Magnetization Transfer Micro Imaging Characterization of Axonal Degeneration and Regeneration in Live Excised Lamprey Spinal Cord

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Introduction

Magnetization transfer (MT) can enhance tissue contrast and provide information on neural tissue composition based on the exchange of ^{1}H magnetization between pools of relatively free water and protons with restricted motion. (1-3). Among the tissue components that may play a key role in conferring the MT effect are the membrane constituents (4) in the axolemmal lipid bilayer. In a previous study on normal live excised lamprey cord (8) we determined that calculated magnetization transfer ratios (MTRs) are determined by the membrane density. We used features of the sea lamprey spinal cord, characterized by rapid spontaneous axonal regeneration after injury (5), to determine whether changes in MTRs may correlate with changes in axon density in response to trauma and repair.

Materials and Methods

Twenty nine larval sea lamprey spinal cords were studied (six normal spinal cords, five at 2 weeks and six for each 5 weeks, 10 weeks and 15 weeks after injury). After immersing the animals in a solution of 0.1% tricaine methansulfonate, the spinal cords were exposed via a dorsal incision and the transection was performed at the level of the fifth gill. The excised cords were placed in a capillary tube in fish Ringer solution and then in a home built solenoid transmit-receive RF coil (1.5 mm i.d. diameter) (6,7). Magnetization transfer micro-MRI was performed on a 9.4 T system. Measurements were conducted by using a conventional spin-echo sequence with and without a pre-saturation MT pulse applied 6500 Hz off water resonance. Two 0.25-mm-thick sections were selected for the MT study, at 2.5-mm rostral and caudal to the injury site. The parameters were: repetition time/ echo time = 4000/15 ms, 3 mm field of view, 128 x 128 matrix, 23 x 23- μm^2 in-plane resolution, four averages, resulting in a total scan time of approx. 8 minutes for each slice. The magnetization transfer pulse was a block pulse, 40 µT power, 55 dB gain, 3.9 ms long, applied 6500 Hz off resonance. In order to preserve the spinal cord viability scans were performed at 8°C and scan time limited as much as possible. Histological studies performed after the MR scan proved the absence of postmortem changes. The analysis regions of interest (ROIs) were placed on different regions of the white matter (note that lamprey lacks myelin and WM is used only as a convention for the regions of SC that comprise axons). Signal intensities were also analyzed in the surrounding buffer and used to calculate the MTR for each of the ROI. Intensities were measured in air to provide an estimate of noise in each image. Magnetization transfer ratio was computed as MTR= [Ringer(M_{sat}/M_0)- tissue(M_{sat}/M_0)] x 100, where M_0 and M_{sat} are the signal intensity without and with the MT pulse applied, respectively. Statistical review of the data was performed using the two tailed unpaired t test and ANOVA.

Results and Discussion

When placing our ROIs we focused on the dorsal columns of WM situated above the injury site and containing sensitive fibers passing toward the brain and also on the ventral columns of the WM situated below the transection site and containing motor fibers headed to the caudal part of the animal. The calculated MTRs in all drawn ROIs decreased progressively, reaching their minimum ($P<0.05$) at 10 weeks after injury, and then returned toward the values before injury. These changes in MTR correspond on the NF immunostained sections to axonal degeneration and regeneration. All the imaged spinal cords were analyzed histologically and the results were expressed semi-quantitatively. **Fig.1** shows representative histological microphotographs.

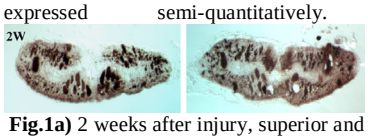
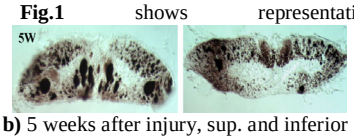
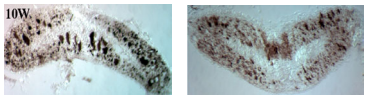


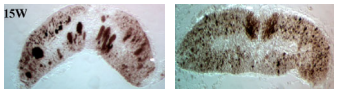
Fig.1a) 2 weeks after injury, superior and inferior to the transection site



b) 5 weeks after injury, sup. and inferior



c) 10 weeks after injury, sup. and inferior



d) 15 weeks after injury, sup. and inferior

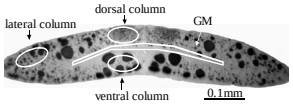
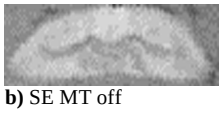
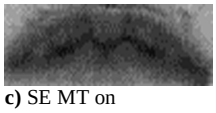


Fig. 2.a) Microphotograph



b) SE MT off



c) SE MT on

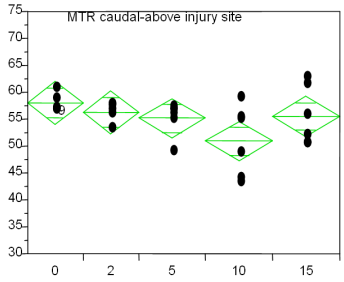


Fig.3. Regional MTR decreased progressively at 2, 5 and 10 weeks when compared to the normal cord values, and increased back at 15 weeks.

Conclusion

The present study results imply that MTR measurements may be useful to semi-quantitatively assess the degeneration and regeneration of axons after injury. In our animal model the MTR variance appears to be determined by the change in axolemmal density that follows transection. Our results - MTR decreasing as axons degenerate and recovering toward normal values while axons regenerate and the animal functionally recovers - support our hypothesis that MTR can be used to detect changes in axon density in response to trauma and repair. The method may have potential for monitoring the severity of axonal injury and response to therapy, in animal models and possible in humans.

References

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