Electrophysiological and behavioural impairments in the cuprizone demyelination/remyelination mouse model

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Background

Central myelin, produced by oligodendrocytes in the central nervous system (CNS), is a lipid-rich membrane protecting and electrically isolating neuronal axons. In multiple sclerosis, myelin membranes and oligodendrocytes are destroyed through chronic inflammation in the CNS[1]. The cuprizone murine model is a toxic model of demyelination, in which neurotoxin cuprizone is fed to mice, inducing death of oligodendrocytes and consequent CNS demyelination[2]. If after 5 weeks of cuprizone administration, when demyelination is over 90%, mice are returned to a regular diet, spontaneous remyelination occurs, with myelination returning to 90% after 4 weeks of recovery[3]. Hence cuprizone-fed mice represent a model of demyelination/remyelination[4].

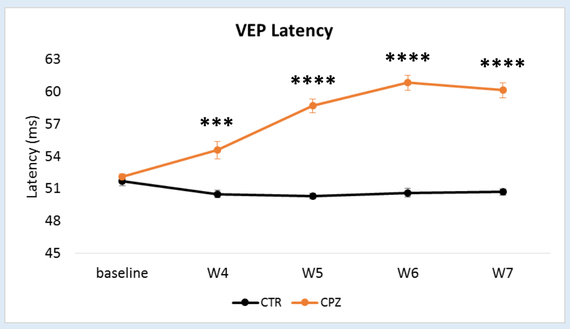
Aim of the Study

To characterize electrophysiological and behavioral impairments of cuprizone-fed mice over the course of 7 weeks and subsequently, after cuprizone diet suspension, to evaluate the possible recovery of these impairments during the remyelinization phase.

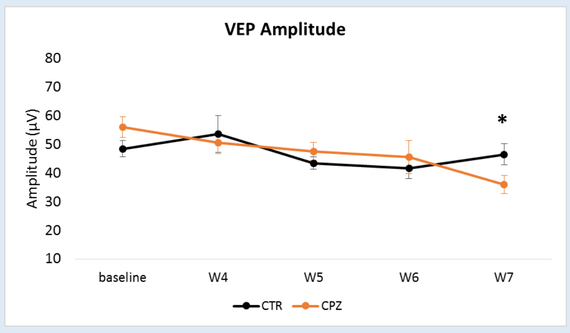
Materials and Methods

Male wild-type C57BL/6 mice were fed cuprizone (CPZ) or regular diet (CTR) for 7 weeks (W0-W7), after which toxin was removed and 2 weeks of recovery were allowed (RW1-2). Visual evoked potential (VEP) under ketamine/xylazine (80/10 mg/kg) was used to evaluate optic nerve function. Epidermal VEP[5] (Ag/AgCl cup electrode on scalp) was recorded in response to flash stimulation (3 trains of 20 stimuli of 10 µs duration and 1 Hz frequency) from both eyes of 9 cuprizone mice and 6 control mice (n = 18 and 12, respectively). One eye at a time was stimulated (with the other eye covered through a black silicon band) and VEP was recorded from the contralateral visual cortex. Cup active electrode was placed on the left or right visual cortex (+3.5 mm anterior to interaural line, ±4 mm lateral to midline). A needle electrode in the cheek was used as reference, while a needle in the hindlimb was used as ground. Body temperature was maintained at 37°C using a homeothermic heating pad. Pupils were dilated with tropicamide 1% and hydroxypropylmethylcellulose 2% was applied to prevent eye drying. Electrodes were connected through flexible cables to an amplifier (Micromed, Mogliano Veneto, Italy). Latencies and amplitudes of ERG b-wave and VEP N1 were recorded. Forelimb grip strength was measured through a spring dynamometer (Kern & Sohn GmbH, Balingen, Germany). Mean and maximal grip strength over 6 consecutive trials were acquired. Motor coordination was evaluated by recording time to touch down in the vertical pole test (diameter: 1 cm; height: 50 cm). Mean latency of 2 consecutive trials was utilized for the analysis.

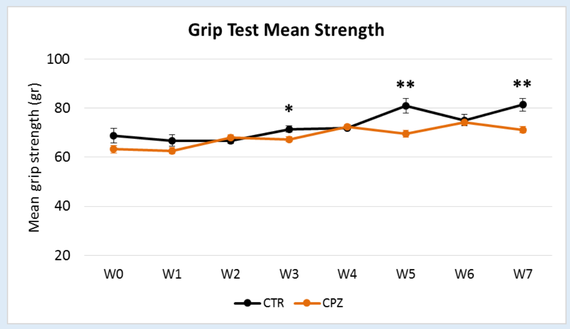
Results



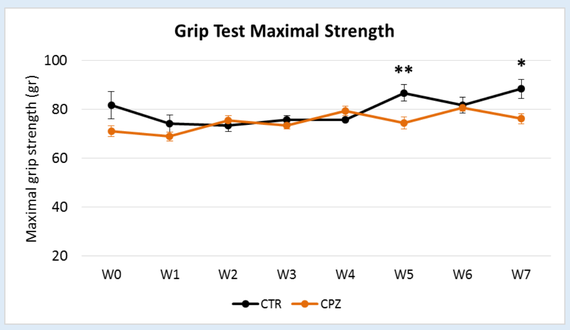
Two-way ANOVA for repeated measures revealed a significant effect of diet ($p < 0.0001$). Starting from W4, VEP latency was significantly increased in CPZ mice compared with CTR (W4: $p = 0.0001$; W5: $p < 0.0001$; W6: $p < 0.0001$; W7: $p < 0.0001$). Mean values ± SEM are represented.



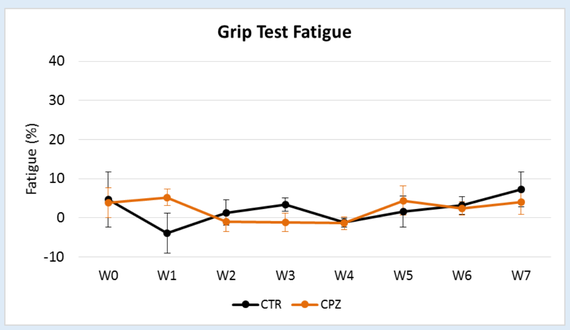
A significant effect of time on VEP amplitude in the CPZ group was found (One-way ANOVA for repeated measures: $p = 0.020$). At W7 amplitude was significantly reduced compared with baseline ($p < 0.0001$) and with CTR ($p = 0.042$). Asterisk highlights between groups comparison. Mean values ± SEM are represented.



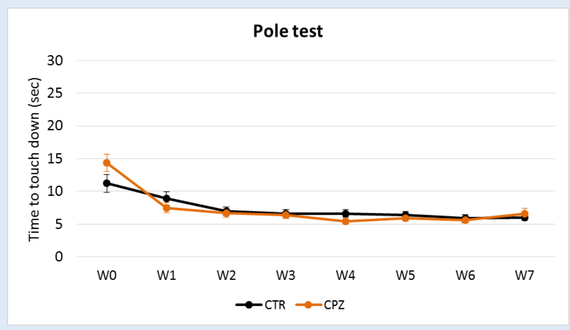
Two-way ANOVA for repeated measures revealed a significant effect of diet ($p = 0.004$). Compared with CTR, mean grip strength across the 6 trials was significantly reduced for CPZ at W3 ($p = 0.025$), W5 ($p = 0.002$) and W7 ($p = 0.001$). Mean values ± SEM are represented.



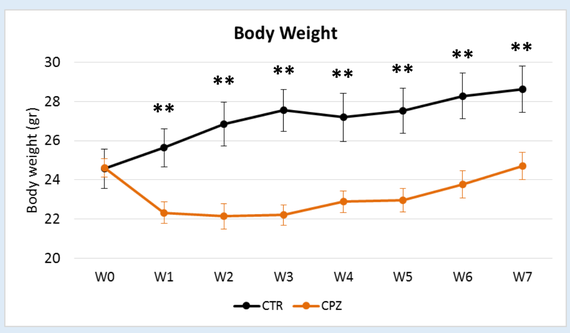
Two-way ANOVA for repeated measures detected a significant effect of diet ($p = 0.019$). Compared with CTR, maximal grip strength across the 6 trials was significantly reduced for CPZ at W5 ($p = 0.009$) and W7 ($p = 0.010$). Mean values ± SEM are represented.



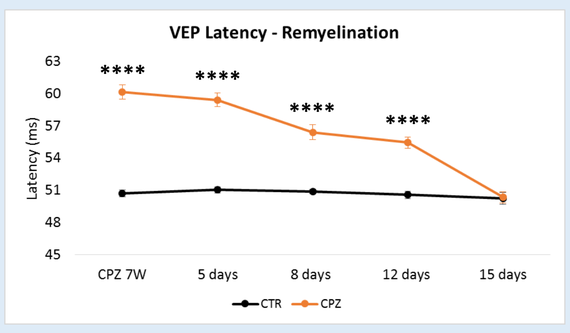
No difference between CPZ and CTR was observed during the 7 weeks of diet in grip test fatigue across the 6 trials (Two-way ANOVA for repeated measures: $p = 0.980$). Fatigue was calculated as percentage of decrement between strength I pulls 1+2 and in pulls 5+6. Mean values ± SEM are represented.



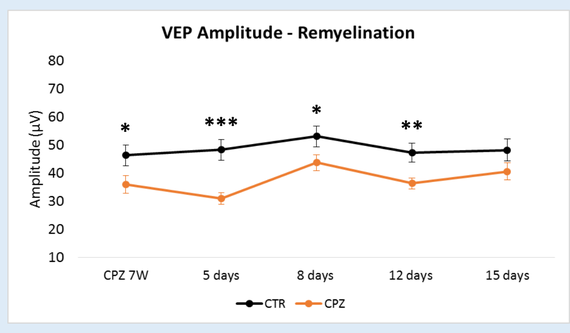
No difference between CPZ and CTR was observed during the 7 weeks of diet in pole test motor coordination (Two-way ANOVA for repeated measures: $p = 0.971$). Mean values ± SEM are represented.



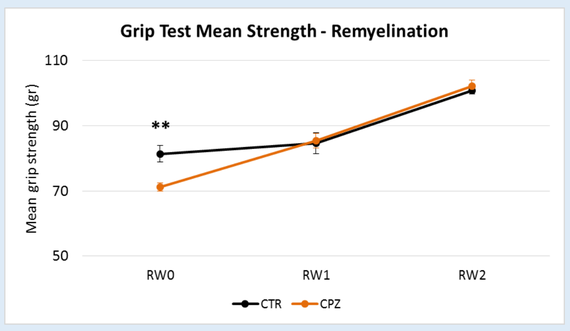
Two-way ANOVA for repeated measures found a significant effect of diet ($p < 0.005$). Starting from W1, body weight significantly decreased in CPZ compared with CTR (W1: $p = 0.007$; W2: $p = 0.002$; W3: $p = 0.003$; W4: $p = 0.003$; W5: $p = 0.002$; W6: $p = 0.003$; W7: $p = 0.009$). Mean values ± SEM are represented.



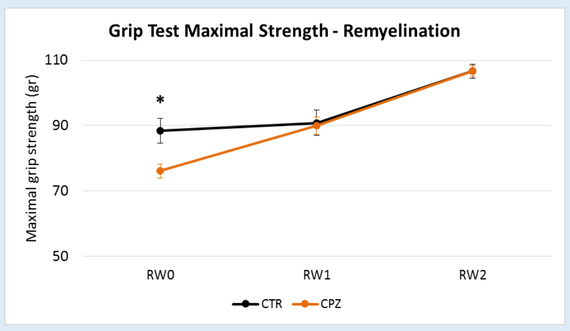
CPZ mice showed a significant recovery of latency during the remyelination period (One-way ANOVA: $p = 0.001$). Compared with CTR, CPZ exhibited significantly higher latencies at CPZ 7W ($p < 0.0001$), 5 days ($p < 0.0001$), 8 days ($p < 0.0001$) and 12 days ($p < 0.0001$). Mean values ± SEM are represented.



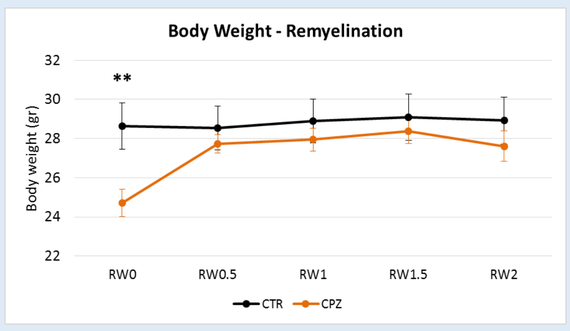
CPZ mice displayed a significant rescue of amplitude during the remyelination period (One-way ANOVA: $p = 0.009$). Compared with CTR, CPZ exhibited significantly lower amplitudes at CPZ 7W ($p = 0.042$), 5 days ($p = 0.0002$), 8 days ($p = 0.0495$) and 12 days ($p = 0.004$). Mean values ± SEM are represented.



Mean strength of CPZ in grip test significantly improved during the remyelination period (One-way ANOVA: $p < 0.0001$). Compared with CTR, CPZ showed significantly lower mean strength at RW0 ($p = 0.001$). Mean values ± SEM are represented.



Maximal strength of CPZ in grip test significantly ameliorated over the remyelination period (One-way ANOVA: $p < 0.0001$). Compared with CTR, CPZ displayed significantly lower maximal strength at RW0 ($p = 0.010$). Mean values ± SEM are represented.



Body weight of CPZ mice significantly increased during the remyelination period (One-way ANOVA: $p < 0.0001$). Compared with CTR, CPZ showed lower body weight at RW0 ($p = 0.009$), while starting from RW0.5 no difference was observed. Mean values ± SEM are represented.

Conclusions

Body weight was reduced at very early stages (since W1), followed by loss of grip strength at W3. VEP latency delay was found from W4, suggesting damage to optic nerve myelin. Reduction of VEP amplitude appeared only later at W7. Both VEP abnormalities were reverted 2 weeks after cuprizone suspension, indicating that remyelination-mediated rescue is possible. Indeed, VEP evaluation of optic nerve function in the cuprizone model could be used to test treatments aiming at boosting and/or speeding up remyelination.

Bibliography

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