Low-field magnetic stimulation improved cuprizone-induced depression-like symptoms and demyelination in female mice

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Abstract. Depression is a common and disabling comorbidity of multiple sclerosis (MS), with currently no clear guidelines for treatment. Low-field magnetic stimulation (LFMS), a novel non-invasive neuromodulation intervention, has been previously demonstrated to rapidly alleviate mood disorders. The aim of the present study was to investigate the effects of LFMS on depression-like behaviors and demyelination in a well-established mouse model of MS. C57BL/6 female mice were fed a 0.2% cuprizone (CPZ) diet for 3 or 6 weeks to induce acute demyelination. During this time, the mice were treated with either sham or LFMS for 20 min/day, 5 days/week. After 3 or 6 weeks of treatment, behavior was assessed with the open field task, Y-maze and the forced swim test. The prefrontal cortex and hippocampus were then collected to perform immunohistochemistry and western blot analysis to verify myelination status. The CPZ diet did not cause significant locomotor deficits; however, working memory, measured using the Y maze, depression-like behavior and adaptive learning, assayed using the forced swim test, were significantly impaired in these animals. LFMS treatment demonstrated a significant antidepressant-like effect and markedly attenuated the CPZ-induced demyelination in the prefrontal cortex after 3- and 6-weeks of treatment, as observed by changes in myelin basic protein immunostaining and western blot analysis. Therefore, the results of the present study indicated that LFMS may be a promising therapy for demyelinating diseases due to the improvement of depressive symptoms via regulation of myelination in cortical areas.

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Abbreviations: MS, multiple sclerosis; CNS, central nervous system; CI, cognitive impairment; CPZ, cuprizone; LFMS, low-field magnetic stimulation; CTL, control sham treatment; PFC, prefrontal cortex; HPC, hippocampus; MBP, myelin basic protein; OFT, open field test; FST, forced swim test; RT, room temperature

Key words: LFMS, depression, multiple sclerosis, remyelination

Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating and neurodegenerative disease of the central nervous system (CNS) (1,2). It is estimated that a total of 2.8 million people live with MS worldwide (35.9 cases per 100,000 globally); representing a significant socioeconomic impact (3). MS is the main cause of disability in young adults (20-40 years old) and is one of the leading causes of neurological disability in this age group (4). Depression is a common and significant comorbidity in MS (5), whereby ~50% of patients with MS will experience major depression and disease-related psychosocial challenges (6). Depression and the associated symptoms, including fatigue and anxiety, are strong determinants of a patients quality-of-life with MS (6), with depression in patients increasing the risk of suicidal ideation and death by suicide in this population (7,8). Treating depression in patients with MS is a challenge and there is therefore an urgent need for effective, safe and better-tolerated therapies for depression in patients with MS (9).

Moreover, another disabling consequence of MS is cognitive impairment (CI). This can affect 40-70% of patients at any time throughout disease progression (10,11). Attention, working memory and executive function are commonly affected in patients with MS (12,13). Disease-modifying therapies, memory-enhancing agents, physical exercise and cognitive restraint have demonstrated limited and inconsistent benefits (11,14). As a result, efficient treatments for depression and CI in patients with MS are urgently needed.

Rodent models to study MS are generally divided into autoimmune, viral-induced and toxin-induced models (15). The cuprizone (CPZ) model has been widely used to study demyelination and remyelination in the context of MS (16), as it is capable of recapitulating the demyelination-induced reorganization of brain circuitry observed in patients with

MS (16,17). Previous CPZ studies have demonstrated memory impairment and depression like-symptoms following a CPZ diet of 5-6 weeks (18-20). Hence, strategies that promote intrinsic repair of myelin may help restore brain function and consequently alleviate depression and improve cognition in animal models, and ultimately, patients with MS.

In the present study the effects of a novel technology that delivers low-intensity magnetic stimulation to multiple cortical areas (21), called low-field magnetic stimulation (LFMS), were investigated. Over 25 years ago, a population of individuals with bipolar disorder, reported mood improvements following a magnetic resonance spectroscopic imaging procedure (22). Since then, numerous studies have sought to investigate the ability of magnetic stimulation on neuromodulation (23-26). LFMS is of interest as a non-invasive neuromodulation therapy, which has been shown to rapidly improve mood in patients diagnosed with depression (27). In our previous study, we demonstrated that 40 Hz LFMS restored cognitive and motor functions in an animal model of traumatic brain injury (28) and enhanced the differentiation of oligodendrocyte progenitor cells *in vitro* (29). More recently, LFMS stimulation has been reported to promote myelin repair in the prefrontal cortex (PFC) and improves cognition and depression-like symptoms in a chronic CPZ mouse model (30). Together, these findings support the hypothesis that LFMS will improve depressive symptoms and CI, while restoring demyelination in an acute CPZ-induced mouse model of MS.

In the present study, we aimed to investigate locomotor activity, working memory, depression-like behavior and adaptability following a 3- or 6-week CPZ diet with concurrent sham or LFMS treatment. Following the behavioral analysis, the levels of demyelination within the PFC and hippocampus (HPC) were examined through immunostaining and immunoblotting for myelin-basic protein (MBP).

Materials and methods

Animals. Female C57BL/6 mice (age, 7 weeks; weight, 18-20 g) were purchased from Charles River Laboratories, Inc. and were acclimatized for one week in the vivarium (12 h light-dark cycles; 22±0.5˚C at 60% humidity; *ad libitum* access to food and water). As MS is predominantly diagnosed in women, we chose to complete our experiments in female mice (31,32). Furthermore, a previous study examining sex differences in a CPZ model of C57BL/6 mice demonstrated no differences in terms of demyelination (33). All animal procedures were performed in accordance with the guidelines of the Canadian Council on Animal Care (34,35) and approved by the University of Saskatchewan's Animal Research Ethics Board in 2016 (Saskatoon, Canada; approval no. 20160103).

Experimental design and CPZ treatment. After the acclimatization period, mice were randomly divided into four groups in a 2x2 experimental design: Normal diet healthy mice with either sham treatment (CTL) or LFMS treatment (LFMS) and CPZ-treated mice received either the sham treatment (CPZ) or LFMS treatment (CPZ + LFMS). CPZ was purchased from Sigma-Aldrich (Merck KGaA). The CPZ groups received rodent chow supplemented with 0.2% CPZ for 3 or 6 weeks to induce acute demyelination. The effect of LFMS treatment was studied at two time points: i) After 3 weeks CPZ diet, marking the peak inflammatory response within the brain; and ii) at the end of 6 weeks, when acute demyelination is fully established (36). At the end of week 3, half of the mice from each group underwent behavioral tests followed by euthanasia, the remaining animals were treated until the end of week 6, at which point they underwent behavioral analysis and were euthanized (the experimental design is presented in Fig. 1A). For immunohistochemistry, a deep plane of surgical anesthesia was induced at 5% isoflurane and the mice were maintained in this state with 2% isoflurane. Deep anesthesia was confirmed by loss of pedal reflexes prior to the perfusion of 1X PBS followed by 4% paraformaldehyde via transcardiac injection through the left ventricle of the heart. The animals were maintained in a deep surgical plane of anesthesia until death was confirmed by cessation of breathing, no palpable heartbeat and pale/blue/grey colored mucous membranes. For protein extraction and western blotting, a deep plane of surgical anesthesia was reached as described prior to decapitation, tissue dissection and collection of the specified brain regions.

LFMS treatment. The LFMS device is composed of two 360 mm-diameter coils and a control center that generates intermittent gamma stimulation waves (Beijing Antis Biotech Co., Ltd.). The LFMS parameter settings were based on our previous *in vivo* and *in vitro* studies (28-30,37). Briefly, the magnetic field alternated every 2 min between approximate and linear gradients. Cycles consisting of 2 sec on and 8 sec off were applied for 20 min. Every 2-sec output was composed of 80 trains of stimulation, producing a 40-Hz rhythm. Each train had 6 pulses (6 msec) at 1,000 Hz frequency and 19 msec intervals. The maximal magnetic flux density was less than 2 mT and the peaked induced electric field was less than 0.5 V/m.

Following the removal of any metal components, mice in their home cages were placed on the LFMS device and received 20-min of LFMS treatment daily, 5 days a week (Monday to Friday) for 3 weeks and up to 6 weeks. Animals in the sham group underwent the same treatment routine without LFMS stimulation.

Behavioral tests

Open Field Test (OFT). The OFT is frequently used to assess a rodent's locomotor activity and anxiety levels in an open space (38). Briefly, mice were placed in the center of a white PVC plastic box (50x50x38 cm; built in house) and allowed to freely explore the apparatus for 5 min. The surface of the box was divided into 25 equal squares. In the middle, nine squares were appointed as the central zone and the remaining squares adjacent to the wall were designated the peripheral zone using ANY-maze software (version 6.35; Stoelting Co.). The following parameters were collected for each mouse during the test: the time spent in the central and peripheral zones (sec), the total distance travelled (m), the mean freezing score and the mean speed velocity (mm/sec). The OFT data were quantified using ANY-maze tracking system software.

Y-maze spontaneous alternation test. The Y-Maze is widely used to evaluate spatial working memory (39) and has been

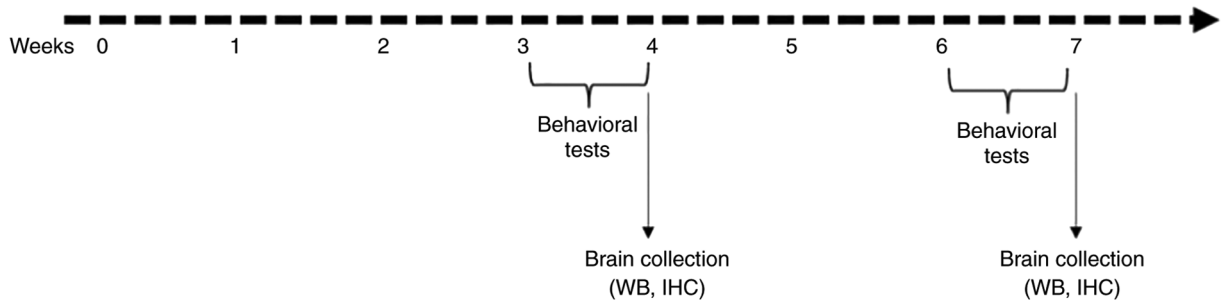

Figure 1. Schematic representation of the experimental procedures. Groups of mice were fed and treated as follows: Control group were fed with a normal diet (0% CPZ) + sham treatment; LFMS mice group were fed with a normal diet (0% CPZ) + LFMS treatment; CPZ mice were fed with a CPZ diet (0.2% cuprizone) + sham treatment; and CPZ + LFMS mice group were fed with cuprizone diet (0.2% cuprizone) + LFMS treatment. Behavioral tests were performed after 3 and 6 weeks of treatment (week 4 and 7, respectively) follow by euthanasia and brain collection. CPZ, cuprizone; LFMS, low-field magnetic stimulation; WB, western blotting; IHC, immunohistochemistry.

previously used to detect memory deficits in CPZ models in numerous studies (18,30,40,41). Mice exhibiting normal behavior remember the arm they have already explored and will enter one of the other arms of the maze (19). The Y maze apparatus was built in house and previously described (19,30). Briefly, each mouse was placed at the end of arm A and allowed to freely explore all three arms for 5 min. The number and sequence of arms entered was evaluated and the results were calculated as the percentage of the spontaneous alternations (%)=(number of alternations)/(total number of arm entries-2) x100 (19). If a mouse re-entered an arm immediately after exiting it but before entering another arm, it was not counted as a separate entry and was not included in the final analysis. If an animal entered <8 arms throughout the duration of the test, the animal was excluded from statistical analysis to avoid the potential effect that low numbers of entries may have on the spontaneous alternation score (42). Distance travelled (m) was measured during the test and used as a measure of mobility. The Y-maze data were quantified using ANY-maze tracking system software.

Forced Swim Test (FST). The FST is one of the most commonly used animal models for assessing antidepression-like behavior (43) and has been used to test the efficacy of existing and novel antidepressant drugs (44). The FST test is based on the assumption that when a rodent is placed in a container filled with water it will first attempt to escape, but will eventually stop or become immobile, reflecting a measure of 'behavioral despair' (45). While there has been controversy regarding the use of the forced swim test in mice (46), numerous studies have utilized this behavior assay in mice and have concluded that this test can be used with good reliability (47,48). Behavioral despair analysis is usually measured after one FST. The FST can also be used to measure adaptive learned behavioral responses, behaviors that promote survival and coping skill abilities (49,50). Repeated exposure to the FST can progressively increase the passive coping strategies evidenced by floating behavior (immobility time) over time (49,50). The shift from active (climbing and swimming) to passive (floating) coping strategies is a normal response in rodents repeatedly exposed to an inescapable water environment, where animals deficient in their adaptive response will continue to struggle (50).

On the third day of the week following the 3-week treatment, mice were individually placed in a plexiglass cylinder (10 cm internal diameter, 20 cm high) filled with water to a depth of 10 cm (25-26˚C). Each mouse was allowed to swim for 6 min; however, total immobility time was only recorded for the last 5 min of the session to analyze depression-like behavioral despair. Mobility time was measured using a stopwatch by researchers blinded to experimental conditions. The mobile time was then subtracted from the total test time of 300 seconds to determine the immobility time. To analyze adaptive learned behavioral responses and long-term memory coping skills, mice were tested 24 h after the initial FST. A third and fourth test were run 24 h apart following the completion of the 6-week treatment.

Immunohistochemistry. Perfused brains were cut into 30 μm sections coronally using the Leica Vibrating Microtome (model VT1200; Leica Microsystems, Inc.). Floating sections were quenched with 0.3% hydrogen peroxide in 0.01 M PBS at room temperature (RT) for 30 min to remove endogenous peroxidase activity. The sections were subsequently incubated in 0.01 M PBS with 10% goat serum (Abcam) blocking solution for 1 h at RT and then incubated overnight with rabbit anti-myelin basic protein (MBP; 1:250; cat. no. 78896; Cell Signaling Technology, Inc.) primary antibody diluted in the blocking solution. After washing in 0.01 M PBS, the sections were incubated with goat anti-rabbit IgG biotin-conjugated secondary antibody (1:1,000; cat. no. BA-1000-1.5; Vector Laboratories, Inc.) for 1 h at RT. The Avidin-Biotin Complex Kit (cat. no. PK-6100; Vector Laboratories, Inc.) was used according to manufacturer's instructions and visualized by incubating for 1-2 min at room temperature in DAB chromogen (Sigma-Aldrich; Merck KGaA), monitoring for color development.

Image analysis. All images were obtained using an Aperio Scanscope CS Digital Pathology Scanner (Leica Microsystems Inc.). The integrated optical density of MBP-positive staining was analyzed using ImageJ software (version 1.51; National Institutes of Health), which was calibrated using an optical density step tablet (Stouffer Industries). Uniform areas within the images were selected and the integrated density of the image was calculated and compared between groups.

A minimum of 20 coronal sections were examined, with a minimum of 3 animals per group.

Western blotting. After the removal of the brain, one hemisphere from randomly selected mice in each group was dissected into the PFC and HPC using a brain matrix. Samples were then stored at -80˚C until further use. Frozen brain samples were homogenized and total protein was extracted using a Tris-EDTA lysis buffer (1% Triton X-100, 10% glycerol, 20 mM Tris, pH 7.5, 1 mM EDTA) with a freshly added protease inhibitor cocktail (Sigma-Aldrich; Merck KGaA). Protein quantification was completed using the Bio-Rad Protein Assay (cat. no. 500-0006; Bio-Rad Laboratories, Inc.) as per manufacturer's instructions. Equal quantities of protein (40 μg protein/well) were separated on a 10% SDS-PAGE gel and transferred onto a 0.45 μm nitrocellulose membrane (Bio-Rad Laboratories, Inc.) prior to blocking in 5% milk in TBS for 1 h at room temperature. β-actin was used as the internal protein loading control. Membranes were incubated overnight at 4˚C with primary antibodies against β-actin (1:1,000; cat. no. A3854; Sigma-Aldrich; Merck KGaA) and MBP (1:1,000; cat. no. 78896; Cell Signaling Technology, Inc.) diluted in 10% BSA in TBS + 0.1% Tween-20 (TBST; Sigma-Aldrich, Merck KGaA). The membranes were washed in TBST, prior to incubation for 1 h at room temperature with the appropriate HRP-conjugated horse anti-mouse IgG (1:10,000; cat. no. 7076; Cell Signaling Technology, Inc.) and goat anti-rabbit IgG (1:10,000; cat. no. 7074, Cell Signaling Technology, Inc.) secondary antibodies. Protein bands were visualized using an ECL detection kit (Amersham; Cytiva). Band densities were semi-quantified using ImageJ (version 1.51; National Institutes of Health), with MBP band intensities normalized to the corresponding β-actin band intensity, prior to being expressed as a ratio to the control (CTL) band.

Statistical analysis. Each animal was considered to be a single biological replicate (the number of animals analyzed are indicated in the figure legend for each assay). Data are presented as the mean ± SEM. Statistical analysis was completed using GraphPad PRISM software (version 8.0; GraphPad Software, Inc.) using unpaired student's t-test or one- and two-way ANOVAs followed by Tukey's post hoc test. Two-way ANOVA was used to evaluate the behavioral tasks with factors of treatment (CTL, LFMS, CPZ and CPZ + LFMS) and time (3- and 6-weeks). For immunohistochemistry and western blotting data, one-way ANOVA was used to compare differences among more than two groups. The unpaired two-tailed t-test was used to statistically compare the demyelination data between the CPZ and CPZ + LFMS. Data that did not reach normality and/or equal variances were analyzed using a nonparametric Kruskal-Wallis test, which was followed by Dunn's post hoc test. All data were tested for outliers using Grubb's test and identified outliers were removed. $P<0.05$ was considered to indicate a statistically significant difference.

Results

LFMS treatment has no effect on locomotion or anxiety parameters in CPZ mice. The impact of 3- and 6-week LFMS treatment on locomotor activity was quantified using the OFT, which evaluated the total distance travelled (Fig. 2A). The two-way ANOVA demonstrated a significant effect of time ($F_{(1,85)}=16.26$; $P=0.0001$). However, no significant effect was seen with types of treatment ($F_{(3,85)}=0.18$; $P=0.90$) or treatment by time interaction ($F_{(3,85)}=1.97$; $P=0.12$) among the groups. Statistical analysis on the mean speed velocity, demonstrated a significant effect of time ($F_{(1,84)}=31.31$; $P=0.0001$; Fig. 2D). However, there was no statistically significant difference with treatment effect or interaction. These results suggested that the animals remembered the open field arena and therefore exhibited reduced exploration at 6 weeks, however, neither the CPZ diet nor LFMS treatment had an impact on locomotor ability.

The OFT can also be used to assess anxiety-like behaviors. Time spent in the inner zone of the arena demonstrated a significant main effect of treatment ($F_{(3,83)}=3.60$; $P=0.0167$), without time differences or interaction between factors. Tukey's post hoc test demonstrated that CPZ-treated mice spent markedly more time ($P<0.05$) in the inner zone compared with the LFMS group after 6 weeks (Fig. 2B), which suggested these mice potentially exhibited lower anxiety levels than the controls. The two-way ANOVA also demonstrated that the mean freezing score was significantly affected by treatment ($F_{(3,85)}=6.06$; $P=0.0009$) and time ($F_{(1,85)}=20.58$; $P=0.0001$), but not by interaction ($F_{(3,85)}=6.24$; $P=0.86$) The post hoc multiple comparisons test demonstrated that mice fed the CPZ diet with LFMS treatment exhibited significantly lower mean freezing scores ($P<0.05$) compared with the LFMS mice (Fig. 2C). These findings indicated that the CPZ diet may inhibit anxiety-like behaviors compared with the controls; however, LFMS treatment alone has no impact on anxiety levels.

LFMS treatment improves spatial memory in CPZ mice. In support of the OFT results, locomotor activity was not significantly different between groups in the Y maze (Fig. 3B). Analysis of the spontaneous alternation revealed a statistically significant result in treatment effect ($F_{(3,80)}=3.9574$; $P<0.0175$). However, there were no significant results for time ($F_{(1,80)}=3.262$; $P=0.0777$) or interaction ($F_{(3,80)}=0.4641$; $P<0.7081$). Moreover, while CPZ diet mice demonstrated a significant spatial memory deficit ($P<0.05$) compared with the control group at 6 weeks, LFMS treatment markedly improved spontaneous alteration compared with the CPZ group; however, this trend was not significant (Fig. 3A).

LFMS treatment significantly improves depression-like symptoms and impacts the adaptive learning response in CPZ mice. The FST was used to assess depression-like symptoms and evaluate adaptive learning behavior. Analysis of the latency to first immobility (time to start floating) revealed a significant time effect ($F_{(3,164)}=185.9$; $P<0.0001$) without treatment effect ($F_{(3,164)}=0.2419$; $P=0.8670$) but with interaction ($F_{(3,164)}=0.2419$; $P=0.8670$; Fig. 4A). Post hoc analysis revealed that the CPZ diet mice exhibited a significant lower latency to start floating ($P<0.01$) when compared to the CTL, LFMS and CPZ + LFMS groups (Fig. 4A; 1st swim at 3rd week). LFMS treatment significantly reversed the CPZ effect on the latency to the first immobility instance compared with the CPZ only group ($P<0.001$), suggesting that LFMS may have a potential antidepressant effect on the animal demyelination model.

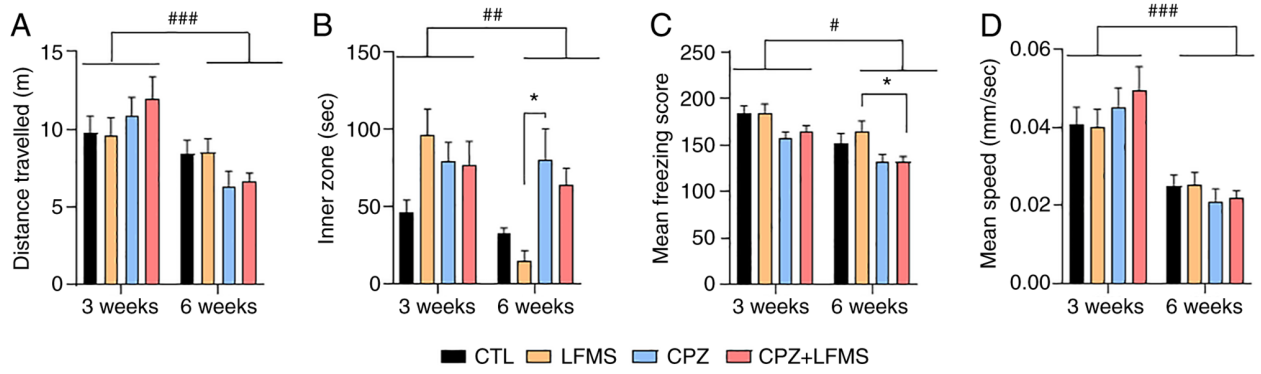

Figure 2. Effects of LFMS on behavioral parameters observed in the OFT in an acute CPZ mouse model of MS after 3 or 6 weeks of treatment. The following OFT parameters were quantified: (A) Distance travelled; (B) time spent in the inner zone; (C) mean freezing score; and (D) mean speed. n=9-16 mice/group. *P<0.05 vs. treatment; #P<0.05, ##P<0.01 and ###P<0.001. OFT, open field test; CPZ, cuprizone; LFMS, low-field magnetic stimulation; CTL, control.

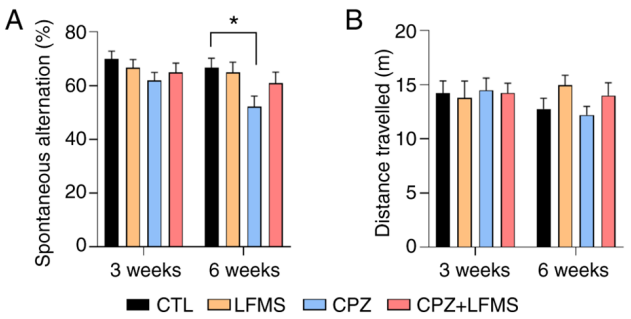

Figure 3. Effects of LFMS on behavioral parameters observed in the Y maze test in an acute CPZ mouse model of multiple sclerosis after 3 or 6 weeks of treatment. The following Y maze test parameters were quantified: (A) Spontaneous alternation; and (B) distance travelled. n=10-14 mice/group. *P<0.05. LFMS, low-field magnetic stimulation; CPZ, cuprizone; CTL, control.

Repeated exposure to the FST (2nd, 3rd and 4th swim at week 3 and 6) revealed a significant reduction in the latency to the first instance of immobility (P<0.0001) as determined by two-way ANOVA with Tukey's post hoc multiple comparisons, suggesting that all animals remembered their previous experience.

Results from total immobility time revealed a significant main treatment effect ($F_{(3,168)}$=9.927; P<0.0001) and significant differences with interaction ($F_{(9,168)}$=6.095; P<0.0001), but not with time ($F_{(3,168)}$=2.423; P=0.10). The CPZ group exhibited a significantly higher total immobility time compared with the LFMS treatment group (P<0.05) in the first FST (Fig. 4B), which indicated a depressive-like behavior in CPZ mice. Total immobility time did not show significant differences on the second trial day; however, in the third trial, the CPZ diet mice presented reduced immobility time compared with the CTL and LFMS groups (P<0.0001). This trend continued in the fourth trial, although the CPZ differed from the CTL group only (P<0.001; Fig. 4B). LFMS treatment demonstrated a slight improvement on the passive behavior compared with the CPZ fed mice during the third and fourth FST trials; however, this effect was not significant. Furthermore, there was a significant difference between the CPZ + LFMS and CTL groups in the third (P<0.001) and fourth trials (P<0.05) (Fig. 4B), suggesting that LFMS was unable to completely ameliorate this adaptive learning impairment.

LFMS treatment significantly impacts myelination in the PFC in CPZ mice. CPZ has previously been demonstrated to cause oligodendrocyte death leading to demyelination (51). Using western blotting, the relative protein expression levels of MBP, a major protein involved in myelin function, within the PFC was compared following 3 or 6 weeks of treatment. The results demonstrated that MBP protein expression levels were not significantly difference between groups after 3 weeks (Fig. 5A and C), whereas statistically significant differences were observed following 6 weeks on the CPZ diet (Kruskal-Wallis 4,19; P<0.0003; Fig. 5D). The CPZ diet mice exhibited a decrease (P<0.001) in relative MBP protein expression levels compared with the control groups (CTL and LFMS treatment only), which demonstrated that there was potentially a significant loss of myelin in the PFC of CPZ diet mice after 6 weeks. The two-tailed unpaired t-test (t=2.665; degrees of freedom=10; P<0.0237) revealed a significant difference between CPZ and CPZ + LFMS mice, whereby LMFS treatment significantly increased the relative protein expression levels of MBP in CPZ treated mice. These results therefore indicated that LFMS treatment either protected the PFC from demyelination or promoted remyelination following 6 weeks of treatment.

One-way ANOVA demonstrated significant differences in MBP staining density between diet in the PFC ($F_{(3,20)}$=19.25; P<0.0001) and HPC ($F_{(3,16)}$=18.67; P<0.0001) at 3 weeks (Fig. 6A and B). The CPZ diet mice exhibited significantly decreased MBP staining density compared with the CTL or LFMS treatment group in both the PFC and HPC (Fig. 6E-G), which represented a potential loss of myelin in mice fed with the CPZ diet. Mice that received the LFMS treatment (CPZ + LFMS) exhibited significantly increased MBP staining density in the PFC compared with the CPZ group (t=2.246; degrees of freedom=15; P<0.0402) and HPC (P<0.0025).

Following 6 weeks of treatment, one-way ANOVA demonstrated significant differences between groups in the PFC ($F_{(3,21)}$=4.472; P<0.0141) and HPC ($F_{(3,19)}$=60.57; P<0.0001). Reduction of MBP staining was sustained at 6 weeks in the mice on the CPZ diet, with significant reductions within the

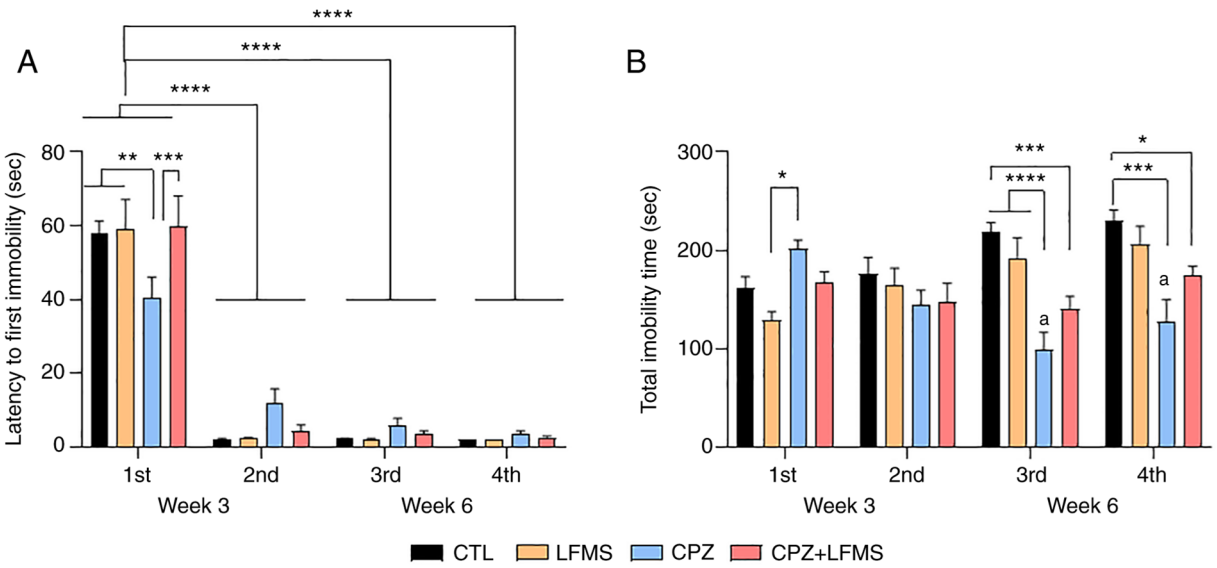

Figure 4. Effects of LFMS on behavioral parameters observed in the repeated FST in an acute CPZ mouse model of multiple sclerosis. The following FST parameters were quantified: (A) Latency to first period of immobility; and (B) total immobility time during the FST1, FST2, FST3 and FST4. n=10-15 mice/group. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. ^{a}P<0.05 vs. CPZ (1st FST tests). LFMS, low-field magnetic stimulation; FST, forced swim test; CPZ, cuprizone; CTL, control.

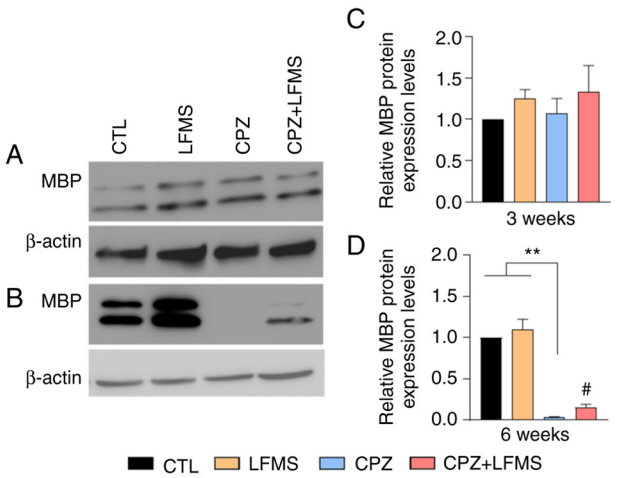

Figure 5. Effects of LFMS on the relative protein expression levels of MBP in the PFC of an acute CPZ mouse model of multiple sclerosis. Representative western blots of MBP and β-actin expression in the PFC after (A) 3 weeks and (B) 6 weeks of treatment. MBP band intensity was semi-quantified and normalized to the β-actin band intensity for each mouse at (C) 3 weeks and (D) 6 weeks. Data are presented as a ratio to the control group. n=5-6 mice/group. **P<0.01. $^{\#}$P<0.05 vs. CPZ. LFMS, low-field magnetic stimulation; MBP, myelin basic protein; PFC, prefrontal cortex; CTL, control; CPZ, cuprizone.

HPC (P<0.001) compared with the CTL and LFMS groups, but not in the PFC. Moreover, LFMS treatment did not impact MBP staining density in CPZ diet mice within the HPC, whereas LFMS treatment significantly increased MBP staining density in CPZ diet mice within the PFC compared with the CPZ only group (P<0.05).

Discussion

In the present study, it was demonstrated, to the best of our knowledge for the first time, the beneficial effects of LFMS, a non-invasive and deep brain stimulation, after 3 or 6 weeks of treatment in a CPZ-induced demyelination animal model. Mice treated with LFMS exhibited significantly improved depression-like symptoms and demonstrated modest enhancements in cognitive function and adaptive learning skills. Furthermore, in the demyelination model, LFMS treatment was able to potentially protect from or reverse the demyelination processes, evidenced by the markedly increased immunostaining density of MBP in the PFC after 3 and 6 weeks of treatment.

It was also observed that following 3 weeks on the CPZ diet, mice demonstrated depressive-like behaviors, while cognitive deficits were observed following 6 weeks on the CPZ diet. These behavioral changes were associated with demyelination, as quantified by significantly reduced immunostaining of MBP in the PFC and HPC evident at 3 and 6 weeks and confirmed by western blot analysis in the PFC at 6 weeks. These results indicated that the CPZ-induced acute demyelination model may be useful for studying the effects of LFMS on myelination.

To the best of our knowledge, this is the first study to have demonstrated early depression-like behavior following 3 weeks of CPZ feeding. Previous studies have demonstrated an increase in depression-like symptoms in CPZ models after 5, 6 and 12 weeks on a CPZ diet (19,30,52). A significantly increased latency to the first immobile episode was observed in the CPZ group compared to the control and LFMS groups after only 3 weeks on the CPZ diet (FST1), which was ameliorated by LFMS treatment. This therefore demonstrated that LFMS has an antidepressant-like effect in this demyelination model.

Almost one-half of patients with MS display symptoms of depression (53). The depressive symptoms observed in patients with MS can precede the onset of neurological symptoms, suggesting that depression may be related to early disease-specific processes (54). Both MS and major depressive disorder share the common pathophysiology

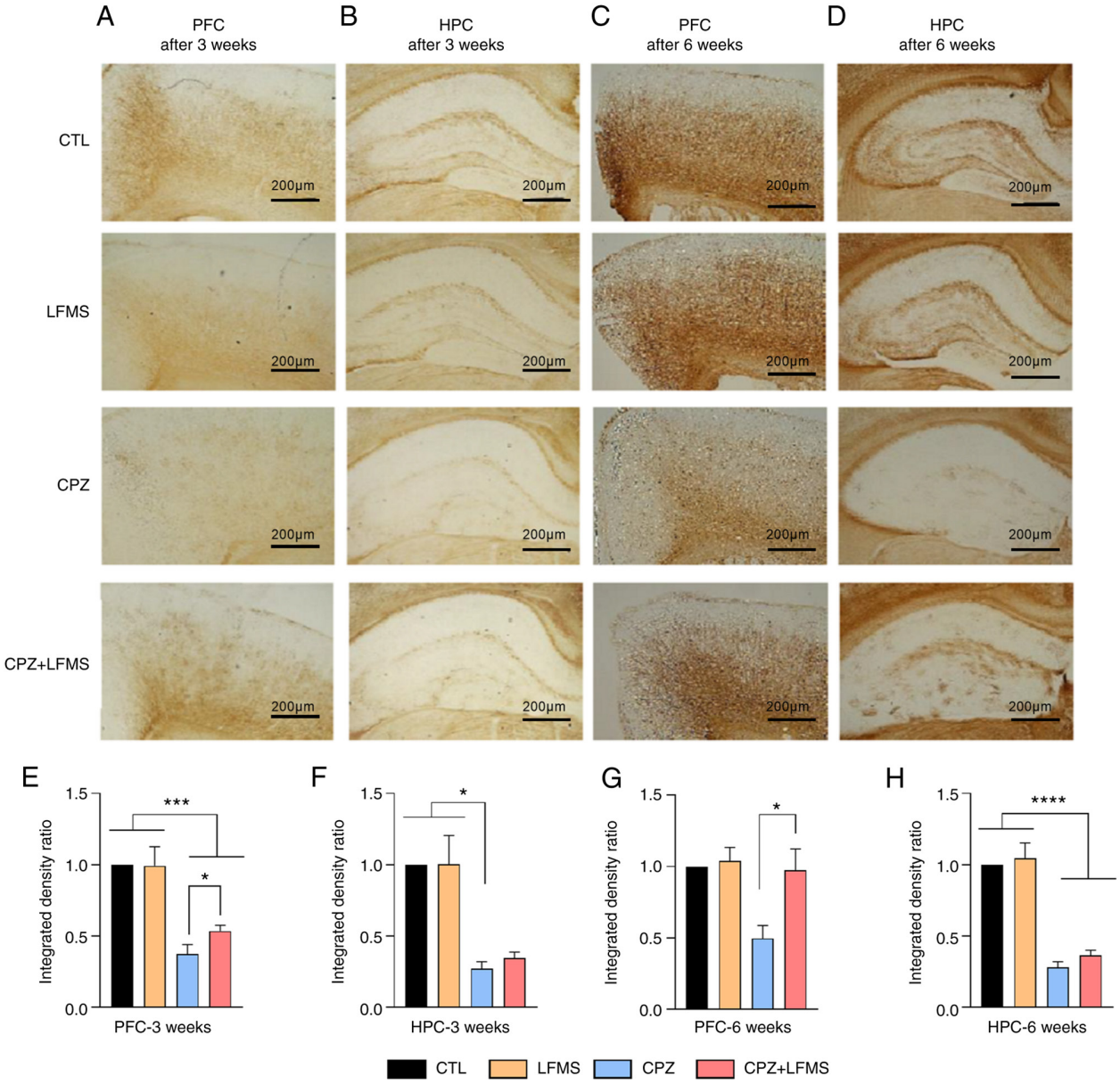

Figure 6. Impact of LFMS on the staining intensity of MBP in the PFC and HPC of an acute CPZ mouse model of multiple sclerosis. Representative images of MBP immunostaining in the PFC after (A) 3 weeks and (C) 6 weeks and in the HPC at (B) 3 weeks and (D) 6 weeks following the CPZ diet and treatment. MBP staining intensity for the PFC at (E) 3 weeks and (G) 6 weeks and the HPC at (F) 3 weeks and (H) 6 weeks was determined for a minimum of 20 coronal sections per mouse, averaged between groups then compared with the CTL. Scale bar, 200 μm. Data are presented as a ratio to the CTL group. n=3-11 mice/group. *P<0.05, ***P<0.001 and ****P<0.0001. LFMS, low-field magnetic stimulation; MBP, myelin basic protein; PFC, prefrontal cortex; HPC, hippocampus; CPZ, cuprizone; CTL, control.

of demyelination of CNS regions and are associated with neuro-inflammation processes (55,56). Indeed, previous CPZ mouse models have reported depression-like symptoms and myelin deficiency (19,30) demonstrating a possible correlation between depression and demyelination of the CNS. In the present study, it was demonstrated that mice fed with CPZ exhibited markedly reduced MBP staining density within the PFC and HPC after only 3 weeks, which suggested that there was a possible link between early depression and myelin damage in this demyelinating disease model. Most importantly, the LFMS antidepressant-like effect observed after 3 weeks of treatment during the FST1 may be associated with markedly greater levels of MBP in the PFC. The significant increase in MBP levels following LFMS treatment after 6 weeks suggested that myelin is either protected by or its loss is reversed by LFMS treatment. These findings indicated that myelination is an important factor to improve depression in patients with MS and possibly in other demyelinating diseases. Future studies are needed to elucidate the mechanism by which LFMS impacts myelination, either via protecting myelin from demyelination or stimulating remyelination, and to examine the integrity of the protected/restored myelin, including investigating the re-establishment of the nodes of Ranvier.

Demyelination is associated with axon damage, leading to cognitive deficits, including in memory and attention (52). In the present study, CPZ fed mice demonstrated a significantly lower percentage of spontaneous alternations in the Y maze after 6 weeks, which demonstrated a working memory deficit.

Spontaneous alternations were not altered after 3 weeks of CPZ feeding, which indicated that short-term CPZ exposure may not impair working memory. In support of this finding, previous studies have reported no changes in the Y-maze test after 0.4% CPZ-feeding for 3 weeks or 0.2% CPZ for 1 week (57,58). Together, these findings suggested that short-term exposure (1-3 weeks) to the CPZ diet may not cause working memory dysregulation. LFMS treatment demonstrated a trend in repairing the cognitive impairment caused by the 6-week CPZ diet. A previous study demonstrated that LFMS treatment improved cognition in the CPZ mouse model after twelve weeks of 0.2% CPZ exposure followed by four weeks of CPZ withdrawal with sham or LFMS treatment (29). The difference between treatment time and procedure may explain the discrepancy between the observed effect of LFMS treatment on working memory.

Evidence has also suggested that patients with MS have demonstrated higher levels of anxiety (59,60). In the present study, we used the time spent in the central area of the OFT arena to measure anxiety-like behavior. The results demonstrated that mice treated with CPZ for 6 weeks had a significantly higher center-area activity compared with the LFMS group, which indicated diminished anxiety. Previous studies have demonstrated similar behavior after CPZ treatment for 3 and 4 weeks (61,62). This result can be associated with an inhibited anxiogenic response to novel environments or increased impulsiveness and could be related to white matter alterations (61). Further studies investigating anxiety, such as the elevated plus maze or the light-dark box, will need to be conducted to obtain a clearer understanding of the impact of CPZ on anxiety levels.

CPZ mice displayed significantly decreased immobility at the FST3 and FST4 timepoints, whereas the control groups exhibited an adaptative learning behavior response or intact coping strategies following exposure to a stressful situation. These results suggested that the CPZ mice failed to display normal adaptation from active to passive coping. To the best of our knowledge this is the first study to have demonstrated that the CPZ model impairs adaptive coping strategies. LFMS treatment resulted in a marked improvement in the CPZ mice coping strategy but was not sufficient to reverse this effect. Coping strategies serve an important role in challenging conditions ensuring the ability to adapt to stressful life conditions (63,64). Patients with MS and other neuropsychiatric conditions are less able to integrate adaptive coping abilities compared with healthy individuals (65,66). As coping strategies are important factors that can affect a patients' quality of life (67) further investigation in this area is required.

Depression and defective working memory (68), have both been shown to have a negative impact on the ability to utilize coping strategies (69,70). Furthermore, the PFC coordinates processes which enable effective coping skills (71). Considering these factors, the results of the present study indicated that failure to engage in coping strategies by mice exposed to CPZ, may be associated with the depressive-like symptoms and white matter impairment and demyelination observed in the PFC. It can therefore be hypothesized that these elements are required for a healthy coping mechanism.

The results of the present study have provided the first evidence for the short-term effect of LFMS in attenuating early-stage depressive-like behavior in a demyelination animal model. It was also demonstrated that LFMS treatment either provided protection against demyelination or promoted remyelination with increased immunostaining of MBP in the PFC after 3 and 6 weeks of treatment, confirmed by western blot analysis at 6 weeks. Therefore, these results indicated that LFMS may have the potential to be a novel therapy to manage depression in patients with MS. The present study has raised the possibility for the future application of this non-pharmacological therapy for MS and has encouraged the exploration of technological advances in manipulating brain activity in a non-invasive manner to treat different neurological and neuropsychiatric diseases. The present study's design did not determine the mechanism by which LFMS increased MBP levels and whether the treatment protected oligodendrocytes from damage or whether the treatment stimulated remyelination. The ability for LFMS to trigger remyelination has been previously reported (30), but it is unknown if LFMS treatment is sufficient to mitigate the toxic effects of CPZ in oligodendrocytes, protecting them from damage, or if the LFMS treatment could stimulate repair at a rate faster than CPZ destruction. Further investigation into the mechanism by which LFMS acts is needed to better understand the impact on myelination and how this treatment can be used in clinical conditions.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AM developed the project, collected the data and performed formal analysis. TS analyzed and interpreted the data and wrote and edited the manuscript. RVB assisted with data interpretation, writing, review and editing of the manuscript. HL and ZW contributed towards research data collection. XML made substantial contributions to the conception and design of the study. YZ provided funding acquisition, project development and conception, supervision, review and editing. AM and

YZ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Animal Research Ethics Board from University of Saskatchewan (Saskatoon, Canada) in 2016 (approval no. 20160103).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Dendrou CA, Fugger L and Friese MA: Immunopathology of multiple sclerosis. Nat Rev Immunol 15: 545-558, 2015.
2. Islas MÁM and Ciampi E: Assessment and impact of cognitive impairment in multiple sclerosis: An overview. Biomedicines 7: 22, 2019.
3. Walton C, King R, Rechtman L, Kaye W, Leray E, Marrie RA, Robertson N, La Rocca N, Uitdehaag B, van der Mei I, *et al*: Rising prevalence of multiple sclerosis worldwide: Insights from the ATLAS of MS, third edition. Mult Scler 26: 1816-1821, 2020.
4. Eijlers AJC, Van Geest Q, Dekker I, Steenwijk MD, Meijer KA, Hulst HE, Barkhof F, Uitdehaag BMJ, Schoonheim MM and Geurts JJG: Predicting cognitive decline in multiple sclerosis: A 5-year follow-up study. Brain 141: 2605-2618, 2018.
5. Patten SB, Marrie RA and Carta MG: Depression in multiple sclerosis. Int Rev Psychiatry 29: 463-472, 2017.
6. Salehpoor G, Rezaei S and Hosseininezhad M: Quality of life in multiple sclerosis (MS) and role of fatigue, depression, anxiety, and stress: A bicenter study from north of Iran. Iran J Nurs Midwifery Res 19: 593-599, 2014.
7. Kalb R, Feinstein A, Rohrig A, Sankary L and Willis A: Depression and suicidality in multiple sclerosis: Red flags, management strategies, and ethical considerations. Curr Neurol Neurosci Rep 19: 77, 2019.
8. Shen Q, Lu H, Xie D, Wang H, Zhao Q and Xu Y: Association between suicide and multiple sclerosis: An updated meta-analysis. Mult Scler Relat Disord 34: 83-90, 2019.
9. Carta MG, Paribello P, Anastasia A, De Berardis D, Nardi AE and Fornaro M: Pharmacological management of depression in patients with multiple sclerosis. Expert Opin Pharmacother 19: 1533-1540, 2018.
10. Vanotti S and Caceres FJ: Cognitive and neuropsychiatric disorders among MS patients from Latin America. Mult Scler J Exp Transl Clin 3: 2055217317717508, 2017.
11. Sumowski JF, Benedict R, Enzinger C, Filippi M, Geurts JJ, Hamalainen P, Hulst H, Inglese M, Leavitt VM, Rocca MA, *et al*: Cognition in multiple sclerosis: State of the field and priorities for the future. Neurology 90: 278-288, 2018.
12. Nebel K, Wiese H, Seyfarth J, Gizewski ER, Stude P, Diener HC and Limmroth V: Activity of attention related structures in multiple sclerosis patients. Brain Res 1151: 150-160, 2007.
13. Guimarães J and Sá MJ: Cognitive dysfunction in multiple sclerosis. Front Neurol 3: 74, 2012.
14. Feinstein A, Freeman J and Lo AC: Treatment of progressive multiple sclerosis: What works, what does not, and what is needed. Lancet Neurol 14: 194-207, 2015.
15. Procaccini C, De Rosa V, Pucino V, Formisano L and Matarese G: Animal models of multiple sclerosis. Eur J Pharmacol 759: 182-191, 2015.
16. Zhan J, Mann T, Joost S, Behrangi N, Frank M and Kipp M: The cuprizone model: Dos and do nots. Cells 9: 843, 2020.
17. Hübner NS, Mechling AE, Lee HL, Reisert M, Bienert T, Hennig J, von Elverfeldt D and Harsan LA: The connectomics of brain demyelination: Functional and structural patterns in the cuprizone mouse model. Neuroimage 146: 1-18, 2017.
18. Hibbits N, Pannu R, Wu TJ and Armstrong RC: Cuprizone demyelination of the corpus callosum in mice correlates with altered social interaction and impaired bilateral sensorimotor coordination. ASN Neuro 1: e00013, 2009.
19. Zhang Y, Bi Y, Adebiyi O, Wang J, Mooshekhian A, Cohen J, Wei Z, Wang F and Li XM: Venlafaxine improves the cognitive impairment and depression-like behaviors in a cuprizone mouse model by alleviating demyelination and neuroinflammation in the brain. Front Pharmacol 10: 332, 2019.
20. Mi G, Gao Y, Liu S, Ye E, Li Y, Jin X, Yang H and Yang Z: Cyclin-dependent kinase inhibitor flavopiridol promotes remyelination in a cuprizone induced demyelination model. Cell Cycle 15: 2780-2791, 2016.
21. Shafi M, Stern AP and Pascual-Leone A: Adding low-field magnetic stimulation to noninvasive electromagnetic neuromodulatory therapies. Biol Psychiatry 76: 170-171, 2014.
22. Posse S, Dager SR, Richards TL, Yuan C, Ogg R, Artru AA, Müller-Gärtner HW and Hayes C: In vivo measurement of regional brain metabolic response to hyperventilation using magnetic resonance: Proton echo planar spectroscopic imaging (PEPSI). Magn Reson Med 37: 858-865, 1997.
23. Becker JE, Shultz EKB and Maley CT: Transcranial magnetic stimulation in conditions other than major depressive disorder. Child Adolesc Psychiatr Clin N Am 28: 45-52, 2019.
24. Machado S, Arias-Carrion O, Paes F, Vieira RT, Caixeta L, Novaes F, Marinho T, Almada LF, Silva AC and Nardi AE: Repetitive transcranial magnetic stimulation for clinical applications in neurological and psychiatric disorders: An overview. Eurasian J Med 45: 191-206, 2013.
25. Singh A, Erwin-Grabner T, Goya-Maldonado R and Antal A: Transcranial magnetic and direct current stimulation in the treatment of depression: Basic mechanisms and challenges of two commonly used brain stimulation methods in interventional psychiatry. Neuropsychobiology 79: 397-407, 2020.
26. Camprodon JA: Therapeutic neuromodulation for bipolar disorder-The case for biomarker-driven treatment development. JAMA Netw Open 4: e211055, 2021.
27. Rohan ML, Yamamoto RT, Ravichandran CT, Cayetano KR, Morales OG, Olson DP, Vitaliano G, Paul SM and Cohen BM: Rapid mood-elevating effects of low field magnetic stimulation in depression. Biol Psychiatry 76: 186-193, 2014.
28. Sekar S, Zhang Y, Mahabadi HM, Parvizi A and Taghibiglou C: Low-field magnetic stimulation restores cognitive and motor functions in the mouse model of repeated traumatic brain injury: Role of cellular prion protein. J Neurotrauma 36: 3103-3114, 2019.
29. Dolgova N, Wei Z, Spink B, Gui L, Hua Q, Truong D, Zhang Z and Zhang Y: Low-field magnetic stimulation accelerates the differentiation of oligodendrocyte precursor cells via non-canonical TGF-β signaling pathways. Mol Neurobiol 58: 855-866, 2021.
30. Wang Z, Baharani A, Wei Z, Truong D, Bi X, Wang F, Li XM, Verge VMK and Zhang Y: Low field magnetic stimulation promotes myelin repair and cognitive recovery in chronic cuprizone mouse model. Clin Exp Pharmacol Physiol 48: 1090-1102, 2021.
31. Harbo HF, Gold R and Tintoré M: Sex and gender issues in multiple sclerosis. Ther Adv Neurol Disord 6: 237-248, 2013.
32. Voskuhl RR, Sawalha AH and Itoh Y: Sex chromosome contributions to sex differences in multiple sclerosis susceptibility and progression. Mult Scler 24: 22-31, 2018.
33. Taylor LC, Gilmore W, Ting JPY and Matsushima GK: Cuprizone induces similar demyelination in male and female C57BL/6 mice and results in disruption of the estrous cycle. J Neurosci Res 88: 391-402, 2010.
34. Schiefer HB: Guide to the care and use of experimental animals, volume 2. Can J Comp Med 49: 49, 1985.
35. Canadian Council on Care (CCAC): Guide to the Care and Use of Experimental Animals. Vol 1. 2nd edition. Publication date: 1993, Revision date: 2020. CCAC, Ottawa, ON, 2013. https://ccac.ca/Documents/Standards/Guidelines/Experimental_Animals_Vol1.pdf.
36. Gudi V, Moharregh-Khiabani D, Skripuletz T, Koutsoudaki PN, Kotsiari A, Skuljec J, Trebst C and Stangel M: Regional differences between grey and white matter in cuprizone induced demyelination. Brain Res 1283: 127-138, 2009.
37. Zhang Y, Adebiyi O, Wei Z, Mooshekhian A, Truong D, Lavoie C, Cohen J, Zhang Z, Wang F, Bowen R and Li XM: Low-field magnetic stimulation (LFMS) decreases cuprizone-induced cognitive impairment and brain pathology in mice. Eur Neuropsychopharmacol 29: S227-S228, 2019.

38. Seibenhener ML and Wooten MC: Use of the open field maze to measure locomotor and anxiety-like behavior in mice. J Vis Exp 6: e52434, 2015.
39. Cleal M, Fontana BD, Ranson DC, McBride SD, Swinny JD, Redhead ES and Parker MO: The Free-movement pattern Y-maze: A cross-species measure of working memory and executive functio. Behav Res Methods 53: 536-557, 2021.
40. Yan G, Xuan Y, Dai Z, Shen Z, Zhang G, Xu H and Wu R: Brain metabolite changes in subcortical regions after exposure to cuprizone for 6 weeks: Potential implications for schizophrenia. Neurochem Res 40: 49-58, 2015.
41. Xu H, Yang HJ, Rose GM and Li XM: Recovery of behavioral changes and compromised white matter in C57BL/6 mice exposed to cuprizone: Effects of antipsychotic drugs. Front Behav Neurosci 5: 31, 2011.
42. Rustay NR, Cronin EA, Curzon P, Markosyan S, Bitner RS, Ellis TA, Waring JF, Decker MW, Rueter LE and Browman KE: Mice expressing the Swedish APP mutation on a 129 genetic background demonstrate consistent behavioral deficits and pathological markers of Alzheimer's disease. Brain Res 1311: 136-147, 2010.
43. Slattery DA and Cryan JF: Using the rat forced swim test to assess antidepressant-like activity in rodents. Nat Protoc 7: 1009-1014, 2012.
44. Kraeuter AK, Guest PC and Sarnyai Z: The forced swim test for depression-like behavior in rodents. Methods Mol Biol 1916: 75-80, 2019.
45. Porsolt PR, Le Pichon M and Jalfre M: Depression: A new animal model sensitive to antidepressant treatments. Nature 266: 730-732, 1977.
46. Borsini F and Meli A: Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berl) 94: 147-160, 1988.
47. Petit-Demouliere B, Chenu F and Bourin M: Forced swimming test in mice: A review of antidepressant activity. Psychopharmacology (Berl) 177: 245-255, 2005.
48. Hascoët M and Bourin M: The forced swimming test in mice: A suitable model to study antidepressants. Neuromethods 1: 85-118, 2009.
49. Mul JD, Zheng L and Goodyear LJ: Validity assessment of 5 day repeated forced-swim stress to model human depression in young-adult C57BL/6J and BALB/CJ mice. eNeuro 29: ENEURO.0201-16.2016, 2016.
50. Commons KG, Cholanians AB, Babb JA and Ehlinger DG: The rodent forced swim test measures stress-coping strategy, not depression-like behavior. ACS Chem Neurosci 8: 955-960, 2017.
51. Titus HE, Chen Y, Podojil JR, Robinson AP, Balabanov R, Popko B and Miller SD: Pre-clinical and clinical implications of 'Inside-Out' vs. 'Outside-In' paradigms in multiple sclerosis etiopathogenesis. Front Cell Neurosci 14: 599717, 2020.
52. Liu Q, Lv HW, Yang S, He YQ, Ma QR and Liu J: NEP1-40 alleviates behavioral phenotypes and promote oligodendrocyte progenitor cell differentiation in the hippocampus of cuprizone-induced demyelination mouse model. Neurosci Lett 725: 134872, 2020.
53. Khalilian B, Madadi S, Fattahi N and Abouhamzeh B: Coenzyme Q10 enhances remyelination and regulate inflammation effects of cuprizone in corpus callosum of chronic model of multiple sclerosis. J Mol Histol 52: 125-134, 2021.
54. Vattakatuchery JJ, Rickards H and Cavanna AE: Pathogenic mechanisms of depression in multiple sclerosis. J Neuropsychiatry Clin Neurosci 23: 261-276, 2011.
55. Shail MS: Neuropsychiatry in demyelination disease: Using depression as a prodrome for early diagnosis and treatment of multiple sclerosis. Cureus 9: e1813, 2017.
56. Shin JS, Kwon YN, Choi Y, Lee JY, Lee YI, Hwang JH, Choi SH and Kim SM: Comparison of psychiatric disturbances in patients with multiple sclerosis and neuromyelitis optica. Medicine (Baltimore) 98: e1718, 2019.
57. Shao Y, Peng H, Huang Q, Kong J and Xu H: Quetiapine mitigates the neuroinflammation and oligodendrocyte loss in the brain of C57BL/6 mouse following cuprizone exposure for one week. Eur J Pharmacol 765: 249-257, 2015.
58. Chang H, Liu J, Zhang Y, Wang F, Wu Y, Zhang L, Ai H, Chen G and Yin L: Increased central dopaminergic activity might be involved in the behavioral abnormality of cuprizone exposure mice. Behav Brain Res 331: 143-150, 2017.
59. Wood B, Van Der Mei IAF, Ponsonby AL, Pittas F, Quinn S, Dwyer T, Lucas RM and Taylor BV: Prevalence and concurrence of anxiety, depression and fatigue over time in multiple sclerosis. Mult Scler 19: 217-224, 2013.
60. Serra-de-Oliveira N, Boilesen SN, de França Carvalho CP, LeSueur-Maluf L, de Lima Zollner R, Spadari RC, Medalha CC and de Castro GM: Behavioural changes observed in demyelination model shares similarities with white matter abnormalities in humans. Behav Brain Res 287: 265-275, 2015.
61. Franco-Pons N, Torrente M, Colomina MT and Vilella E: Behavioral deficits in the cuprizone-induced murine model of demyelination/remyelination. Toxicol Lett 169: 205-213, 2007.
62. Xu H, Yang HJ, Zhang Y, Clough R, Browning R and Li XM: Behavioral and neurobiological changes in C57BL/6 mice exposed to cuprizone. Behav Neurosci 123: 418-429, 2009.
63. Milanlioglu A, Özdemir PG, Cilingir V, Gülec TÇ, Aydin MN and Tombul T: Coping strategies and mood profiles in patients with multiple sclerosis. Arq Neuropsiquiatr 72: 490-495, 2014.
64. Holubova M, Prasko J, Hruby R, Latalova K, Kamaradova D, Marackova M, Slepecky M and Gubova T: Coping strategies and self-stigma in patients with schizophrenia-spectrum disorders. Patient Prefer Adherence 10: 1151-1158, 2016.
65. Cotton SM, McCann TV, Gleeson JF, Crisp K, Murphy BP and Lubman DI: Coping strategies in carers of young people with a first episode of psychosis. Schizophr Res 146: 118-124, 2013.
66. Grech LB, Kiropoulos LA, Kirby KM, Butler E, Paine M and Hester R: Target coping strategies for interventions aimed at maximizing psychosocial adjustment in people with multiple sclerosis. Int J MS Care 20: 109-119, 2018.
67. McCabe MP, McKern S and McDonald E: Coping and psychological adjustment among people with multiple sclerosis. J Psychosom Res 56: 355-361, 2004.
68. Reising MM, Bettis AH, Dunbar JP, Watson KH, Gruhn M, Hoskinson KR and Compas BE: Stress, coping, executive function, and brain activation in adolescent offspring of depressed and nondepressed mother. Child Neuropsychol 24: 638-656, 2018.
69. Arnett PA, Higginson CI, Voss WD, Randolph JJ and Grandey AA: Relationship between coping, cognitive dysfunction and depression in multiple sclerosis. Clin Neuropsychol 16: 341-355, 2002.
70. Goretti B, Portaccio E, Zipoli V, Hakiki B, Siracusa G, Sorbi S and Amato MP: Impact of cognitive impairment on coping strategies in multiple sclerosis. Clin Neurol Neurosurg 112: 127-130, 2010.
71. Bradshaw SD, Shumway ST, Dsauza CM, Morris N and Hayes ND: Hope, coping skills, and the prefrontal cortex in alcohol use disorder recovery. Am J Drug Alcohol Abuse 43: 591-601, 2017.

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