

In-Vivo Mouse Brain DT-MRI: Assessment of Gender Specific Response to the Thyroid Hormone Remyelinating Treatment

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Introduction: *Multiple Sclerosis (MS)* is one of the most common neurological disorders, characterized by extensive loss of myelin, axonal damage and inflammation of the central nervous system (CNS) axonal tracts (1). It affects twice more women than men and the progression is gender dependent; the male patients having a faster and more severe outcome. Various features of the MS are mimicked in animal models. One of the extensively used mouse model of demyelination, underlying the progression of the pathology from acute to chronic states is the cuprizone diet model (2). Long-term 0.2% cuprizone feeding in mice produces severe brain demyelination, accompanied by gliosis, astrocytic hypertrophy and axonal injury. We recently evidenced by DT-MRI of the cuprizone demyelinated mouse brains; gender related differences in the development of the white matter pathology (3), with more severe axonal damage in the male animals. Therefore, the response to different therapeutic strategies might be gender specific.

The main goal of the present study was to comparatively investigate the response of the cuprizone demyelinated male and female mice to a remyelinating therapy based on thyroid hormones (T3, tri-iodothyronine). This therapy was very efficient in one of our previous studies (4) for inducing remyelination in female mice, but no longitudinal investigation was performed in male animals so far.

Materials and Methods: Six groups (n=10) of male and female 8-week old C57BL/6 mice were used for DT-MRI and T2-weighted imaging at different time points (w0, w12, w12+3, w12+6, w12+12), as shown in Fig. 1, a). Duplicates for each group, subjected to similar treatments were used for histology. **MRI:** The mice were scanned under isoflurane anesthesia, using a 9.4T small bore animal Scanner (Biospec 94/20, Bruker), a transmit/receive 1H mouse quadrature birdcage resonator and the ParaVision 5 software interface. After localized shimming procedure on a volume of interest (4.8 x 5.3 x 9 mm³), inside the mouse brain, brain DT-MRI data was acquired with a 4-shots DT-EPI sequence. Diffusion gradients were applied in 45 non-collinear directions (b factor = 1000s/mm²), TR/TE = 5000/30 ms, time Δ=17ms, diffusion gradient duration δ=7ms. The in-plane image resolution was 156 x 156 μm², for 20 slices (500μm). Respiratory gating was performed in order to avoid respiratory movement artifacts. The diffusion tensor was calculated and fractional anisotropy (FA), mean diffusivity <D>, radial diffusivity (D_⊥), axial diffusivity (D_∥) as well as directional encoded maps were generated. The values of these parameters were assessed in different ROIs of white and gray matter, comparatively in males and females, and statistical analysis was performed. DT-MRI results were further correlated with the histological evaluation, assessing the myelin and axonal state and the modifications in the glial cell s.

Results and Discussion: 12 weeks of cuprizone feeding induced marked white matter (WM) pathology in both male and female mouse brains. The drastic increase of the radial diffusivity and the reduced values of FA at w12 (Fig. 1,b, w12 and Fig.2 a, w12) were consistent with oligodendrocyte and myelin loss, axonal injury and inflammation. These pathological features were maintained in both male and female animals which were not subjected to hormonal therapy. The mice receiving thyroid hormone for 3 weeks showed progressive recovery towards normal radial diffusivity values during the 12 weeks of observation (Fig. 2, a). Both males and female mice positively responded to the remyelinating therapy. However, a certain trend of faster recovery was observed in the female mice, which shown consistent decrease of the radial diffusivity towards control values at already w12+6 (Fig. 2, a). The efficacy of the T3 treatment was obvious at w12+12 timepoint in the in-vivo longitudinal examination (Fig. 2-a) of the male animals. Similar features of faster myelin recovery were observed in the histopathological investigation, although a strong effect of induced oligodendrogenesis in both genders was quantified at already week w12+3 (Fig.2-b-C,F). This might suggest greater myelinating potential of the newly formed female oligodendrocytes or greater sensitivity to the thyroid hormone actions. Further investigations of the possible mechanisms involved in the faster recovery observed in females animals are warranted using cellular and molecular biology based techniques.

Conclusion: The present study unveiled the great potential of the T3 based therapy for inducing recovery in both male and female mouse demyelinated brains. The results are valuable for understanding the role of gender in the physiopathology and the remyelination of the white matter. In the view of future translation of the preclinical assays in the clinical environment, DT-MRI investigation is of high value, allowing the quantitative survey of the same individuals overtime.

(1). Confavreux and Vukusic, 2006; Brain 129:606-616. (2) Matsushima and Morell 2001; Brain Pathol 11:107-116, (3) Harsan et al., ISMRM 2009; (4) Harsan et al., J. Neurosci 2008; 28(52):14189-201

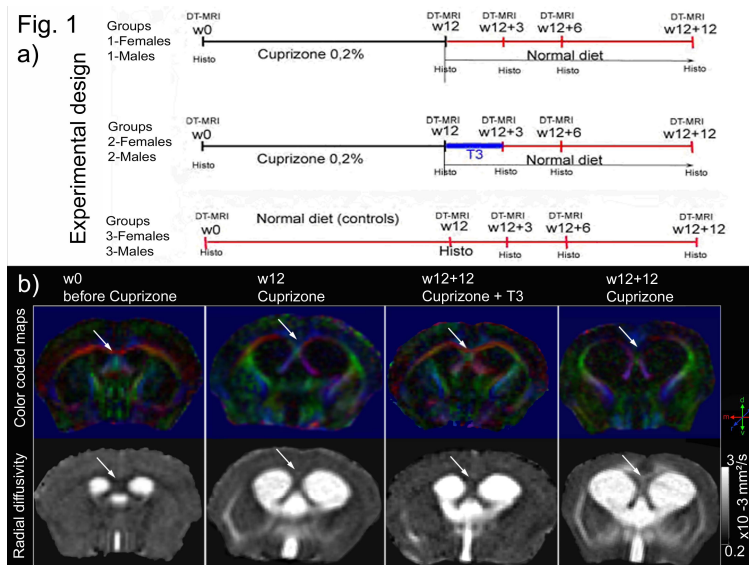


Fig. 1: a) Experimental design: Animal groups, treatments, and timepoints for the MRI investigation (week 0 – w0 - before cuprizone treatment; w12 – 12 weeks of cuprizone diet; w12+3, w12+6, w12+12 (3, 6, 12 weeks after stopping the cuprizone diet). **b) Color-coded and radial diffusivity maps** showing the cuprizone (w12) and thyroid hormone effects in the brain of male animals (w12+12). Notice the high radial diffusivity values at w12 (arrow), consistent with demyelination. Important recovery is observed in animals receiving T3 (w12+12 Cuprizone+T3), but not in the animal not receiving the hormonal therapy (w12+12 Cuprizone).

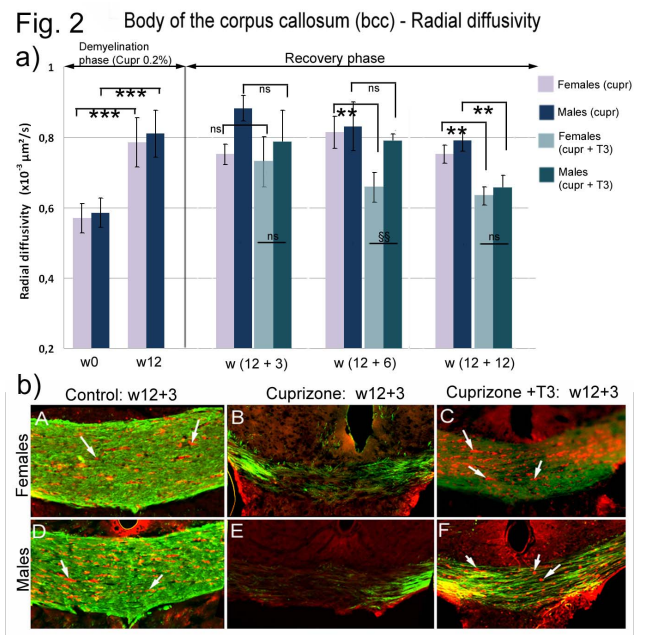


Fig. 2: a) Comparative (females vs males) longitudinal assessment of the radial diffusivity values in the body of the corpus callosum in cuprizone demyelinated mice receiving T3 (Cuprizone+T3) or not receiving T3 (Cuprizone). **b)** T3 induced oligodendrogenesis in male and female mice. Staining of myelin (green) and oligodendrocytes (red) in brain sections at w12+3, from female (A, B, C) and male (D, E, F) control (A, D), cuprizone (B, E) and T3 treated (C, F) mice. Arrows point the oligodendrocytes cells.