Cuprizone demyelination of the corpus callosum in mice as a model of the neurodegenerative aspects of the human demyelinating disease, multiple sclerosis (MS)

***Species*:** mice

Fields of application: Autoimmune diseases, Neurodegeneration, Demyelination and Remyelination

Multiple sclerosis (MS) is a chronic demyelinating, inflammatory and neurodegenerative neurological disease often occurring in early adulthood. It results in disabling physical symptoms. With progression of the disease, the likelihood increases that comorbid conditions occur (such as cognitive deficits, depression and fatigue).

Cuprizone intoxication is a commonly used model to study experimental remyelination, with the corpus callosum being the most frequently investigated white matter tract. In this model mice are fed with the copper chelator cuprizone (bis-cyclohexanone oxaldihydrazone), which leads to a primary oligodendrocyte (OL) apoptosis and secondary demyelination within weeks. After removal of the toxin spontaneous remyelination occurs, thus making the cuprizone model appropriate for studying compounds which can prevent demyelination and/or stimulate remyelination.

Endpoints/Outcome parameters: It is important to consider which *in vivo* readouts are relevant for the evaluation of drug effects. We develop animal models for MS and try to mimic the symptoms of MS in animals in order to better predict efficacy of compounds in patients. Our approach is not only to use standard readouts, but we also offer the possibility to study more subtle and objective readouts that potentially could have a higher translational value.

Readout parameters

The myelin staining method is used as a primary measure for demyelination and remyeliniation. To assess the fluorescence, brain sections are stained with fluoromyelin green using a fluorescent microscope for image acquisition. All images from the same area of interest (AIO), the corpus callosum at the level of the hippocampus, are taken for fluoromyelin green detection. To evaluate the fluoromyelin green, the integrated optical density (the product of area and mean gray value) of the AIO is measured.

In addition to the myelin measure, we assess other variables that may be more predictive for the study of drug efficacy. More subtle and objective measurements of balance, motor coordination and muscle strength can be investigated using (semi) automated setups, rotarod, threadmills and grip strength meter. For example, we use the rotarod test which is based on a rotating rod with forced motor activity being applied. The test measures parameters such as riding time (seconds) and endurance. Some of the functions of the test include evaluating balance and coordination of the subjects; these are especially useful in testing the effect of experimental drugs or in disease animal models. In the test, a rodent is placed on a horizontally oriented, rotating cylinder (rod) suspended above the floor/table. Rodents naturally try to stay on the rotating cylinder, or rotarod, and avoid falling. The latency for a given animal stays to fall is a

measure of their balance, coordination, physical condition, and motor-planning. The speed of the rotarod is mechanically driven, and may either be held constant (fixed) or accelerated.

MS co-morbid symptoms such as cognitive impairment, fatigue and mood disturbances are often untreated and therefore represent potential therapeutic targets. For example, clinical studies have revealed discrete cognitive dysfunction in MS patients already at an early stage of the disease. About 60% of individuals with MS experience significant cognitive dysfunction. We offer the opportunity to test effects of compounds on cognitive impairment in the cuprizone animal model using setups such as the Mouse Touch Screen Chambers (Campden Instruments Ltd.).

Quality management and validation: All current drugs work by decreasing inflammation through modulation of the immune system and while they have been very efficient in reducing the rate of relapses, their impact on the chronic disease course is unknown. A current thought in the MS therapeutics community is that drugs that enhance remyelination may be more effective in reducing long-term disability. We are presently testing existing and novel compounds in the model in order to investigate their effects on remyelination.

References:

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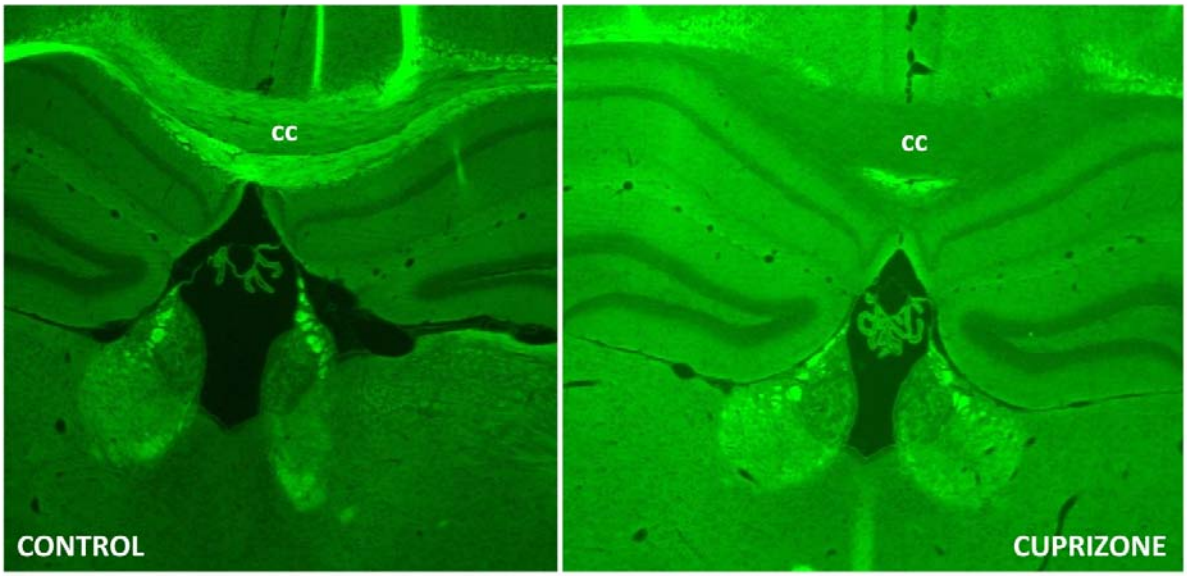

Figure: Demyelination of the corpus callosum (cc) in the cuprizone animal model

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