Magnetization EXchange (MEX) MRI Reveals Myelin Content in ex-vivo Rat Spinal Cord of Genetic Dysmyelination Mutants

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Synopsis

Keywords: Spinal Cord, Magnetization transfer, Myelin, MEX

Motivation: Developing an MT based sequence to quantify myelin in normal and abnormal brain tissue.

Goal(s): (1) Demonstrate the sensitivity of the MEX sequence to minor and severe changes in myelin fraction. (2) Study the maturation process of a lesser- known *Taiep* model, that bears much resemblance to human disorders.

Approach: Acquiring data by MRI and EM at 4 time-points, from 2 different genetic dysmyelination models, and controls.

Results: The results reveal significant myelin decreased (36%) in impacted animals and lack of myelination with age. Values also establish the normal maturation and aging process of controls. Finally, the results highly correlate with EM values (*R=0.88,p<0.0001).*

Impact: The results validate the MEX sequence capabilities to quantify myelin content, with the prospective of clinical use. The main challenge is reducing scan time. Additionally, the use of the *Taiep* model shows great promise for further studying genetic dysmyelination disorders.

Introduction

Inherited white matter (WM) disorders constitute a large group of rare genetic diseases[1,2], which primarily affect children, many of whom die young or suffer from long-term disability. These WM disorders are related to abnormal myelin production or myelin loss. The *Taiep*[3] and Long Evans Shaker (*LES*)[4] rat models are two important myelin mutants of differing severity of which there are few previous MR studies. The Magnetization EXchange (MEX) pulse sequence allows the acquisition of a signal originating from macromolecular components of the tissue that enables quantification of the volume fraction of these macromolecules[5-7]. The MEX signal is acquired using selective saturation pulses and spoiling gradients, followed by a recovery period. The magnetization detected is divided into two contributions: free water interaction (the fitted T_1 value), and the magnetization transferred from non- aqueous protons (Fig. 1). The purpose of this study is to apply the MEX sequence and its derived quantitative macromolecular fraction to evaluate impaired myelin development of inherited white matter disorders in rat spinal cords.

Methods

Eighteen spinal cords from *LES, Taiep*, and control animals, ages 28 days, 3, 6, and 9 months (n=2) were used. E*x-vivo* MEX scans (Fig. 1), were acquired on a 9.4T MRI scanner (Bruker, Germany). The preparation MEX block was individually calibrated to ensure maximal water suppression. Thirteen delay times (t_{LM}) ranging 1-5000 ms were used to acquire three slices with TE/TR=2.5/3000 ms.
Images were fitted to Eq.1, assuming fast exchange:

$$M_{zw}(t_{LM}) = M_{zw}^{eq}(F(1 - \exp(-\frac{t_{LM}}{\tau_{exc}})) + (1 - F)(1 - \exp(-\frac{t_{LM}}{T_1})))$$

F is the myelin fraction, τ_{exc} is the exchange time, and T_1 is the longitudinal relaxation time. Fitting was conducted using nonlinear least-squares Trust-Region algorithm with $R^2 > 0.95$ threshold. 3 WM ROI's and 2 GM ROI's were segmented (Fig. 2m). A Welch's t-test was used for group analysis and paired t-test for time-points progression. Pearson's correlation for EM comparison.
EM images, in corresponding dorsal, lateral and ventral WM ROIs (Fig. 2m), were captured. Myelin fraction per area was calculated:

$$\text{MyelinFraction} = \pi(r_{out}^2 - r_{in}^2) * \frac{N_{axons}}{area}$$

Results

Figure 2 shows the quantitative MEX myelin fraction maps of the three groups at 4 time points, and corresponding EM images.
Figure 3 depicts the F values (mean±SD) in each ROI for all three animal groups at 28 days time point. The myelin content of the *LES* animals in WM ROIs is significantly lower than the values of *Taiep* and control, by: 46.4% and 65.8%, respectively. GM ROIs is not significantly different between *LES* and *Taiep* but significant decrease of 17% and 33.6% in ROI4&5 respectively, is shown compared to controls.
Figure 4 follows the animals maturation. For controls there is a steady increase in myelin between 28 days and 6 months and then a slight decrease at 9 months, all significant. Gray matter areas show initial significant increase and then maintain an even trend. The myelin content of *Taiep* rats are lower in WM areas at 28 days compared to controls and remain steady through age 9 months. GM does not behave monotonically and its values vary. Figure 5 shows a very good correlation between the MEX F values at all time points for all 3 WM ROIs, and the calculated EM myelin fraction.

Discussion

Qualitatively, in Figure 2, it can be appreciated that the LES animal model is dramatically affected. It has very low myelin content throughout the cord, with no WM/GM separation. When comparing the controls and the less severe model of *Taiep*, differences are also very distinct, in all 4 time-points. It is also visible that there is an increase with age in myelin content in the WM area of controls, as to be expected in normal maturation. This is almost not

evident in the *Taiep* dysmyelination model. At the last data point for controls there is a decrease in values hinting at the dependance of aging. These qualitative observations are supported by the quantitative group analysis for all five ROIs, at the first data point (Fig. 3), and along the maturation and aging values in Figure 4.
Although the comparison to EM is of dramatically different in sensitivity and resolution, the correlation shown in Figure 5 is strong and the separation between the three animal groups is substantial, thus supporting the merit of this method.

Conclusions

The results show the benefit of using the MEX method for studying myelin development in varying pathologies longitudinally. The severity of the *LES* model makes it highly detectable even in early stages, The value of the method proposed here are in following mild myelin changes of the spinal cord in the *Taiep* model disease progression.

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References

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Figures

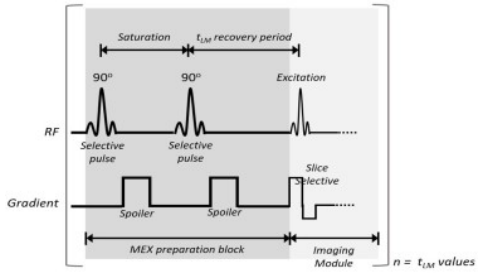

Fig. 1: MEX pulse sequence. Two selective pulses are applied at the water resonance, each followed by a spoiler. The delay time, t_{LM}, between the saturation and the imaging module, was varied between repetitions.

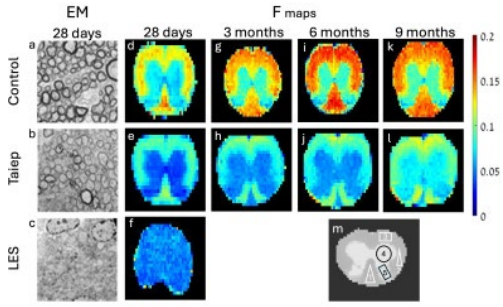

Fig. 2: (a)-(c) are the EM images taken at 28 days, for three representative animals from each group: control, *Taiep* and LES. (d)-(l) are the MEX myelin fraction analysis for the spinal cords of the animal groups at ages of: 28 days (d-f), 3 months (g-h), 6 months (i-j), and 9 months (k-l). (m) shows the segmented ROIs, where WM ROIs are in white, and GM ROIs are in black.

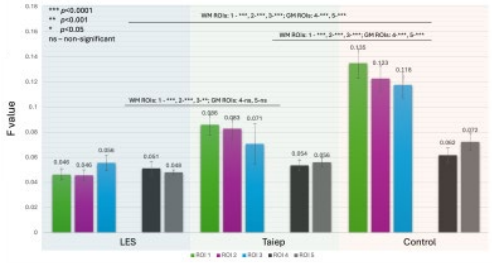

Fig. 3: F values at 28 days, as mean±SD of the pixels in each of the 5 segmented ROIs, for LES, *Taiep* and control groups, compared using Welch's t-test. ROIs 1,2,3 are in WM areas (green, purple and blue, respectively); ROIs 4, 5 are in GM areas (dark and light gray, respectively). Significance was deemed based on: p<0.05, p<0.001, p<0.0001 (marked: *,**,***, respectively).

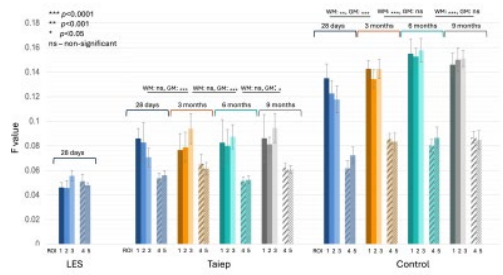

Fig. 4: F values, as mean±SD of the pixels in each of the 5 previously shown ROIs, for LES, *Taiep* and control groups at all 4 time-points. Values compared using Welch's t-test. Significance was deemed based on: p<0.05, p<0.001, p<0.0001 (marked: *,**,***, respectively).

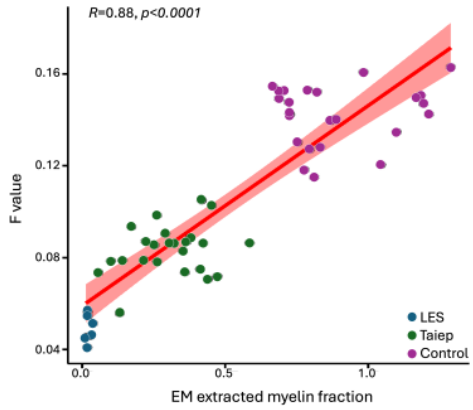

Fig. 5: Pearson's correlation between MEX F value (y-axis) and EM myelin fraction calculation (x-axis). The data points are colored to match all three animal groups: LES, *Taiep* and control (blue, green and purple, respectively), at 4 time-points and the 3 WM ROIs.