

REVIEW ARTICLE

Five Decades of Cuprizone, an Updated Model to Replicate Demyelinating Diseases

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Abstract: Introduction: Demyelinating diseases of the central nervous system (CNS) comprise a group of neurological disorders characterized by progressive (and eventually irreversible) loss of oligodendrocytes and myelin sheaths in the white matter tracts. Some of myelin disorders include: Multiple sclerosis, Guillain-Barré syndrome, peripheral nerve polyneuropathy and others. To date, the etiology of these disorders is not well known and no effective treatments are currently available against them. Therefore, further research is needed to gain a better understand and treat these patients. To accomplish this goal, it is necessary to have appropriate animal models that closely resemble the pathophysiology and clinical signs of these diseases. Herein, we describe the model of toxic demyelination induced by cuprizone (CPZ), a copper chelator that reduces the cytochrome and monoamine oxidase activity into the brain, produces mitochondrial stress and triggers the local immune response. These biochemical and cellular responses ultimately result in selective loss of oligodendrocytes and microglia accumulation, which conveys to extensive areas of demyelination and gliosis in corpus callosum, superior cerebellar peduncles and cerebral cortex. Remarkably, some aspects of the histological pattern induced by CPZ are similar to those found in multiple sclerosis. CPZ exposure provokes behavioral changes, impairs motor skills and affects mood as that observed in several demyelinating diseases. Upon CPZ removal, the pathological and histological changes gradually revert. Therefore, some authors have postulated that the CPZ model allows to partially mimic the disease relapses observed in some demyelinating diseases.

Conclusion: for five decades, the model of CPZ-induced demyelination is a good experimental approach to study demyelinating diseases that has maintained its validity, and is a suitable pharmacological model for reproducing some key features of demyelinating diseases, including multiple sclerosis.

Keywords: Cuprizone, myelin, oligodendrocyte, demyelination, remyelination, white matter, neuroinflammation, multiple sclerosis, demyelinating disease, microglia.

1. INTRODUCTION

Demyelinating diseases are neural disorders characterized by inflammation and damage or selective destruction of myelin associated with diffuse primary synaptic loss and progressive axonal degeneration [1-3]. Clinical deficits include ataxia, loss of dexterity, myoclonus, spasticity,

paraparesis or hemiparesis, vision loss and cognitive deficits. These alterations are due to disrupted electrical transmission along axons because of myelin loss. A crucial characteristic of demyelinating diseases is the limited ability to rapidly regenerate myelin and the extension of secondary damage of axons. There are several causes of myelin destruction, including immunological action, chemicals and infections [4]. Multiple sclerosis (MS) is associated with focal white matter plaques of demyelination, which are partially repaired during remyelination. Reappearance of oligodendrocytes within active lesions is frequently seen in patients at early stages of

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MS. Some authors suggest that remyelination is a transient phenomenon and remyelinated shadow plaques may be affected by new bouts of demyelination, which ultimately lead to incomplete myelin repair [5, 6]. There are several animal models that resemble the clinical and pathophysiological course of MS. To date, four experimental approaches to induce demyelination have been well-characterized and include: genetic myelin mutations, autoimmune inflammatory-induced demyelination (experimental autoimmune encephalomyelitis -EAE), virus-induced demyelination and toxic demyelination (cuprizone and lyssolecithin models). All these models partially mimic the MS pathology. Although EAE is a commonly used model that reproduces some of the autoimmune aspects of MS, the toxic demyelination with cuprizone can be still considered an appropriate model to study the remyelination process [7, 8].

Cuprizone (CPZ) is a copper chelator that targets many metalloenzymes as ceruloplasmin, impairs the activity of the copper dependent cytochrome oxidase, decreases oxidative phosphorylation and produces degenerative changes in oligodendrocytes (OLs). This cascade of events eventually end in demyelination [9, 10]. In 2007, Zucconi, *et al.* found the copper deficiency induced by CPZ was related to microglial activation in cerebral cortex and thalamus [11]. This evidence suggests that CPZ may reproduce different pathological aspects of some neurodegenerative diseases [11]. The supplementation of animal diet with CPZ has been used as demyelinating model that mimics some histological hallmarks of demyelinating diseases [12]. Cuprizone intoxication induces apoptosis of oligodendrocyte cell population that leads to extensive demyelination of white matter tracts in the corpus callosum, internal capsule, the thalamus, anterior commissure and cerebellar peduncles [13, 14]. Interestingly, when CPZ treatment is withdrawn, there is rapid remyelination and myelin proteins re-expression [15].

The cuprizone-induced damage appears to be mediated by certain molecules, such as tumor necrosis factor alpha (TNF α), interleukin 1 β (IL-1 β) and interferon gamma (IFN γ) that are secreted by microglia/macrophages in the brain. These events are associated with a decrease in mitochondrial activity in OLs, low energy production, and high activity of reactive oxygen species [16]. The administration of CPZ *in vivo* leads to fast proliferation of microglia/macrophages surrounding the lesion area. These immune cells are known to produce TNF α , which seems to exacerbates acute demyelination and remains undetectable on untreated mice, as reported by Arnett *et al.* [17]. However, some important features of MS, such as inflamed blood vessels and the presence of CD3+ T cells, have not been described in the CPZ model [18].

2. CUPRIZONE

CPZ, also referred as to cyclohexylidene hydrazide (Fig. 1), is a condensation product of oxalylhydrazide and cyclohexanone. CPZ is an effective and selective copper chelating agent. The first description of CPZ was made by Gustav Nilsson in the 1950s, he discovered that CPZ induced a subtle blue color reaction upon chelating copper (Cu²⁺) salts [19]. In 1966, Carlton observed that CPZ administration in

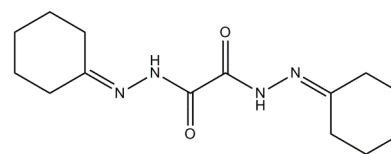


Fig. (1). Molecular structure of N,N'-bis(cyclohexylideneamino)oxamide (cuprizone). Molecular formula: C₁₄H₂₂N₄O₂; molecular weight: 278.45008g/mol.

mice produced low serum Cu²⁺ levels and demyelination [20]. Later, it was found that oral administration of CPZ also produced severe status spongiosus, hydrocephalus and hepatic lesions [21, 22].

3. COPPER METABOLISM

Copper is an essential element in all mammal's nutrition. This element is a component of metalloenzymes in which it acts as an electron donor or acceptor [23]. Thus, copper is required for preserving tissue growth, cardiovascular integrity, neuroendocrine function, neovascularization, lung elasticity and iron metabolism. Copper acts as a coenzyme in aerobic metabolism for cytochrome c oxidase in the mitochondria, lysyl-oxidase in the connective tissue, dopamine monooxygenase in the brain, and ceruloplasmin in the liver. Copper is also a cofactor for apo-copper-zinc superoxide dismutase (ApoCuZnSOD) that protects against oxygen-free-radical damage to proteins, membrane lipids, and nucleic acids in several systems. Severe copper deficiencies are relatively rare in humans and may occur by gene mutations or low dietary copper intake. Copper deficit may produce mental retardation, anemia, hypothermia, neutropenia, diarrhea, cardiac hypertrophy, bone fragility, immune deficiencies, connective tissue weakened, brain function impaired, peripheral neuropathy, and accelerate hair loss [24]. Therefore, the copper metabolism is strongly regulated by a complex homeostatic process [25].

In mammals, absorption of copper primarily occurs in the small intestine. Copper absorption is performed by the brush border of the intestinal mucosa and transferred across the basolateral membrane into interstitial fluid and blood. Once copper is absorbed from the intestine, it binds to albumin and copper transport protein (transcuprein), and reaches serum levels of 40 mg Cu/L, approximately. Most of the serum copper is then deposited into the liver (Fig. 2). Nonetheless, only \approx 180 μ g Cu²⁺ remains associated with albumin in human plasma. The rest of the copper (\approx 1000 μ g per liter plasma) is bound to ceruloplasmin (65%, approximately), transcuprein (12%), and other components of low molecular weight. Most of the incoming copper rapidly finds its way into the hepatic cells (Fig. 2), and minimal amounts of this metal enter the kidney. There are two phases of copper distribution into the blood serum. The first phase is mediated by transcuprein that facilitates copper intake into the liver. The second phase is mediated by ceruloplasmin that helps increase the copper levels into systemic organs (Fig. 2). The main role of copper is to function as a cofactor for specific enzymes and electron transport proteins involved in energetic or anti-oxidative metabolism [26].

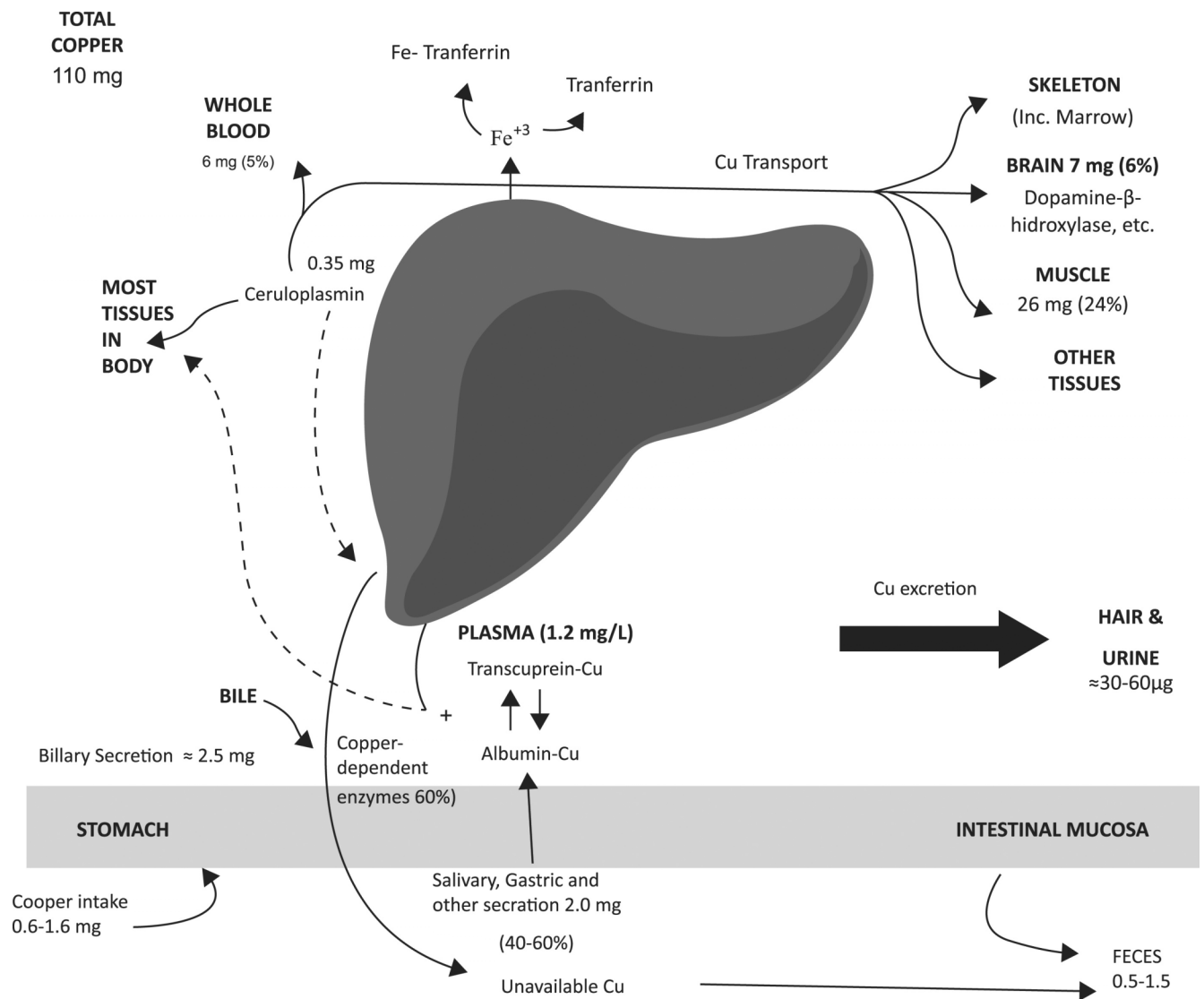


Fig. (2). Copper absorption and distribution. Copper values indicate the average of daily amounts of copper ingested, absorbed, secreted or excreted through different tissues. Modified from Linder & Hazeg-Azam [105].

4. CUPRIZONE AS DEMYELINATION MODEL

Since 1960s, several studies have been designed to determine the precise dose of CPZ to produce significant changes in the CNS. Significant myelin changes have been observed when 0.2% - 0.6% CPZ is mixed with standard rodent chow [22]. The most common protocol consists in feeding 8-week-old mice with 0.2 % CPZ (w/w) for 5-6 weeks (Fig. 3). Lindner *et al.* in 2008 demonstrated that high levels of demyelination can be achieved by increasing the dosage to 0.3% CPZ (w/w) [27]. However, as the CPZ concentration increases (from 0.2 % to 0.3%) the mortality rate rises from < 5 % to more than 10 % or 15 % [28]. Thus, the 0.2% CPZ is the preferred concentration because it produces extensive demyelination with less side effects [29-34].

Oligodendrocyte apoptosis begins a few days after cuprizone administration. Recently, Hesse and colleagues demonstrated that oligodendroglial cell death and myelin gene downregulation start a few days after CPZ supplementation,

but demyelination is only evident a few weeks after [35]. Recent evidence has shown that short-term exposure to CPZ (3 weeks) is enough to induce demyelination [29]. Astrogliosis and depletion of mature OLs is observed at 5th week. Similar histological changes can be observed with CPZ exposure for five weeks. Hence, it seems that once the mature OLs are perturbed the demyelinating activity progresses without needing additional exposure. Interestingly, early withdrawal of CPZ did not accelerate the recovery process, which suggests that a mild white matter insult triggers a cascade of demyelinating events in the following weeks [36]. Although matter degeneration in the CC is a key neuro-anatomical sign of the CPZ model [33, 37, 38], several brain regions are also affected by CPZ (Table 1) [14, 32, 33, 37, 39-52]. The histopathological changes produced by CPZ include oligodendroglial cell death, microglia activation, astrocyte reactivity and grey matter demyelination [53].

Recent studies have demonstrated that juvenile mice are more vulnerable to CPZ. In fact, aged mice require high

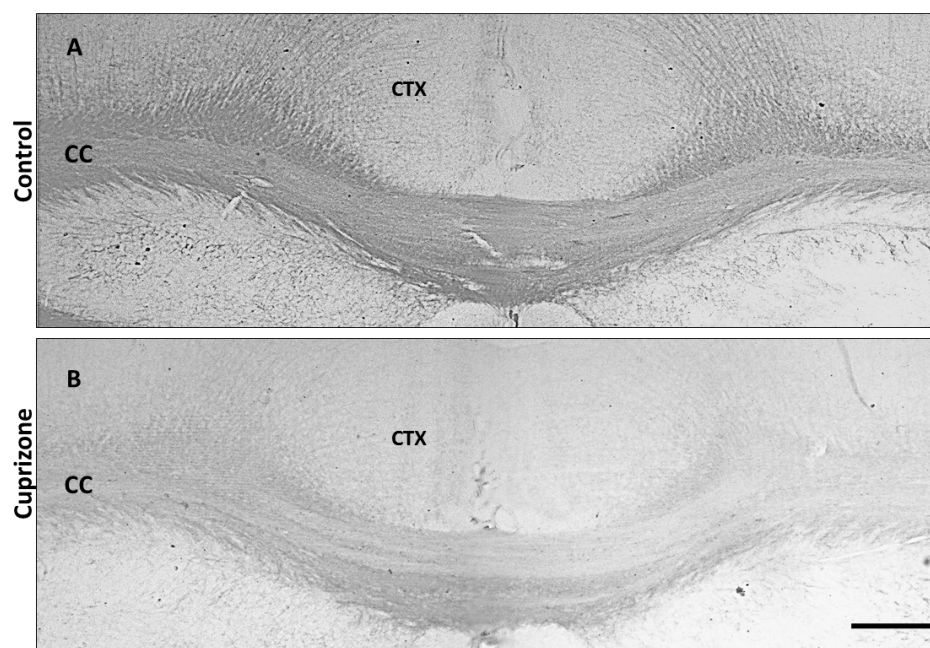


Fig. (3). Cuprizone effects in the CD1 mouse brain. CD1 mice received 0.2% CPZ for 6 weeks. 30- μ m-thick coronal sections immunostained with anti-myelin basic protein (MBP) and revealed with 3,3'-Diaminobenzidine. The control animal (A) shows a strong expression of MBP in the corpus callosum (CC), whereas the cuprizone-treated mouse (B) expresses low levels of MBP. CTX: cortex. Bar = 5 μ m.

Table 1. Cuprizone demyelination over susceptible regions.

Brain Region	CPZ Concentration	Initial Demyelination (Week)	Complete Demyelination (Week)	Microgliosis Initiation (Week)	Astrogliosis Initiation (Week)	Refs.
Corpus callosum	0.1% – 0.5%	3	4.5	1	3	[32, 33, 39]
Anterior commissure	0.2%, 0.25%	5	N/R	5	N/R	[40-42]
Basal ganglia (caudoputamen, globus pallidus)	0.2% – 0.4%	2	5	5	2	[40-44]
Brainstem	0.2%	N/R	5	N/R	N/R	[41, 106]
Cerebellum	0.2%, 0.5%	2	5	2-3	3-4	[20, 47, 48, 106-108]
Cortex	0.2% – 0.5%	4	5	3	3	[39-41, 49, 109-111]
External Capsule	0.2%	N/R	6	N/R	N/R	[110]
Fornix	0.2%, 0.25%	5	12	5	N/R	[40, 111]
Hippocampus	0.2% – 0.4%	3	5	N/R	5	[41, 51, 109-112]
Hypothalamus	0.2%	6	N/R	N/R	N/R	[110]
Internal capsule	0.25%	5	N/R	5	5	[40]
Olfactory bulb-tract	0.2%, 0.25%	5	5	5	N/R	[40, 41, 110, 111]
Optic chiasm	0.2%	N/R	5	N/R	N/R	[41]
Optic tract	0.25%	5	N/R	5	N/R	[40]
Septal nucleus	0.25%	5	N/R	5	N/R	[40]
Substantia innominata	0.25%	N/R	5	5	N/R	[40]
Thalamus	0.2%, 0.25%	6	5-6	5	5	[40, 110]

Several brain regions have been reported to be susceptible to CPZ induced demyelination and show microgliosis and astrogliosis. N/R, Not reported.

doses of CPZ to achieve a similar degree of demyelination as compared to young mice, but the precise mechanism underlying this effect is still unknown. CPZ-induced demyelination in the corpus callosum of juvenile mice is more severe than that of middle-aged animals. Young mice (<57 days) exposed to CPZ show working memory deficit throughout both the CPZ intoxication period and the remyelination process (CPZ removal). In contrast, old mice (>57 days) only show working memory deficit immediately after CPZ exposure [54]. This age-related vulnerability also results in profound behavioral dysfunctions in young-adult mice, including anxiety, attention deficit hyperactivity disorder and schizophrenia-like phenotypes. [55, 56]. At week four after CPZ exposure, motor deficits are also observed as observed in the rotoroad test and the open field maze [57-59]. The proclivity of young mice to demyelination may be explained by age-related changes in the expression of PDGFR α , Nkx-2.2 and Olig2 [60] that, in turn, modify the recruitment of oligodendrocyte progenitor cells and drive the cell fate of neural progenitors toward the astrocyte lineage [61-64]. Interestingly, short-term exposure to cuprizone do not produce evident demyelination, but induces astrocyte and microglia activation that have been associated with work memory impairment and social behavior disruption [65, 66]. Further evidence indicates some sex-related differences in CPZ-induced demyelination. In this regard, Ludwin reported that Swiss and SJL/J female mice did not show demyelination as compared to males. However, no significant intersex differences were observed in C57BL/6 mouse strain [67, 68]. C57BL/6 is the mouse strain most commonly used for the CPZ demyelination model [33, 69]; however, many other mouse strains and animals species have been used to study different aspects of the demyelinating effect of CPZ (Table 2).

Recently, a modification in the CPZ model was described and consisted in the addition of 10 mg/kg i.p. rapamycin five times per week. This mTOR inhibitor produces extensive demyelination and prolongs the remyelination period, which help evaluate the effect of remyelinating treatments and downstream mechanisms involved in remyelination [70, 71]. Besides the CPZ model, other demyelinating models have been designed to reproduce the pathological course of demyelination and investigate possible treatments against MS (Table 3). Patients with MS may display three different clinical presentations: the relapsing-remitting form (RRMS) that is characterized by exacerbations of the symptoms (relapses), followed by periods of complete or partial remission; the primary progressive form (PPMS) that shows a continuous and irreversible evolution of the disease; and the secondary progressive form (SPMS) in which there is a progressive worsening of neurologic function over time, probably as a transition from RRMS [72]. To date, there are several experimental models that mimic some of the MS features and all of them have different characteristics. The autoimmune or allergic experimental encephalomyelitis (EAE) model in SJL/J mice is useful to study the relapsing forms. The MOG₃₅₋₅₅ immunization of C57BL/6 mice is a good model to study a chronic-progressive disease [73] and it properly resembles the physiopathology of MS patten-II lesions [74]. The Theiler's murine encephalomyelitis virus (TMEV) model, in which mice develop a chronic progres-

Table 2. CPZ susceptibility of diverse mouse/rat strains and other animal species.

Mice Strain/ Species	Demyelination Severity	Refs.
C57BL/6 mice	Male ++ / Female ++	[32-33, 39, 43]
ICI Mice	Male ++	[14]
Swiss-Webster Mice	Male ++	[113]
Swiss Mice	Male ++ / Female -	[61]
Albino Mice	Male ++ / Female ++	[114]
SJL Mice	Male ++ / Female +	[69]
129/SVJ Mice	Male ++	[43]
BALB/cJ Mice	Male +	[49]
CD1 Mice	Male ++	[115]
Cynomolgus Macaque	Male - / Female -	[116]
Wistar rat	Male ++ / Female ++	[117-120]
Guinea Pig	Male ++	[119]

The CPZ demyelination pattern depends on the concentration, exposure time, sex, strain, and specie. (++) Acute; (+) Mild; (-) Not found.

sive demyelinating disease without remissions [75] and their histopathological patterns look alike the patterns III and IV of MS lesions [76]. The lysolecithin injection model that produces focal and delimited demyelinating lesion with microglia/macrophage activation and recruitment, which provides a fast and well-defined remyelination pattern comparable to the patten III of MS lesions [38, 77, 78]. Finally, the CPZ model can be considered as a suitable model to reproduce extensive pattern-III lesions of MS [79], which are characterized by the presence of apoptotic OLs and early down regulation of myelin-associated glycoproteins (MAG) [80, 81]. The CPZ model also allows to the study of mechanism of spontaneous remyelination, because the mechanism of demyelination/remyelination occurs simultaneously and the white-matter damage reverts after CPZ withdrawal resembling the RRMS [82]. In summary, the choice of the best model depend on the aim of study and the characteristics of the treatment to be evaluated (Table 3).

5. MECHANISM OF ACTION OF CPZ-INDUCED DEMYELINATION

5.1. Mitochondrial Response to CPZ Intoxication

The oral administration of CPZ *via* daily diet in adult mice produces a specific insult to mature oligodendrocytes by impairing their metabolic demands to support myelin production and by triggering oligodendroglia apoptosis [83]. These events are followed by microglia recruitment and myelin phagocytosis. Morphological and gene-expression studies indicate that during the CPZ administration some oligodendrocyte progenitor cells keep proliferating and invading demyelinated areas, but the magnitude of CPZ effect always ends in severe copper deficiency and secondary demyelination [14, 84-86]. Remarkably, demyelination and

Table 3. Comparison of the most commonly used demyelination models.

Model	Specie	Chemical Agent	Demyelination	Administration Route	Spontaneous Remyelination	Complexity	Disadvantages	Refs.
Cuprizone	Rat, Mouse	Cuprizone	Initial: 3 weeks Complete: 4.5 weeks	Oral	Yes (completed at week 4)	Low [mixed with chow]	Not validated for functional readouts in some strains	[32, 39, 86]
Lysolecithin	Rat, Mouse	Lysophosphatidylcholine	Initial: 2 days Complete: 1 week	Intracerebral injection	Yes (completed at week 4)	High [Stereotactic surgery]	High technical complexity	[121-123]
EAE*	Rat, Mouse, Guinea pigs	CFA+MOG/MBP/PLP peptides or rSCH	Initial: 11 days Complete: 2 weeks	Intraperitoneally	No	Low [i.p. injection]	More related to ADEM than MS. Not for investigating remyelination process	[124-125]
TMEV	Mouse	Theilovirus (cardiovirus/piconaviridae). TO and GDVII subtypes	Initial: 6 weeks Complete: 35 weeks	Oral, intranasal, footpad inoculation, intracerebral injection	No	High [Stereotactic surgery, virus culture]	The proportion of the disease caused by the virus activity or the inflammatory response is hard to determine	[8, 126-127]

EAE: experimental autoimmune encephalomyelitis; TMEV: Theiler's murine encephalomyelitis virus; CFA: complete Freund adjuvant; rSCH: rat spinal cord homogenate; MOG: myelin oligodendrocyte glycoprotein; MBP: myelin basic protein; PLP: proteolipid protein; W: weeks; ADEM: acute disseminated encephalomyelitis. * Demyelination pattern reported in the EAE model study was carried out on optic nerve of SJL/J mice, but demyelination pattern may vary between specie, strain and brain region.

oligodendrocyte damage produced by CPZ is not associated with injury of other neural cell types [85]. These findings contrast with those observed in liver, where the formation of megamitochondria (mitochondria enlargements or clusters) has been observed in hepatocytes. This alteration in liver mitochondria may be a consequence of deficient activity of cytochrome oxidase [16, 61, 85, 87, 88]. Interestingly, CPZ leads to a reduction in brain activity of cytochrome oxidase, monoamine oxidase (MAO) and inhibition of complexes I, II and III of the respiratory chain. After the CPZ intoxication, the formation of megamitochondria has been observed in liver, but not in neurons, astrocytes and other neural cells [16, 87, 89]. Remarkably, studies have confirmed that CPZ only affects mature oligodendrocytes, without modifying the absolute number of oligodendrocyte progenitor cells (OPCs) [85]. Consequently, CPZ does not affect *de novo* formation of oligodendrocytes.

Oxygen free radicals appear to be responsible for mitochondria enlargement. This process may be a protective reaction to reduce and suppress intracellular reactive oxygen species (ROS) levels, which restore normal cellular functions and organelle structure. However, if ROS levels dramatically increase into the oligodendrocytes and the resting membrane potential decreases with a concomitant caspase-3 activation and demyelination. During the first 3 weeks of CPZ intoxication the caspase-3 is strongly active. Next, the caspase-3 activity decreases, whereas the activity of poly ADP-ribose polymerase (PARP) increases and induces apoptosis *via* the apoptosis inducing factor (AIF) [29, 90, 91]. Hence, this evidence indicates that CPZ intoxication increases oxidative stress that, in turn, triggers apoptosis in mature OLs (Fig. 5) [83].

5.2. Lipid Metabolism Disturbance

Besides the inhibition of myelin protein synthesis, myelin lipid metabolism is also affected by cuprizone. Myelin sheet consist of 70% lipids, 40% phospholipids (mainly plasmalogens), and 30% proteins [92]. Membrane-bound plasmalogens can be hydrolyzed by plasmalogenase, leading to an increase of free plasmalogens. Phospholipase A2 (PLA2) can degrade both membrane-bound and free plasmalogens into arachidonic acid (AA), a key intermediate of pro-inflammatory signaling. The activity of both plasmalogenase and PLA2 is increased by CPZ intoxication, which contributes to myelin sheath degradation and elevated concentrations of AA [83]. In MS lesions, the AA cascade is activated by increasing activity of PLA2 and cyclooxygenase 1 or 2 (COX-1, COX-2). The produced AAs will be subsequently metabolized by COX-1 or COX-2 into prostaglandin H2 (PGH2) that strongly increase prostaglandin E2 (PGE2) related to OLs apoptosis *via* PGE2-E2 receptor, prostacyclin (PGI2), prostaglandin D2 (PGD2) and prostaglandin F2 α (PGF2 α). Interestingly, this AA cascade is activated by cuprizone and is associated with a substantial involvement of sPLA2 isoform [93].

Other organic compounds involved in the myelin sheet formation are cerebrosides (glycosphingolipids) and cholesterol [92]. These lipidic compounds are drastically reduced by increased activity of plasmalogenase during early stages of CPZ intoxication, and these findings are comparable to the increased phospholipase (PL) A₁ and A₂ activity found in MS and other neurodegenerative diseases [94-96]. These events produce myelin vacuolation and fluid accumulation between myelin lamellae with extensive space-occupying lesions, which cause axoplasmic displacement and axonal

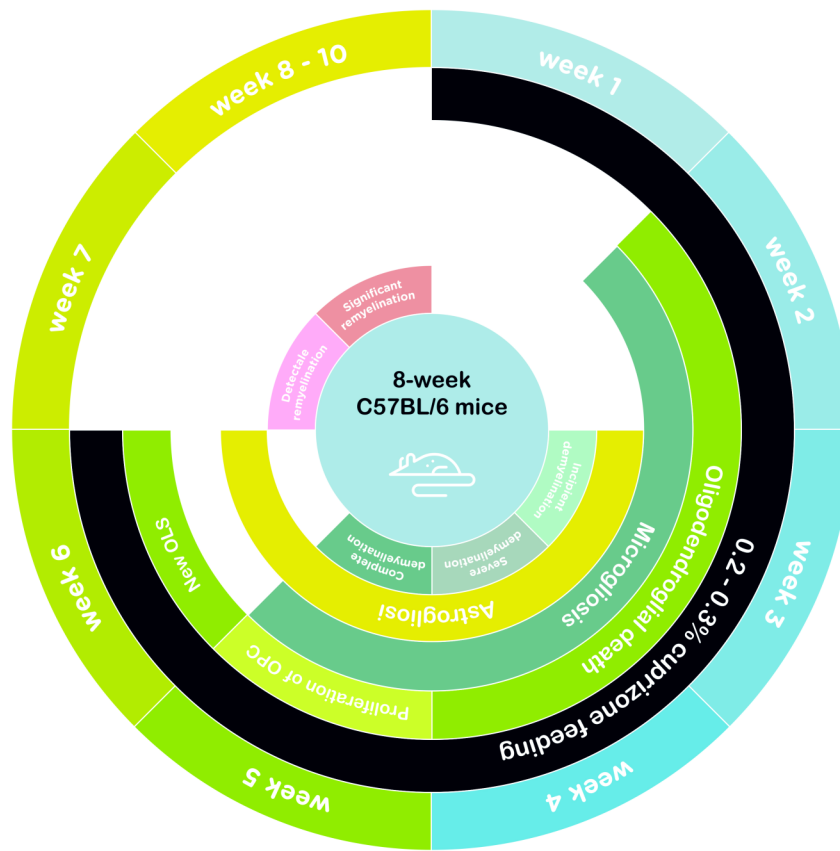


Fig. (4). Time course of demyelination induced by 0.2% cuprizone feeding. Oligodendrocyte death begins at day three to seven and this event is followed by myelin protein degradation that approximately occurs at week one to three (early demyelination). At 4th week, the severe demyelination period begins and culminates at 5th - 6th weeks, when a complete demyelination is observed in many brain regions. The recovery process (remyelination phase) is detectable in the first week after cuprizone withdrawal, but it is more significant between the weeks two and four.

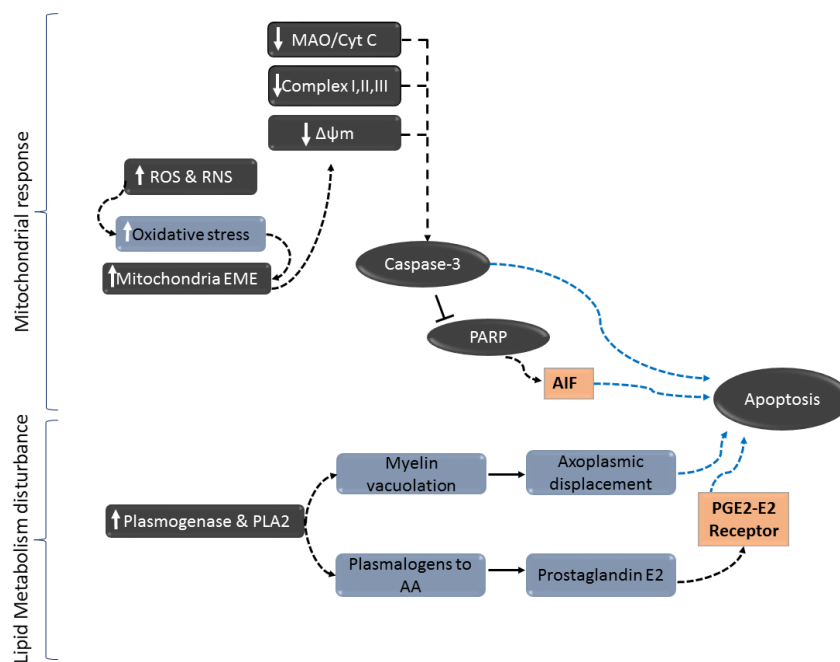


Fig. (5). Mitochondrial response to CPZ and lipid metabolism disturbance. Cuprizone induces alterations in many Cu dependent enzymes, generate increased oxidative stress leading to apoptosis and impairing the synthesis of key compounds in the myelin formation. ($\Delta\psi_m$, mitochondrial membrane potential).

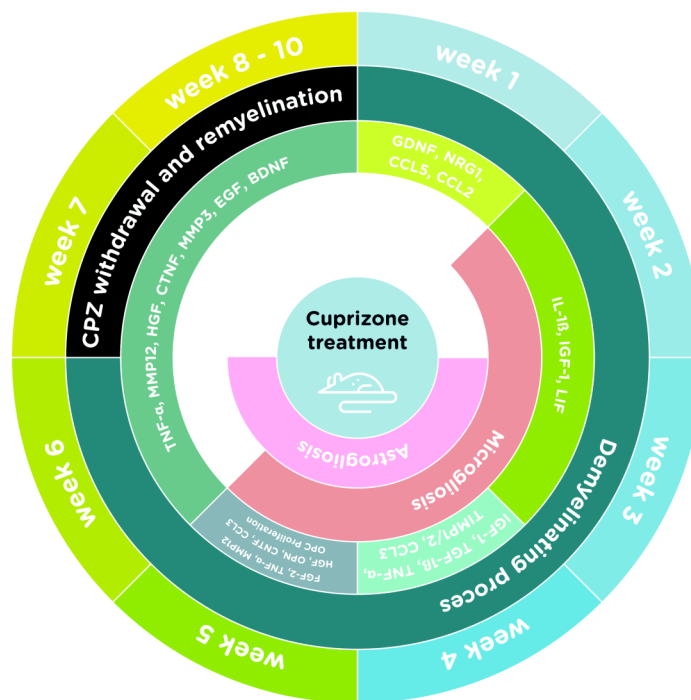


Fig. (6). Growth factors, cytokines, chemokines, and matrix metalloproteinases expressed in the medial corpus callosum during demyelination and remyelination in cuprizone treatment. The diagram depicts the expression levels of several molecules at different time points of the CPZ-induced demyelination. GDNF: Glial cell-derived neurotrophic factor; NRG1: neuroregulin -1; CCL: chemokine (C-C motif) ligand (2, 3 & 5); IL-1 β : interleukin-1 β ; IGF: insulin like growth factor-1; LIF: Leukemia inhibitory factor; TGF-1 β : transforming growth factor 1 β ; TNF- α : Tumor necrosis factor- α ; TIMP1/2: tissue inhibitor of metalloproteinase 1 & 2; FGF-2: fibroblast growth factor 2; MMP: matrix metalloproteinase (3 & 12); HGF: hepatocyte growth factor; OPN: osteopontin; CNTF: Ciliary neurotrophic factor; EGF: epidermal growth factor; BDNF: brain-derived neurotrophic factor.

disruption [14, 97]. Although myelin vacuolation is reversible, demyelination becomes irreversible by cell-body degeneration of mature oligodendrocyte [67]. OLs degeneration and apoptosis precede the CPZ-induced demyelination. This sequence of events suggests that the initial deterioration of OLs is not linked to autoimmune response against myelin proteins. A few days after CPZ treatment, oxidative stress induces degeneration of oligodendrocyte and myelin sheath that progresses to OLs apoptosis. Once mature OLs are disrupted, a sequence of cellular and inflammatory processes drive caspase-3-triggered apoptosis and demyelination [36]. Caspase-3 is a crucial apoptotic protease [98] that is expressed at initial stages of cuprizone model in most of the injured oligodendrocytes. At later stages, the percentage of caspase-3-expressing oligodendrocytes decreases gradually until it is completely absent at 3th week. This evidence suggests a biological switch in the cell-death mechanism triggered by CPZ, that begins with a caspase 3-dependent mechanism and progressively changes to a caspase 3-independent cell death [29] (Fig. 5).

5.3. Immune System Influence in the CPZ Model

The classical hypothesis indicated that cuprizone intoxication produced a direct cell-autonomous toxicity in mature OLs with the concomitant demyelination. However, inflammatory mediators seem to be involved in the process of cuprizone-induced demyelination (Fig. 6). Some of this evi-

dence includes: 1) Production of NO by upregulating inducible nitric oxide synthase (iNOS) and the neuronal isoform (nNOS), which strongly determine the demyelinating process as demonstrated in eNOS^{-/-} mice [30, 31]. 2) Inflammatory cytokines produced by microglia and astrocytes that have a cytotoxic role and promote the overexpression of the endothelial nitric oxide synthase (eNOS) [16]. 3) Astrocyte production of interleukin-6 (IL-6) and interleukin-17 (IL-17) resulted in functional deficits associated with accelerated demyelination, reduced myelin synthesis and microglia activation [65, 99]. 4) Overexpression of CXCL10 produced by astrocytes that induces reactive microglia and, in turn, increases the levels of TNF α [13]. 5) IFN- γ that is crucial in the demyelinating process as demonstrated in transgenic mice that express low level of IFN- γ under the transcriptional control of MBP gene (MBP/IFN- γ mice) and display almost null evidence of injury in the white matter after CPZ administration [100-102]. 6) Interferon-beta (IFN- β) absence promotes remyelination by increasing the proliferation of oligodendroglial precursors cells [103]. 7) Activation of neutrophils that express the type 2 chemokine receptor (CXCR2) and trigger demyelination, as observed in CXCR2^{-/-} mice that are resistant to CPZ-induced demyelination [104]. Taken together, this evidence suggests that the immune system is a key component in the cascade of events triggered by cuprizone administration. Therefore, this model may represent an plausible tool for studying the role of immune system in the process of demyelination [30].

CONCLUSION

The CPZ model is a highly reproducible and easily implemented animal model that produces very consistent demyelinating lesions. The white matter damage, oligodendrocyte apoptosis and microglial activation induced by CPZ mimics some of the aspects of demyelinating diseases. Thus, CPZ model has some characteristics that help study the interactions between the immune system with the oligodendrocyte damage and myelin disruption, which can provide crucial information to test potential therapies against demyelinating diseases. Although it is generally accepted that CPZ model induces extended demyelination and important histological changes, the complete cascade of biochemical events triggered by CPZ remains unclear. In addition, more research needs to be done to establish these mechanisms and clarify the pathways on how CPZ can reach the brain.

LIST OF ABBREVIATIONS

AA	=	Arachidonic acid
CC	=	Corpus callosum
COX-1, COX-2	=	Cyclooxygenase 1 or 2
CNS	=	Central nervous system
CPZ	=	Cuprizone
CXCR2	=	Type 2 chemokine receptor
EAE	=	Experimental encephalomyelitis
eNOS	=	Endothelial nitric oxide synthase
IFN γ	=	Interferon gamma
iNOS	=	Inducible nitric oxide synthase
MBP	=	Myelin basic protein
MS	=	Multiple sclerosis
nNOS	=	Neuronal isoform
OLs	=	Oligodendrocytes
OPCs	=	Oligodendrocyte progenitor cells
PGI ₂	=	Prostacyclin
PGH ₂ , PGE ₂ , PGD ₂ , PGF ₂	=	Prostaglandin H ₂ , E ₂ , D ₂ or F ₂
PLA ₂	=	Phospholipase A ₂
ROS	=	reactive oxygen species
TMEV	=	Theiler's murine encephalomyelitis virus
TNF α	=	Tumor necrosis factor alpha

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

- [1] Friese, M. A.; Schattling, B.; Fugger, L., Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. *Nat Rev Neurol* **2014**, *10* (4), 225-38.
- [2] Friese, M. A., Widespread synaptic loss in multiple sclerosis. *Brain* **2016**, *139* (Pt 1), 2-4.
- [3] Jurgens, T.; Jafari, M.; Kreutzfeldt, M.; Bahn, E.; Bruck, W.; Kerschsteiner, M.; Merkler, D., Reconstruction of single cortical projection neurons reveals primary spine loss in multiple sclerosis. *Brain* **2016**, *139* (Pt 1), 39-46.
- [4] Deber, C. M.; Reynolds, S. J., Central nervous system myelin: structure, function, and pathology. *Clinical Biochemistry* **1991**, *24* (2), 113-134.
- [5] Patrikios, P.; Stadelmann, C.; Kutzelnigg, A.; Rauschka, H.; Schmidbauer, M.; Laursen, H.; Sorensen, P. S.; Bruck, W.; Lucchinetti, C.; Lassmann, H., Remyelination is extensive in a subset of multiple sclerosis patients. *Brain* **2006**, *129* (Pt 12), 3165-72.
- [6] Goldschmidt, T.; Antel, J.; Konig, F. B.; Bruck, W.; Kuhlmann, T., Remyelination capacity of the MS brain decreases with disease chronicity. *Neurology* **2009**, *72* (22), 1914-21.
- [7] Gudi, V.; Gingele, S.; Skripuletz, T.; Stangel, M., Glial response during cuprizone-induced de- and remyelination in the CNS: lessons learned. *Front Cell Neurosci* **2014**, *8*, 73.
- [8] Denic, A.; Johnson, A. J.; Bieber, A. J.; Warrington, A. E.; Rodriguez, M.; Pirko, I., The relevance of animal models in multiple sclerosis research. *Pathophysiology* **2011**, *18* (1), 21-29.
- [9] Cammer, W., The neurotoxicant, cuprizone, retards the differentiation of oligodendrocytes *in vitro*. *Journal of the Neurological Sciences* **1999**, *168* (2), 116-120.
- [10] Kalman, B.; Laitinen, K.; Komoly, S., The involvement of mitochondria in the pathogenesis of multiple sclerosis. *Journal of Neuroimmunology* **2007**, *188* (1-2), 1-12.
- [11] Zucconi, G. G.; Cipriani, S.; Scattoni, R.; Balgouranidou, I.; Hawkins, D. P.; Ragnarsdottir, K. V., Copper deficiency elicits glial and neuronal response typical of neurodegenerative disorders. *Neuropathol Appl Neurobiol* **2007**, *33* (2), 212-25.
- [12] van der Star, B. J.; Vogel, D. Y. S.; Kipp, M.; Puentes, F.; Baker, D.; Amor, S., *In Vitro* and *in vivo* models of multiple sclerosis. *CNS and Neurological Disorders - Drug Targets* **2012**, *11* (5), 570-588.
- [13] Clarner, T.; Janssen, K.; Nellessen, L.; Stangel, M.; Skripuletz, T.; Krauspe, B.; Hess, F. M.; Denecke, B.; Beutner, C.; Linnartz-Gerlach, B.; Neumann, H.; Vallieres, L.; Amor, S.; Ohl, K.; Tenbrock, K.; Beyer, C.; Kipp, M., CXCL10 triggers early microglial activation in the cuprizone model. *J Immunol* **2015**, *194* (7), 3400-13.
- [14] Blakemore, W. F., Observations on oligodendrocyte degeneration, the resolution of status spongiosus and remyelination in cuprizone intoxication in mice. *Journal of Neurocytology* **1972**, *1* (4), 413-426.
- [15] Komoly, S.; Hudson, L. D.; Webster, H. D.; Bondy, C. A., Insulin-like growth factor I gene expression is induced in astrocytes during experimental demyelination. *Proceedings of the National Academy of Sciences of the United States of America* **1992**, *89* (5), 1894-1898.
- [16] Pasquini, L. A.; Calatayud, C. A.; Bertone Una, A. L.; Millet, V.; Pasquini, J. M.; Soto, E. F., The neurotoxic effect of cuprizone on oligodendrocytes depends on the presence of pro-inflammatory cytokines secreted by microglia. *Neurochem Res* **2007**, *32* (2), 279-92.
- [17] Arnett, H. A.; Mason, J.; Marino, M.; Suzuki, K.; Matsushima, G. K.; Ting, J. P. Y., TNF[alpha] promotes proliferation of oligoden-

- drocyte progenitors and remyelination. *Nat Neurosci* **2001**, *4* (11), 1116-1122.
- [18] Kipp, M.; Clarner, T.; Dang, J.; Copray, S.; Beyer, C., The cuprizone animal model: new insights into an old story. *Acta Neuropathol* **2009**, *118* (6), 723-36.
- [19] Nilsson, G., A New Colour Reaction on Copper and Certain Carbonyl Compounds. *Acta Chemica Scandinavica* **1950**, *4* (1), 205-205.
- [20] Carlton, W. W., Response of mice to the chelating agents sodium diethyldithiocarbamate, alpha-benzoinoxime, and biscyclohexanone oxaldihydrazone. *Toxicol Appl Pharmacol* **1966**, *8* (3), 512-21.
- [21] Benetti, F.; Ventura, M.; Salmi, B.; Ceola, S.; Carbonera, D.; Mammi, S.; Zitolo, A.; D'Angelo, P.; Urso, E.; Maffia, M.; Salvato, B.; Spisni, E., Cuprizone neurotoxicity, copper deficiency and neurodegeneration. *Neurotoxicology* **2010**, *31* (5), 509-17.
- [22] Carlton, W. W., Studies on the induction of hydrocephalus and spongy degeneration by cuprizone feeding and attempts to antidote the toxicity. *Life Sci* **1967**, *6* (1), 11-9.
- [23] Ransom Stern, B.; Solioz, M.; Krewski, D.; Aggett, P.; Aw, T.-C.; Baker, S.; Crump, K.; Dourson, M.; Haber, L.; Hertzberg, R.; Keen, C.; Meek, B.; Rudenko, L.; Schoeny, R.; Slob, W.; Starr, T., Copper and human health: biochemistry, genetics, and strategies for modeling dose-response relationships. *Journal of Toxicology and Environmental Health* **2007**, *B* (10), 157-222.
- [24] In *Copper in Drinking Water*, Washington (DC), 2000.
- [25] Scheiber, I.; Dringen, R.; Mercer, J. F. B., Copper: Effects of Deficiency and Overload. In *Interrelations between Essential Metal Ions and Human Diseases*, Sigel, A.; Sigel, H.; Sigel, K. O. R., Eds. Springer Netherlands: Dordrecht, 2013; pp 359-387.
- [26] Linder, M. C.; Hazegh-Azam, M., Copper biochemistry and molecular biology. *Am J Clin Nutr* **1996**, *63* (5), 797S-811S.
- [27] Lindner, M.; Heine, S.; Haastert, K.; Garde, N.; Fokuhl, J.; Linsmeier, F.; Grothe, C.; Baumgartner, W.; Stangel, M., Sequential myelin protein expression during remyelination reveals fast and efficient repair after central nervous system demyelination. *Neuropathol Appl Neurobiol* **2008**, *34* (1), 105-14.
- [28] Torkildsen, O.; Brunborg, L. A.; Myhr, K. M.; Bo, L., The cuprizone model for demyelination. *Acta Neurol Scand Suppl* **2008**, *188*, 72-6.
- [29] Hesse, A.; Wagner, M.; Held, J.; Bruck, W.; Salinas-Riester, G.; Hao, Z.; Waisman, A.; Kuhlmann, T., In toxic demyelination oligodendroglial cell death occurs early and is FAS independent. *Neurobiol Dis* **2010**, *37* (2), 362-9.
- [30] Linares, D.; Taconis, M.; Mana, P.; Correcha, M.; Fordham, S.; Staykova, M.; Willenborg, D. O., Neuronal nitric oxide synthase plays a key role in CNS demyelination. *J Neurosci* **2006**, *26* (49), 12672-81.
- [31] Arnett, H. A.; Hellendall, R. P.; Matsushima, G. K.; Suzuki, K.; Laubach, V. E.; Sherman, P.; Ting, J. P., The protective role of nitric oxide in a neurotoxicant-induced demyelinating model. *J Immunol* **2002**, *168* (1), 427-33.
- [32] Matsushima, G. K.; Morell, P., The Neurotoxicant, Cuprizone, as a Model to Study Demyelination and Remyelination in the Central Nervous System. *Brain Pathology* **2001**, (11), 107-116.
- [33] Hiremath, M. M.; Saito, Y.; Knapp, G. W.; Ting, J. P. Y.; Suzuki, K.; Matsushima, G. K., Microglial/macrophage accumulation during cuprizone-induced demyelination in C57BL/6 mice. *Journal of Neuroimmunology* **1998**, *92* (1-2), 38-49.
- [34] Skripuletz, T.; Gudi, V.; Hackstette, D.; Stangel, M., De- and remyelination in the CNS white and grey matter induced by cuprizone: the old, the new, and the unexpected. *Histol Histopathol* **2011**, *26* (12), 1585-97.
- [35] Goldberg, J.; Daniel, M.; van Heuvel, Y.; Victor, M.; Beyer, C.; Clarner, T.; Kipp, M., Short-term cuprizone feeding induces selective amino acid deprivation with concomitant activation of an integrated stress response in oligodendrocytes. *Cell Mol Neurobiol* **2013**, *33* (8), 1087-98.
- [36] Doan, V.; Kleindienst, A. M.; McMahon, E. J.; Long, B. R.; Matsushima, G. K.; Taylor, L. C., Abbreviated exposure to cuprizone is sufficient to induce demyelination and oligodendrocyte loss. *J Neurosci Res* **2013**, *91* (3), 363-73.
- [37] Jurevics, H.; Largent, C.; Hostettler, J.; Sammond, D. W.; Matsushima, G. K.; Kleindienst, A.; Toews, A. D.; Morell, P., Alterations in metabolism and gene expression in brain regions during cuprizone-induced demyelination and remyelination. *J Neurochem* **2002**, *82* (1), 126-36.
- [38] Gudi, V.; Gingele, S.; Skripuletz, T.; Stangel, M., Glial response during cuprizone-induced de- and remyelination in the CNS: lessons learned. *Frontiers in Cellular Neuroscience* **2014**, *8* (73), 1-24.
- [39] Gudi, V.; Moharregh-Khiabani, D.; Skripuletz, T.; Koutsoudaki, P. N.; Kotsiari, A.; Skuljec, J.; Trebst, C.; Stangel, M., Regional differences between grey and white matter in cuprizone induced demyelination. *Brain Res* **2009**, *1283*, 127-38.
- [40] Goldberg, J.; Clarner, T.; Beyer, C.; Kipp, M., Anatomical Distribution of Cuprizone-Induced Lesions in C57BL/6 Mice. *J Mol Neurosci* **2015**, *57* (2), 166-75.
- [41] Silvestroff, L.; Bartucci, S.; Soto, E.; Gallo, V.; Pasquini, J.; Franco, P., Cuprizone-induced demyelination in CNP::GFP transgenic mice. *J Comp Neurol* **2010**, *518* (12), 2261-83.
- [42] Hübner, N. S.; Mechling, A. E.; Lee, H.-L.; Reiser, M.; Bienert, T.; Hennig, J.; von Elverfeldt, D.; Harsan, L.-A., The connectomes of brain demyelination: Functional and structural patterns in the cuprizone mouse model. *NeuroImage* **2017**, *146*, 1-18.
- [43] Pott, F.; Gingele, S.; Clarner, T.; Dang, J.; Baumgartner, W.; Beyer, C.; Kipp, M., Cuprizone effect on myelination, astrogliosis and microglia attraction in the mouse basal ganglia. *Brain Res* **2009**, *1305*, 137-49.
- [44] Yang, H.-J.; Wang, H.; Zhang, Y.; Xiao, L.; Clough, R. W.; Browning, R.; Li, X.-M.; Xu, H., Region-specific susceptibilities to cuprizone-induced lesions in the mouse forebrain: Implications for the pathophysiology of schizophrenia. *Brain Research* **2009**, *1270*, 121-130.
- [45] Blakemore, W. F., Demyelination of the superior cerebellar peduncle in the mouse induced by cuprizone. *Journal of the Neurological Sciences* **20** (1), 63-72.
- [46] Carlton, W. W., Response of mice to the chelating agents sodium diethyldithiocarbamate, alpha-benzoinoxime, and biscyclohexanone oxaldihydrazone. *Toxicology and Applied Pharmacology* **1966**, *8* (3), 512-521.
- [47] Groebe, A.; Clarner, T.; Baumgartner, W.; Dang, J.; Beyer, C.; Kipp, M., Cuprizone treatment induces distinct demyelination, astrogliosis, and microglia cell invasion or proliferation in the mouse cerebellum. *Cerebellum* **2009**, *8* (3), 163-74.
- [48] Acs, P.; Komoly, S., Selective ultrastructural vulnerability in the cuprizone-induced experimental demyelination. *Ideggyogy Sz* **2012**, *65* (7-8), 266-70.
- [49] Skripuletz, T.; Lindner, M.; Kotsiari, A.; Garde, N.; Fokuhl, J.; Linsmeier, F.; Trebst, C.; Stangel, M., Cortical demyelination is prominent in the murine cuprizone model and is strain-dependent. *Am J Pathol* **2008**, *172* (4), 1053-61.
- [50] Bai, C. B.; Sun, S.; Roholt, A.; Benson, E.; Edberg, D.; Medicetty, S.; Dutta, R.; Kidd, G.; Macklin, W. B.; Trapp, B., A mouse model for testing remyelinating therapies. *Experimental neurology* **2016**, *283*, 330-340.
- [51] Norkute, A.; Hieble, A.; Braun, A.; Johann, S.; Clarner, T.; Baumgartner, W.; Beyer, C.; Kipp, M., Cuprizone treatment induces demyelination and astrogliosis in the mouse hippocampus. *J Neurosci Res* **2009**, *87* (6), 1343-55.
- [52] Hoffmann, K.; Lindner, M.; Gröticke, I.; Stangel, M.; Löscher, W., Epileptic seizures and hippocampal damage after cuprizone-induced demyelination in C57BL/6 mice. *Experimental neurology* **2008**, *210* (2), 308-321.
- [53] Kipp, M.; Nyamoya, S.; Hochstrasser, T.; Amor, S., Multiple sclerosis animal models: a clinical and histopathological perspective. *Brain Pathology* **2017**.
- [54] Makinodan, M.; Yamauchi, T.; Tatsumi, K.; Okuda, H.; Takeda, T.; Kiuchi, K.; Sadamatsu, M.; Wanaka, A.; Kishimoto, T., Demyelination in the juvenile period, but not in adulthood, leads to long-lasting cognitive impairment and deficient social interaction in mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **2009**, *33* (6), 978-985.
- [55] Wang, H.; Li, C.; Mei, F.; Liu, Z.; Shen, H. Y.; Xiao, L., Cuprizone-induced demyelination in mice: age-related vulnerability and exploratory behavior deficit. *Neurosci Bull* **2013**, *29* (2), 251-9.
- [56] Xuan, Y.; Yan, G.; Peng, H.; Wuc, R.; Xu, H., Concurrent changes in 1H MRS metabolites and antioxidant enzymes in the brain of C57BL/6 mouse short-termly exposed to cuprizone: Possible im-

- plications for schizophrenia. *Neurochemistry International* **2014**, (69), 20–27.
- [57] Armstrong, R. C.; Le, T. Q.; Flint, N. C.; Vana, A. C.; Zhou, Y. X., Endogenous cell repair of chronic demyelination. *J Neuropathol Exp Neurol* **2006**, *65* (3), 245–56.
- [58] Lindner, M.; Fokuhl, J.; Linsmeier, F.; Trebst, C.; Stangel, M., Chronic toxic demyelination in the central nervous system leads to axonal damage despite remyelination. *Neurosci Lett* **2009**, *453* (2), 120–5.
- [59] Franco-Pons, N.; Torrente, M.; Colomina, M. T.; Vilella, E., Behavioral deficits in the cuprizone-induced murine model of demyelination/remyelination. *Toxicol Lett* **2007**, *169* (3), 205–13.
- [60] Doucette, J. R.; Jiao, R.; Nazarali, A. J., Age-related and cuprizone-induced changes in myelin and transcription factor gene expression and in oligodendrocyte cell densities in the rostral corpus callosum of mice. *Cell Mol Neurobiol* **2010**, *30* (4), 607–29.
- [61] Ludwin, S. K., Central nervous system demyelination and remyelination in the mouse: an ultra structural study of cuprizone toxicity. *Laboratory Investigation* **1978**, *36* (6), 597–612.
- [62] Takahashi, N.; Sakurai, T.; Davis, K. L.; Buxbaum, J. D. L., Linking oligodendrocyte and myelin dysfunction to neurocircuitry. *Progress in Neurobiology* **2011**, (93), 13–24.
- [63] Xu, H.; Li, X. M., White matter abnormalities and animal models examining a putative role of altered white matter in schizophrenia. *Schizophrenia Research and Treatment* **2011**, (2011), 826976.
- [64] Zhang, H.; Zhang, Y.; Xu, H.; Wang, L.; Zhao, J.; Wang, J.; Zhang, Z.; Tan, Q.; Kong, J.; Huang, Q.; Li, X. M., Locomotor activity and anxiety status, but not spatial working memory, are affected in mice after brief exposure to cuprizone. *Neurosci Bull* **2013**, *29* (5), 633–41.
- [65] Tezuka, T.; Tamura, M.; Kondo, M. A.; Sakaue, M.; Okada, K.; Takemoto, K.; Fukunari, A.; Miwa, K.; Ohzeki, H.; Kano, S.-i.; Yasumatsu, H.; Sawa, A.; Kajii, Y., Cuprizone short-term exposure: astrocytic IL-6 activation and behavioral changes relevant to psychosis. *Neurobiology of disease* **2013**, *59*, 63–68.
- [66] Xu, H.; Yang, H. J.; Zhang, Y.; Clough, R.; Browning, R.; Li, X. M., Behavioral and neurobiological changes in C57BL/6 mice exposed to cuprizone. *Behav Neurosci* **2009**, *123* (2), 418–29.
- [67] Ludwin, S. K., Central nervous system demyelination and remyelination in the mouse: an ultrastructural study of cuprizone toxicity. *Lab Invest* **1978**, *39* (6), 597–612.
- [68] Taylor, L. C.; Gilmore, W.; Ting, J. P.; Matsushima, G. K., Cuprizone induces similar demyelination in male and female C57BL/6 mice and results in disruption of the estrous cycle. *J Neurosci Res* **2010**, *88* (2), 391–402.
- [69] Taylor, L. C.; Gilmore, W.; Matsushima, G. K., SJL mice exposed to cuprizone intoxication reveal strain and gender pattern differences in demyelination. *Brain pathology (Zurich, Switzerland)* **2009**, *19* (3), 467–79.
- [70] Narayanan, S. P.; Flores, A. I.; Wang, F.; Macklin, W. B., Akt signals through the mammalian target of rapamycin pathway to regulate CNS myelination.
- [71] Sachs, H. H.; Bercury, K. K.; Popescu, D. C.; Narayanan, S. P.; Macklin, W. B., A new model of cuprizone-mediated demyelination/remyelination. *ASN Neuro* **2014**, *6* (5).
- [72] Polman, C. H.; Reingold, S. C.; Banwell, B.; Clanet, M.; Cohen, J. A.; Filippi, M.; Fujihara, K.; Havrdova, E.; Hutchinson, M.; Kappos, L.; Lublin, F. D.; Montalban, X.; O'Connor, P.; Sandberg-Wollheim, M.; Thompson, A. J.; Waubant, E.; Weinshenker, B.; Wolinsky, J. S., Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann neurol* **2011**, *69* (2), 292–302.
- [73] Robinson, A. P.; Harp, C. T.; Noronha, A.; Miller, S. D., The experimental autoimmune encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment. *Handbook of clinical neurology* **2014**, *122*, 173–189.
- [74] Merkler, D.; Schmeltz, B.; Czéh, B.; Fuchs, E.; Stadelmann, C.; Brück, W., Myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis in the common marmoset reflects the immunopathology of pattern II multiple sclerosis lesions. *Multiple Sclerosis Journal* **2006**, *12* (4), 369–374.
- [75] Martinez, N. E.; Sato, F.; Omura, S.; Minagar, A.; Alexander, J. S.; Tsunoda, I., Immunopathological Patterns from EAE and Theiler's Virus Infection: Is Multiple Sclerosis a Homogenous 1-stage or Heterogenous 2-stage Disease? *Pathophysiology: the official journal of the International Society for Pathophysiology / ISP* **2013**, *20* (1), 71–84.
- [76] Oleszak, E. L.; Chang, J. R.; Friedman, H.; Katsetos, C. D.; Platsoucas, C. D., Theiler's Virus Infection: a Model for Multiple Sclerosis. *Clinical Microbiology Reviews* **2004**, *17* (1), 174–207.
- [77] de Paula Faria, D.; de Vries, E. F.; Sijbesma, J. W.; Buchpiguel, C. A.; Dierckx, R. A.; Copray, S. C., PET imaging of glucose metabolism, neuroinflammation and demyelination in the lysolecithin rat model for multiple sclerosis. *Mult Scler* **2014**, *20* (11), 1443–52.
- [78] Rawji, K. S.; Yong, V. W., The benefits and detriments of macrophages/microglia in models of multiple sclerosis. *Clin Dev Immunol* **2013**, *2013*, 948976.
- [79] Veto, S.; Acs, P.; Bauer, J.; Lassmann, H.; Berente, Z.; Setalo, G., Jr.; Borgulya, G.; Sumegi, B.; Komoly, S.; Gallyas, F., Jr.; Illes, Z., Inhibiting poly(ADP-ribose) polymerase: a potential therapy against oligodendrocyte death. *Brain* **2010**, *133* (Pt 3), 822–34.
- [80] Metz, I.; Weigand, S. D.; Popescu, B. F. G.; Frischer, J. M.; Parisi, J. E.; Guo, Y.; Lassmann, H.; Brück, W.; Lucchinetti, C. F., Pathologic heterogeneity persists in early active multiple sclerosis lesions. *Annals of Neurology* **2014**, *75* (5), 728–738.
- [81] Morell, P.; Barrett, C.; Mason, J.; Toews, A.; Hostettler, J.; Knapp, G.; Matsushima, G., Gene expression in brain during cuprizone-induced demyelination and remyelination. *Molecular and Cellular Neuroscience* **1998**, *12* (4), 220–227.
- [82] Ransohoff, R. M., Animal models of multiple sclerosis: the good, the bad and the bottom line. *Nat Neurosci* **2012**, *15* (8), 1074–1077.
- [83] Praet, J.; Guglielmetti, C.; Berneman, Z.; Van der Linden, A.; Ponsaerts, P., Cellular and molecular neuropathology of the cuprizone mouse model: clinical relevance for multiple sclerosis. *Neurosci Biobehav Rev* **2014**, *47*, 485–505.
- [84] Carlton, W. W., Studies on the induction of hydrocephalus and spongy degeneration by cuprizone feeding and attempts to antidote the toxicity. *Life Sciences* **1967**, *6*, 11–19.
- [85] Benardais, K.; Kotsiari, A.; Skuljec, J.; Koutsoudaki, P. N.; Gudi, V.; Singh, V.; Vulinovic, F.; Skripuletz, T.; Stangel, M., Cuprizone [bis(cyclohexylidenedehydrazide)] is selectively toxic for mature oligodendrocytes. *Neurotox Res* **2013**, *24* (2), 244–50.
- [86] Hiremath, M. M.; Saito, Y.; Knapp, G. W.; Ting, J. P.; Suzuki, K.; Matsushima, G. K., Microglial/macrophage accumulation during cuprizone-induced demyelination in C57BL/6 mice. *Journal of Neuroimmunology* **1998**, *92*, 38–49.
- [87] Kesterson, J. W.; Carlton, W. W., Monoamine oxidase inhibition and the activity of other oxidative enzymes in the brains of mice fed cuprizone. *Toxicology and Applied Pharmacology* **1971**, *20* (3), 386–395.
- [88] Venturini, G., Enzymic activities and sodium, potassium and copper concentrations in mouse brain and liver after cuprizone treatment *in vivo*. *Journal of Neurochemistry* **1973**, *21* (5), 1147–1151.
- [89] Suzuki, K., Giant hepatic mitochondria: production in mice fed with cuprizone. *Science* **1969**, *163* (3862), 81–2.
- [90] Wakabayashi, T., Megamitochondria formation - physiology and pathology. *J Cell Mol Med* **2002**, *6* (4), 497–538.
- [91] Veto, S.; Acs, P.; Bauer, J.; Lassmann, H.; Berente, Z.; Setalo, J. G.; Borgulya, G.; Sumegi, B.; Komoly, S.; Gallyas, J. F.; Illes, Z., Inhibiting poly(ADP-ribose) polymerase: a potential therapy against oligodendrocyte death. *Brain* **2010**, *133* (3), 822–834.
- [92] Baumann, N.; Pham-Dinh, D., Biology of oligodendrocyte and myelin in the mammalian central nervous system. *Physiol Rev* **2001**, *81* (2), 871–927.
- [93] Palumbo, S.; Toscano, C. D.; Parente, L.; Weigert, R.; Bosetti, F., Time-dependent changes in the brain arachidonic acid cascade during cuprizone-induced demyelination and remyelination. *Prostaglandins Leukot Essent Fatty Acids* **2011**, *85* (1), 29–35.
- [94] Carey, E. M.; Freeman, N. M., Biochemical changes in cuprizone-induced spongiform encephalopathy. *Neurochemical Research* **1983**, *8* (8), 1029–1044.
- [95] Neu, I.; Woelk, H., Investigations of the lipid metabolism of the white matter in multiple sclerosis Changes in glycerophosphatides and lipid-splitting enzymes. *Neurochemical Research* **1982**, *7* (6), 727–735.
- [96] Balboa, M. a. A.; Varela-Nieto, I.; Killermann Lucas, K.; Dennis, E. A., Expression and function of phospholipase A2 in brain. *FEBS Letters* **2002**, *531* (1), 12–17.

- [97] Hemm, R. D.; Carlton, W. W.; Welser, J. R., Ultrastructural changes of cuprizone encephalopathy in mice. *Toxicology and Applied Pharmacology* **1971**, *18* (4), 869-882.
- [98] Porter, A. G.; Janicke, R. U., Emerging roles of caspase-3 in apoptosis. *Cell Death Differ* **1999**, *6* (2), 99-104.
- [99] Tezuka, T.; Tamura, M.; Kondo, M. A.; Sakaue, M.; Okada, K.; Takemoto, K.; Fukunari, A.; Miwa, K.; Ohzeki, H.; Kano, S.; Yasumatsu, H.; Sawa, A.; Kajii, Y., Cuprizone short-term exposure: astrocytic IL-6 activation and behavioral changes relevant to psychosis. *Neurobiol Dis* **2013**, *59*, 63-8.
- [100] Gao, X.; Gillig, T. A.; Ye, P.; D'Ercole, A. J.; Matsushima, G. K.; Popko, B., Interferon-gamma protects against cuprizone-induced demyelination. *Mol Cell Neurosci* **2000**, *16* (4), 338-49.
- [101] Krakowski, M.; Owens, T., Interferon-gamma confers resistance to experimental allergic encephalomyelitis. *Eur J Immunol* **1996**, *26* (7), 1641-6.
- [102] Willenborg, D. O.; Fordham, S.; Bernard, C. C.; Cowden, W. B.; Ramshaw, I. A., IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *J Immunol* **1996**, *157* (8), 3223-7.
- [103] Trebst, C.; Heine, S.; Lienenklaus, S.; Lindner, M.; Baumgartner, W.; Weiss, S.; Stangel, M., Lack of interferon-beta leads to accelerated remyelination in a toxic model of central nervous system demyelination. *Acta Neuropathol* **2007**, *114* (6), 587-96.
- [104] Zendedel, A.; Beyer, C.; Kipp, M., Cuprizone-induced demyelination as a tool to study remyelination and axonal protection. *J Mol Neurosci* **2013**, *51* (2), 567-72.
- [105] Linder, M. C.; Hazegh-Azam, M., Copper biochemistry and molecular biology. *The American Society for Clinical Nutrition* **1996**, *63* (5), 797S-811S.
- [106] Jurevics, H.; Largent, C.; Hostettler, J.; Sammond, D. W.; K. Matsushima, G.; Amber, K.; Toews, A. D.; Morell, P., Alterations in metabolism and gene expression in brain regions during cuprizone-induced demyelination and remyelination. *Journal of Neurochemistry* **2002**, *82* (1), 126-136.
- [107] Blakemore, W. F., Observations on oligodendrocyte degeneration, the resolution of status spongiosus and remyelination in cuprizone intoxication in mice. *Journal of neurocytology* **1972**, *1* (4), 413-26.
- [108] Blakemore, W. F., Demyelination of the superior cerebellar peduncle in the mouse induced by cuprizone. *Journal of the neurological sciences* **1973**, *20* (1), 63-72.
- [109] Bai, C. B.; Sun, S.; Roholt, A.; Benson, E.; Edberg, D.; Medicetty, S.; Dutta, R.; Kidd, G.; Macklin, W. B.; Trapp, B., A mouse model for testing remyelinating therapies. *Exp Neurol* **2016**, *283* (Pt A), 330-40.
- [110] Yang, H. J.; Wang, H.; Zhang, Y.; Xiao, L.; Clough, R. W.; Browning, R.; Li, X. M.; Xu, H., Region-specific susceptibilities to cuprizone-induced lesions in the mouse forebrain: Implications for the pathophysiology of schizophrenia. *Brain Res* **2009**, *1270*, 121-30.
- [111] Hubner, N. S.; Mechling, A. E.; Lee, H. L.; Reiser, M.; Bienert, T.; Hennig, J.; von Elverfeldt, D.; Harsan, L. A., The connectomics of brain demyelination: Functional and structural patterns in the cuprizone mouse model. *NeuroImage* **2017**, *146*, 1-18.
- [112] Hoffmann, K.; Lindner, M.; Groticke, I.; Stangel, M.; Loscher, W., Epileptic seizures and hippocampal damage after cuprizone-induced demyelination in C57BL/6 mice. *Exp Neurol* **2008**, *210* (2), 308-21.
- [113] Suzuki, K.; Kikkawa, Y., Status spongiosus of CNS and hepatic changes induced by cuprizone (biscyclohexanone oxalyldihydrazone). *Am J Pathol* **1969**, *54* (2), 307-25.
- [114] Elsworth, S.; Howell, J. M., Variation in the response of mice to cuprizone. *Res Vet Sci* **1973**, *14* (3), 385-7.
- [115] Bakker, A. D.; Ludwin, S. K., Blood-brain barrier permeability during Cuprizone-induced demyelination: Implications for the pathogenesis of immune-mediated demyelinating diseases. *Journal of the Neurological Sciences* **1987**, *78* (2), 125-137.
- [116] Chen, Z.; Chen, J. T.; Johnson, M.; Gossman, Z. C.; Hendrickson, M.; Sakaie, K.; Martinez-Rubio, C.; Gale, J. T.; Trapp, B. D., Cuprizone does not induce CNS demyelination in nonhuman primates. *Ann Clin Transl Neurol* **2015**, *2* (2), 208-13.
- [117] Silvestroff, L.; Bartucci, S.; Pasquini, J.; Franco, P., Cuprizone-induced demyelination in the rat cerebral cortex and thyroid hormone effects on cortical remyelination. *Exp Neurol* **2012**, *235* (1), 357-67.
- [118] Love, S., Cuprizone neurotoxicity in the rat: Morphologic observations. *J Neurol Sci* **1988**, *84* (2-3), 223-237.
- [119] Carlton, W. W., Spongiform encephalopathy induced in rats and guinea pigs by cuprizone. *Exp Mol Pathol* **1969**, *10* (3), 274-87.
- [120] Valeiras, B.; Rosato Siri, M. V.; Codagnone, M.; Reinés, A.; Pasquini, J. M., Gender influence on schizophrenia-relevant abnormalities in a cuprizone demyelination model. *Glia* **2014**, *62* (10), 1629-1644.
- [121] HALL, S. M., The effect of injections of lysophosphatidyl choline into white matter of the adult mouse spinal cord. *Journal of cell science* **1972**, *10* (2), 535-546.
- [122] Jeffery, N.; Blakemore, W., Remyelination of mouse spinal cord axons demyelinated by local injection of lysolecithin. *Journal of neurocytology* **1995**, *24* (10), 775-781.
- [123] Cerina, M.; Narayanan, V.; Göbel, K.; Bittner, S.; Ruck, T.; Meuth, P.; Herrmann, A. M.; Stangel, M.; Gudi, V.; Skripuletz, T.; Daldrup, T.; Wiendl, H.; Seidenbecher, T.; Ehling, P.; Kleinschnitz, C.; Pape, H.-C.; Budde, T.; Meuth, S. G., The quality of cortical network function recovery depends on localization and degree of axonal demyelination. *Brain, Behavior, and Immunity* **2017**, *59*, 103-117.
- [124] J van der Star, B.; YS Vogel, D.; Kipp, M.; Puentes, F.; Baker, D.; Amor, S., *In vitro* and *in vivo* models of multiple sclerosis. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)* **2012**, *11* (5), 570-588.
- [125] Shindler, K. S.; Ventura, E.; Dutt, M.; Rostami, A., Inflammatory demyelination induces axonal injury and retinal ganglion cell apoptosis in experimental optic neuritis. *Experimental eye research* **2008**, *87* (3), 208-213.
- [126] Ulrich, R.; Baumgärtner, W.; Gerhauser, I.; Seeliger, F.; Haist, V.; Deschl, U.; Alldinger, S., MMP-12, MMP-3, and TIMP-1 are markedly upregulated in chronic demyelinating theiler murine encephalomyelitis. *Journal of Neuropathology & Experimental Neurology* **2006**, *65* (8), 783-793.
- [127] Gudi, V.; Gai, L.; Herder, V.; Tejedor, L. S.; Kipp, M.; Amor, S.; Suhs, K. W.; Hansmann, F.; Beineke, A.; Baumgartner, W.; Stangel, M.; Skripuletz, T., Synaptophysin Is a Reliable Marker for Axonal Damage. *J Neuropathol Exp Neurol* **2017**.