Cuprizone model - Correlation Between Fine Motor Performance and White Matter Changes

Kimmo K. Lehtimäki, Sunggun Kim, Anna-Mari Zainana, Antti Nurmi
Charles River Discovery Services, Kuopio, Finland

693.13

1 INTRODUCTION & STUDY DESIGN

Background: The cuprizone exposure has been used to model a wide range of pathological and behavioral deficits endpoints that recapitulate demyelinating diseases and multiple sclerosis (MS). We and others have described the behavioral, pathological and immunological phenotype of mouse cuprizone model at different stages during and after cuprizone challenge. Besides traditional behavioral tools, we have applied kinematic analysis to understand more in detail affected motor behavior due relatively mild phenotype during and after cuprizone exposure. In addition, we have also evaluated the extent of the demyelination, both with classical histological/immunohistochemical tools, but also by applying non-invasive imaging. DTI-MRI revealed progressive demyelination in the major white matter structures during cuprizone exposure. Given that many of the behavioral end-points demonstrate recovery following discontinuation of cuprizone treatment, we wanted in the present study evaluate correlation between white matter changes and fine motor behavior more in detail, during and after cuprizone challenge.

In this study we looked at demyelination during the active cuprizone exposure period like previously, but also examined recovery period after the discontinuation of cuprizone challenge. We specifically looked at changes in the white matter, here mainly corpus callosum but also other whte matter rich structures.We also extended our knowledge about the cuprizone model with kinematic gait analysis, which we have previously seen to show gait changes during the cuprizone challenge period. In this case, we have also evaluated recovery period and looked at how strongly behavioral changes will normalize when reported remyelination process is active.

2 METHODS

Animals Female C57Bl/6 mice at the age of 9-12 weeks were subjected to twice a day p.o. dosing of cuprizone 200 mg/kg corresponding to 0.3% of cuprizone in the diet. Mice were daily treated for 6 weeks and then cuprizone was discontinued for 3 weeks. Control mice were treated with vehicle for cuprizone.

MR experiments: A horizontal 11.7T magnet interfaced to a Bruker Avance III console (Bruker Biospin GmbH, Ettlingen, Germany).

Diffusion Tensor MRI: DTI measurements were performed using 4-segment EPI-sequence with 30 diffusion directions (*b*-values 0 and 870 s/mm^2). TE/TR of 28/6000 ms was used and FOV 20x10 mm^2 with matrix of 256x128 provided 78 microns in-plane resolution in 15 0.5 mm thick slices.

Fine Motor and Gait Kinematic Analysis: The mice were evaluated using an apparatus (Motorater, TSE-systems GmbH, Bad Homburg, Germany) designed for the detection of fine motor skills in rodents. The equipment consists of a brightly illuminated plexiglas corridor (153 x 5 x 10 cm) under which is situated a high-speed camera (300 fps, observations from both sides and under). The gait and fine motor skills were analyzed from three dimensions, first using the Simi Reality Motion Systems (Unterschleissheim, Germany) and the obtained raw data was further analyzed by a custom analysis system. Data were analyzed for distinctive parameters, as well as using principal component analysis (PCA) for the acquired parameters.

All data is mean + SEM and statistics are given as Student's t-test, p-values as indicated in figures.

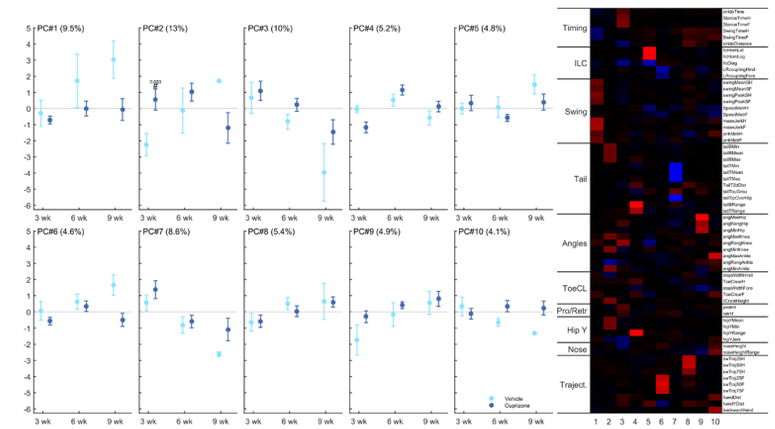


Figure 5. Principal component analysis did not show any consitently affected components in the gait of cuprizone treated mice that were found to be significantly altered during 9 week study, except for 3 week time-point. However, as a whole summary PCA vales showed signficantly altered gait properties.

3 RESULTS

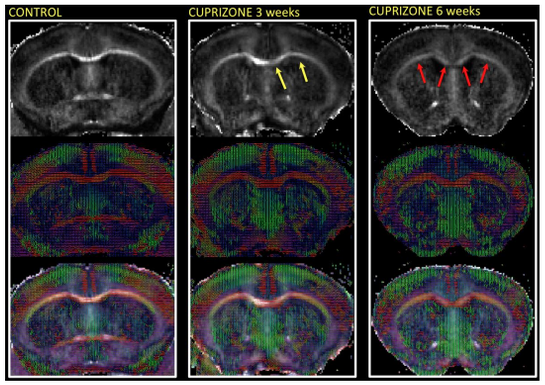
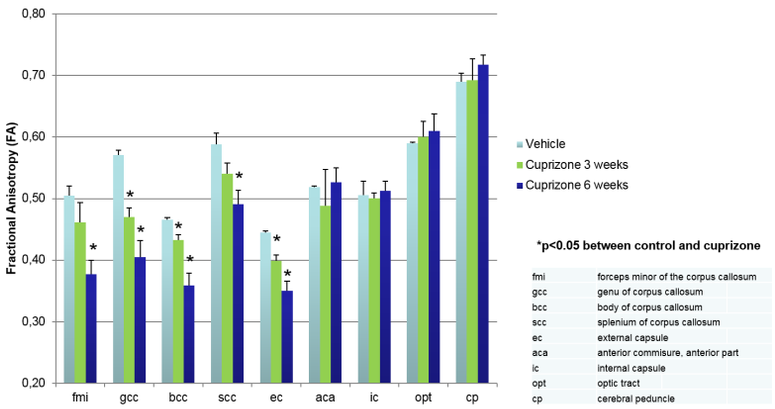


Figure 1. Diffusion tensor magnetic resonance imaging (DTI) from control, 3 weeks and 6 weeks cuprizone challenged animals. Control images show normal homogenous distribution of high FA values in a corpus callosum, whereas cuprizone challenge for 3 weeks show unilateral and partial loss of FA coherence (yellow arrows) and 6 weeks cuprizone treatment leads to a situation, where FA is reduced in whole corpus callosum and this reduction extends to external capsule (red arrows). Colorcoded diffusion principal directions are shown in second row and on bottom row principal directions are overlaid on FA maps



fmi	forceps minor of the corpus callosum
gcc	genu of corpus callosum
bcc	body of corpus callosum
scc	splenium of corpus callosum
ec	external capsule
aca	anterior commisure, anterior part
ic	internal capsule
opt	optic tract
cp	cerebral peduncle

Figure 2. Diffusion tensor magnetic resonance imaging in cuprizone and control mice at 3 and 6 weeks. Quantification of white matter fractional anisotropy (FA) showed signficant decrease in FA values accross multiple white matter rich structures.

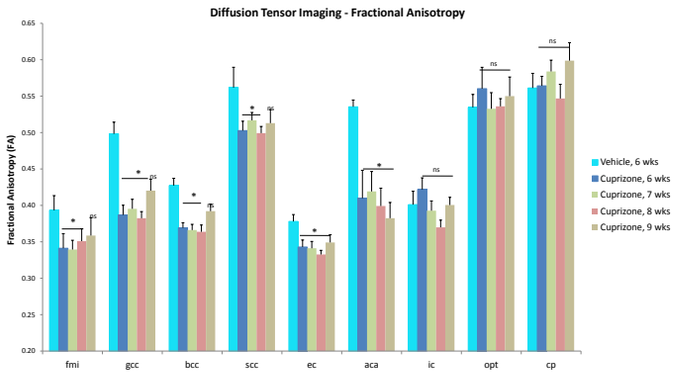


Figure 3. Diffusion tensor magnetic resonance imaging in cuprizone and control mice at 6, 7, 8 and 9 weeks. Quantification of white matter fractional anisotropy (FA) showed signficant decrease in FA values accross multiple white matter rich structures. Recovery period, weeks 7, 8, 9 show only minimal reversal of demyelination. Abbreviations for the anatomical regions, see Figure 2.

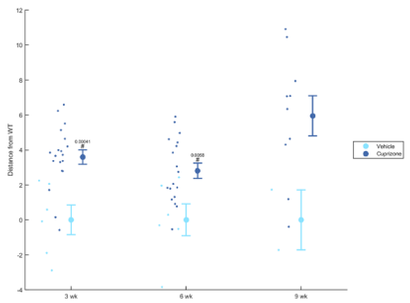


Figure 4. Summary PCA values from kinematic analysis showing differences between control and cuprizone mice. Until 6 weeks mice were twice daily treated with cuprizone p.o. after which cuprizone was discontinued. 9 week time point shows situation with 3 weeks of recovery after 6 weeks of cuprizone challenge.

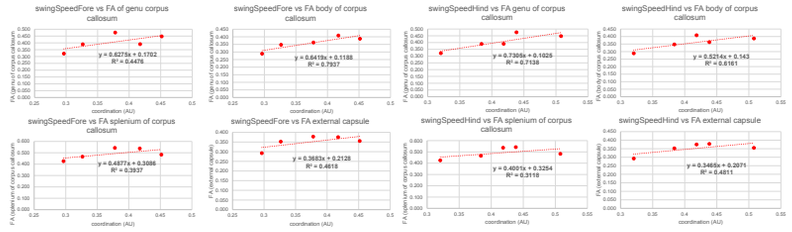


Figure 6. Swing speed and FA change correlation. In some parameters moderate correlations between FA values and gait parameters were found. Structurally FA changes in different subregions were showing varying degree of correlation in individual parameters, here in hind and forelimb swing speed rom randomly selected mice. Some parameters (data not shown) did not show any correlation between FA and parameters.

4 CONCLUSIONS

In this study we looked at demyelination during the active cuprizone exposure period like previously, but also examined recovery period after the discontinuation of cuprizone challenge. We specifically looked at changes in the white matter and gait properties over time and found:

1) DTI-MRI allows assessment of demyelination in cuprizone model longitudinally. Robust demyelination is seen during cuprizone challenge, but interestingly only minimal restoration of white matter changes in cuprizone mice were seen after 3 weeks of recovery.
2) Kinematic analysis confirmed our previously reported fine motor gait changes in cuprizone mice which were extended to recovery period also
3) Moderate correlations between gait parameters and FA values were observed in some cases, some parameters remained not correlated.

Taken together data presented here suggests the benefit of DTI-MRI in the assessment of demyelination longitudinally from same animals without need to perform extensive histology. Kinematic gait analysis results suggests that aftercuprizone recovery period some gait properties are still affected with moderate correlation with white matter readouts.