *Ex vivo* examinations of the spinal cord clearly show regions of demyelination in animal models of MS [2,3]. The goal of this study was to establish high-resolution MRI protocols on the spinal cord specimens of EAE (Experimental Autoimmune Encephalomyelitis) rats in order to effectively identify MS hyperintense lesions using various fixation solutions (since they have shown a significant impact on MR properties in the brain [4]). This protocol has the potential to evaluate the response to possible treatments for MS in the future.

## Methods

Two studies were performed. The first included a total of 19 *ex vivo* rat spines (4 control and 15 EAE animals) preserved in 4% formaldehyde. The second study included 18 spines (5 control and 13 EAE animals) preserved in 4% glutaraldehyde. All of the spines were evaluated using T1-,T2-weighted and Fluid Attenuated Inversion Recovery (FLAIR) MRI protocols. Each spine was removed from the preservative, rinsed with phosphate buffered solution (PBS) and placed in a holder. Anatomic orientation was maintained using Vitamin B gelcaps spaced at 3.5cm intervals and two bed positions (thoracic and lumbar) MR scans were obtained using a Bruker 4.7 Tesla MR scanner equipped with a 68-mm volume RF coils. Multi Slice Multi Echo (MSME) T1- and T2-weighted images were obtained in sagittal plane for both bed positions, followed by MSME IR images in two planes (sagittal and transverse). Images were evaluated by two different MRI investigators (blinded to animal group assignment) and lesions were only reported if they were detected on all MRI protocols (T1-MRI sagittal, T2-MRI sagtittal, IR-MRI sagittal and transversal). Final images are presented as fusion images of thoracic and lumbar segments.

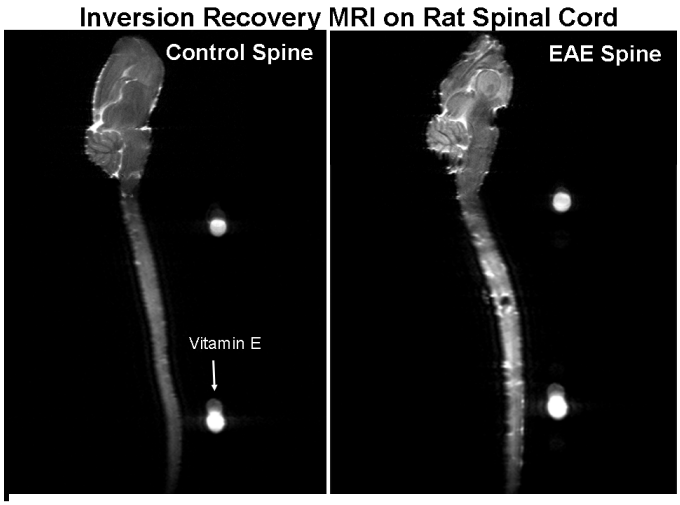
## Results

T1-weighted MRI provided less conclusive information on presence and location of MS lesions in rat spinal cord. On the other hand, we successfully developed T2-weighted and FLAIR (Fluid Attenuated Inversion Recovery) MRI protocols to quantitatively assess MS-lesions in EAE rat spines. Analysis of both study 1 and 2 revealed a clear difference between control and EAE rat spines, the latter presenting with multiple hyperintense lesions that correlate with respective motor scores (Figure 1). The control spines show homogenous T2-weighted signals without hyperintense areas. In study 1, most lesions appeared in the thoracic segments of EAE rats. In general, there was a good correlation between number of lesions and motor score at time of sacrifice (Table 1). However, there was an even stronger correlation between number of lesions and the highest motor score reported at clinical onset. Similar correlations were found in study 2 as well. However, the glutaraldehyde-fixed spines in study 2 showed in general decreased signal-to-noise ratios in all MRI; they also had a slightly increased false positive rate for lesions detection in the control group.

Table 1:

| Rat # | Lesion Number | | MR Intensity | Motor Score | |
|---|---|---|---|---|---|
| | Thoracic | Lumbar | total spine* | sacrifice | highest |
| Control 1 | 0 | 0 | clean | naïve | naïve |
| Control 2 | 0 | 0 | clean | naïve | naïve |
| Control 3 | 0 | 0 | clean | naïve | naïve |
| Control 4 | 0 | 0 | clean | naïve | naïve |
| EAE 1 | 3 | 4 | high | 5 | 5 |
| EAE 2 | 6 | 5 | high | 6 | 6 |
| EAE 3 | 2 | 0 | low | 0 | 1.5 |
| EAE 4 | 0 | 0 | clean | 2 | 5 |
| EAE 5 | 2 | 0 | low | 0 | 0 |
| EAE 6 | 3 | 0 | moderate | 0 | 3 |
| EAE 7 | 2 | 0 | low | 0 | 0 |
| EAE 8 | 3 | 0 | moderate | 0 | 3 |
| EAE 9 | 5 | 3 | high | 3.5 | 5 |
| EAE 10 | 2 | 3 | moderate | 1.5 | 3.5 |
| EAE 11 | 0 | 4 | moderate | 2 | 5 |
| EAE 12 | 1 | 3 | moderate | 3 | 3 |
| EAE 13 | 2 | 1 | low | 1.5 | 3 |
| EAE14 | 4 | 2 | high | 5 | 5.5 |
| EAE15 | 4 | 3 | high | 5 | 5 |

Figure 1:




## Conclusions

Our results show that T2-weighted and IR images are more effective at detecting hypertintense spine lesions than T1-weighted MRI. The lesions found on T2 and IR scans clearly correlate with the motor score of rats, especially the highest scores obtained at the onset of clinical symptoms. Also, 4% formaldehyde appears to be a better preservative than 4% glutaraldehyde when using MRI on *ex vivo* spines due to problems with dehydration. Future studies on possible treatments for MS can utilize these novel protocols to evaluate efficacy.

References: [1] Inglese M, Bester M. NMR Biomed 23: 865-72, 2010. [2] McGeary CR et al. Neuroimage 45: 1173-82, 2009.
[3] Tourdias T et al. Exp Neurol 230: 248-57, 2011. [4] Shepherd TM et al. Magn Reson Med 62: 26-34, 2009.