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·Report·

Clemastine rescues behavioral changes and enhances remyelination in the cuprizone mouse model of demyelination

Zhifang Li[1,*], Yangtao He[2,*], Shuangyi Fan[1], Binbin Sun[1]

[1]*Department of Neurology, Affiliated Hospital of Academy of Military Medical Sciences, Beijing 100071, China*

[2]*Department of Histology and Embryology, Chongqing Key Laboratory of Neurobiology, Third Military Medical University, Chongqing 400038, China*

[*]These authors contributed equally to this work.

Corresponding authors: Zhifang Li and Yangtao He. E-mail: lizhifang@sohu.com, heyangtaotmmu@163.com

ABSTRACT

Increasing evidence suggests that white matter disorders based on myelin sheath impairment may underlie the neuropathological changes in schizophrenia. But it is unknown whether enhancing remyelination is a beneficial approach to schizophrenia. To investigate this hypothesis, we used clemastine, an FDA-approved drug with high potency in promoting oligodendroglial differentiation and myelination, on a cuprizone-induced mouse model of demyelination. The mice exposed to cuprizone (0.2% in chow) for 6 weeks displayed schizophrenia-like behavioral changes, including decreased exploration of the center in the open field test and increased entries into the arms of the Y-maze, as well as evident demyelination in the cortex and corpus callosum. Clemastine treatment was initiated upon cuprizone withdrawal at 10 mg/kg per day for 3 weeks. As expected, myelin repair was greatly enhanced in the demyelinated regions with increased mature oligodendrocytes (APC-positive) and myelin basic protein. More importantly, the clemastine treatment rescued the schizophrenia-like behavioral changes in the open field test and the Y-maze compared to vehicle, suggesting a beneficial effect *via* promoting myelin repair. Our findings indicate that enhancing remyelination may be a potential therapy for schizophrenia.

Keywords: demyelination; myelin basic protein; muscarinic; open-field; Y-maze; antagonist; differentiation; oligodendroglia; oligodendrocyte precursor

INTRODUCTION

Dysfunctional neuronal communication may be the neuropathological basis of schizophrenia[1–4]. Increasing evidence has suggested that abnormalities of myelin sheaths prominently contribute to the disturbance of neuronal circuits[5,6]. A myelin sheath is a structure of compact multiple concentric cell membrane produced by oligodendrocytes (OLs) that wrap an axon segment in the central nervous system (CNS). As an essential component of the plasticity of neuronal circuits, myelin sheaths insulate and propagate electrical signals rapidly along axons[7]. More importantly, properly myelinated nerve fibers allow precise and accurate information to flow within and between neuronal circuits[3,8,9]. A number of imaging studies have shown that the white matter volume of schizophrenic brains is lower than in healthy controls, suggesting that myelin sheath damage or demyelination is involved in the schizophrenic brain[10–15]. In line with this hypothesis, microarray analyses have shown that genes down-regulated in schizophrenic brains are related to OL development and myelination, arguing that myelin repair may be an unmet need that has been overlooked in therapies for schizophrenia[16]. In support of this notion,

recent studies have shown that quetiapine, an atypical antipsychotic, is potent in promoting remyelination in a cuprizone-induced mouse model of demyelination and an inflammatory demyelination model[17–20], suggesting that myelin repair is beneficial in the treatment of schizophrenia.

In response to demyelination or myelin sheath damage, myelin repair or remyelination can be complete, as OL precursors (OPCs) are present in the CNS throughout the entire lifetime and are intrinsically capable of differentiating into mature myelinating OLs[21–23]. Inhibitory cues in the microenvironment could handicap remyelination efficiency of OPCs and result in remyelination failure. For instance, remyelination in the brain of multiple sclerosis patients may be incomplete due to the incapacity of OPC differentiation[24,25]. In an attempt to promote remyelination, a high-throughput functional screening system using micropillar arrays has been developed lately and a cluster of muscarinic antagonists have been identified to be capable of promoting the differentiation and myelination by oligodendroglia *in vivo*[26,27]. Among them, clemastine, an FDA-approved drug that can freely pass the blood-brain barrier, is a potent compound that greatly enhances remyelination in a lysolecithin- or cuprizone-induced mouse model of demyelination[26,27].

Cuprizone-treated rodents display white matter damage and behavioral changes that may reflect anxiety and the cognitive defects induced by demyelination[17,28,29]. Thus, in this study, the cuprizone-induced mouse model of demyelination was used to investigate the potential beneficial effects of remyelination on schizophrenia.

MATERIALS AND METHODS

Animals

C57BL/6 mice (male, 6 weeks old) were purchased from the Animal Facility Center of the Academy of Military Medical Sciences, China. The mice were housed under a 12 h/12 h dark/light cycle at a constant room temperature of 22°C and a relative humidity of 60%. All manipulations were performed in accordance with the protocols approved by the Committee on Animal Care and Use.

Drug Treatments

Clemastine (Cat #: S1847; SelleckChem, Houston, TX) was dissolved in DMSO at 10 mg/mL before further dilution in distilled water. The final concentration of DMSO was 0.004 *v*/*v*. After one week of acclimation, C57BL/6 mice were assigned to receive cuprizone (10 mg/kg per day) for six weeks or regular chow (n = 25 in each group), and five mice from each group were sacrificed for histological examinations (Fig. 1A); the remaining mice were then exposed to either clemastine (n = 10 per group) or vehicle (0.004% DMSO *v*/*v* in distilled water) (n = 10 per group) at 10 mg/kg per day for three weeks. Each group was separately identified and caged, and all mice were weighed weekly.

Immunostaining

Mice were deeply anaesthetized with sodium pentobarbital (150 mg/kg, i.p.) and perfused intracardially with PBS followed by 4% paraformaldehyde (Sigma, St. Louis, MO; in 0.1 mol/L PB, pH 7.4). Brains were collected and post-fixed overnight in the same fixative, followed by 30% sucrose at 4°C overnight. Serial coronal sections were cut on a sliding microtome (Leica Micro system 1900, Wetzlar, Germany) at 20 μm. For immunostaining, the sections were blocked in 5% normal goat serum with 0.1% Triton in PBS for 1 h and incubated with rat anti-myelin basic protein (MBP) (Abcam, Cambridge, MA), rabbit anti-platelet-derived growth factor receptor α (PDGFRα; Santa Cruz, Dallas, TX), or mouse anti-adenomatous polyposis coli (APC) (Millipore, Billerica, MA) primary antibody overnight at 4°C. Then the sections were incubated with Alex488- or Alex568-conjugated secondary antibody for 1 h.

Image Analysis

Measurements were made in the corpus callosum, hippocampus, and cortex. In each brain region, seven sections 300 μm apart were collected. Images of specific areas (the central part of the corpus callosum, the dorsal cerebral cortex, and CA1 of the hippocampus) were digitally recorded using a BX-51 microscope (Olympus, Tokyo, Japan) with fixed exposure time and gain value. The intensity of MBP staining and APC-positive cell numbers were analyzed on the raw images using Image-Pro Plus (version 5.1, Media Cybernetics, Inc., Silver Spring, MD). In these images, MBP-positive myelin was automatically distinguished from the background by Image-Pro plus, which allowed us to calculate the MBP intensity. The APC- or PDGFRα-positive cells were counted using the manual counting function of Image-Pro plus, and one positive cell was counted when positively-stained cytosol was visible.

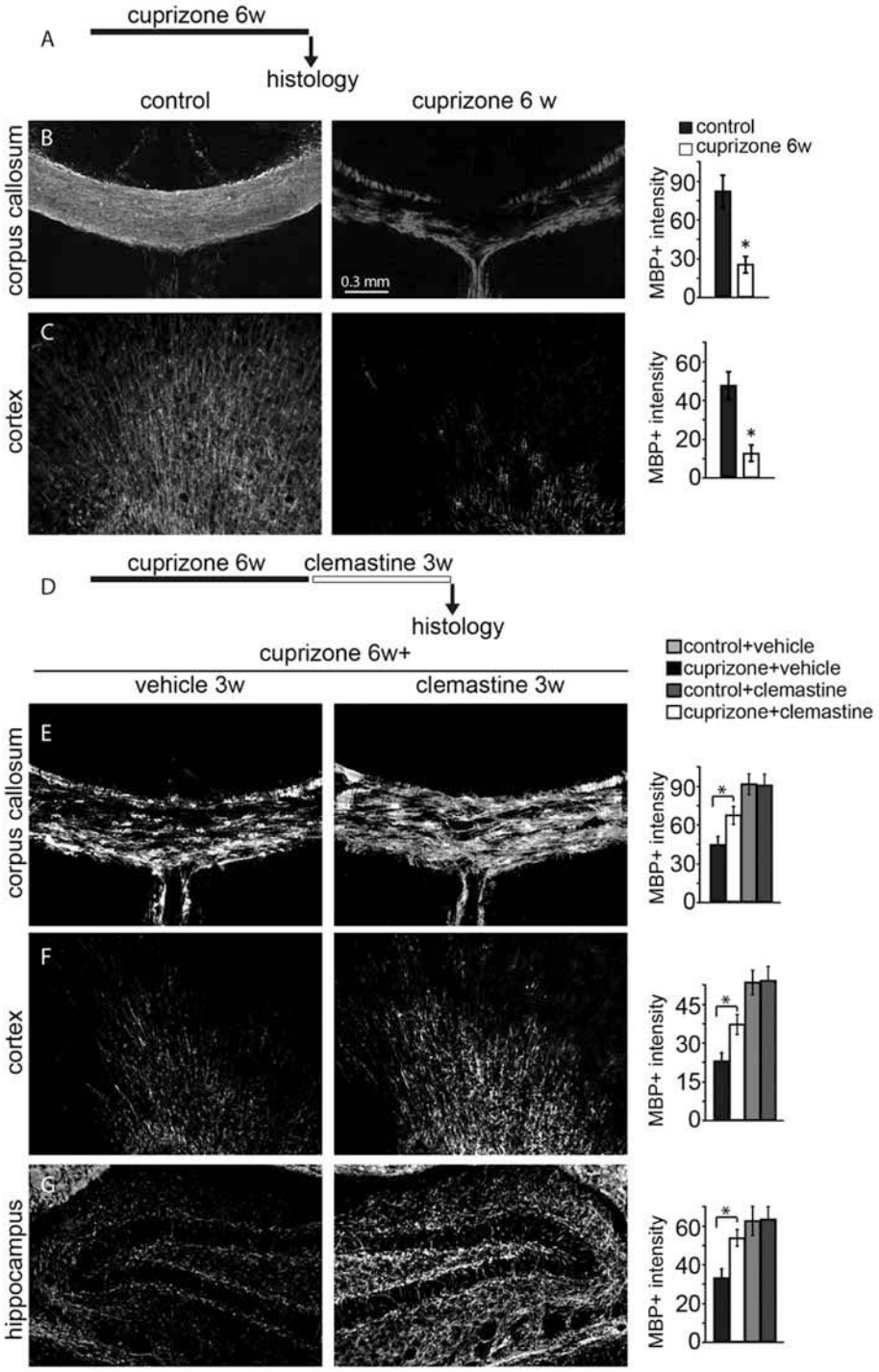

Fig. 1. Clemastine promotes remyelination after cuprizone-induced demyelination. (A, D) Schematic diagrams of the time-course of cuprizone (A) and clemastine treatment (D). (B, C) A dramatic decrease of MBP expression in the corpus callosum (B) and cortex (C) at the end of 6 weeks of exposure to cuprizone. (E–G) Evident recovery of MBP expression in the corpus callosum (E), cortex (F), and hippocampus (G) after 3 weeks of treatment with clemastine. Right panels: quantification of MBP intensity in the corpus callosum, cortex, and hippocampus with 6 weeks of cuprizone exposure (B, C) or treatment with clemastine for 3 weeks (E–G). *$P < 0.05$, $n = 5$ animals/group.

Open-Field Test

Exploratory activity was assessed in an open field, a 25 cm × 25 cm square surrounded by 35-cm high walls. The movement track of each animal was recorded by a video camera placed above the area. A video-tracking program (BW-OF302, Shanghai Biovill Co., Ltd, China) was used to measure the locomotor activity (total distance traveled) and exploratory activity (distance traveled in the central area of 10 cm × 10 cm, a reflection of anxiety-like behavior)[18,20,28,30].

Y-Maze Task

The apparatus consisted of a Y-maze of three arms (Shanghai Biovill Co., Ltd, China). Each arm was 35 cm long, 25 cm high, and 10 cm wide and positioned at equal angles (labeled A, B, and C). Each mouse was placed at the end of one arm and allowed to move freely through the maze during a 10-min session. The sequence of arm entries was recorded by video and calculated manually[17,18,20,30]. Spontaneous alternation is the tendency to explore new arms over the one previously chosen. A set of sequent entries to three individual arms (e.g. ABC, BCA, CAB) was counted as an alternation. The alternation measure was calculated as the percentage: Percent alternation = (number of alternations)/(total number of arm entries−2) ×100%[17].

Statistical Analysis

Data are expressed as mean ± SEM. All quantitative data were analyzed by one-way analysis of variance (ANOVA). Comparisons between two experimental groups were made using Student's *t*-test[18,28]. A significant difference was indicated when the *P*-value was <0.05.

RESULTS AND DISCUSSION

To determine the demyelination induced by cuprizone, we assessed the MBP expression in the corpus callosum and cortex at the end of the 6-week exposure to cuprizone (Fig. 1A). The intensity of MBP expression in the corpus callosum was decreased by 70% and in the cortex by 80% in the cuprizone model compared to the control animals, demonstrating a demyelinating response to cuprizone (Fig. 1B, C). Clemastine was given to the mice for 3 weeks after the 6-week exposure to cuprizone (Fig. 1D). We then found an evident 2-fold increase of MBP expression in the corpus callosum (Fig. 1E), the cortex (Fig. 1F), and the hippocampus (Fig. 1G) compared to the vehicle-treated brain. No significant difference was seen between the control brains with clemastine *versus* those with vehicle treatment (Fig. 1E–G), demonstrating that clemastine enhances remyelination in the cuprizone model of demyelination.

To understand the effect of clemastine on the oligodendroglial lineage, mature OLs were counted by APC immunostaining. The number of mature APC+ OLs was decreased to ~30% in the cortex (Fig. 2A) and corpus callosum (Fig. 2B) after exposure to cuprizone for 6 weeks. After 3 weeks of clemastine treatment, a ~50% increase in the number of mature OLs was found in the cortex (Fig. 2C) and corpus callosum (Fig. 2D) compared to vehicle. Besides, clemastine treatment did not change the number of APC+ cells in control mice (Fig. 2C,D). As the increased number of mature OLs was due to the differentiation of adult OPCs, we evaluated the OPC density in the brain by counting the PDGFRα-positive cells, and the OPC number in the clemastine treatment group did not significantly differ from the vehicle group (Fig. 2E), suggesting that clemastine does not change the OPC population in the brain, and the myelin repair is due to its positive effect on the differentiation of OLs.

Behavioral tests were carried out after 0, 1, and 3 weeks of clemastine treatment (Fig. 3A). The total distance traveled showed no significant difference between the clemastine treatment and vehicle groups, indicating that motor function in the cuprazone-treated animals was independent of the cuprizone-induced CNS demyelination (Fig. 3B). To evaluate whether myelin impairment was positively associated with the behavioral defects in the cuprizone model, the open-field test was used to assess anxiety-like behavioral changes by measuring the distance traveled in the central area[18,20,28,30]. It has been suggested that a decrease of exploration in the central area reflects increased anxiety in rodents. Our results showed that the distance traveled in the central area decreased after 6 weeks of cuprizone exposure, suggesting that anxiety-like behaviors increased, probably due to demyelination (Fig. 3C). Upon treatment with clemastine for 3 weeks, an evident recovery of the central area travel distance occurred in the clemastine-treated mice as compared to

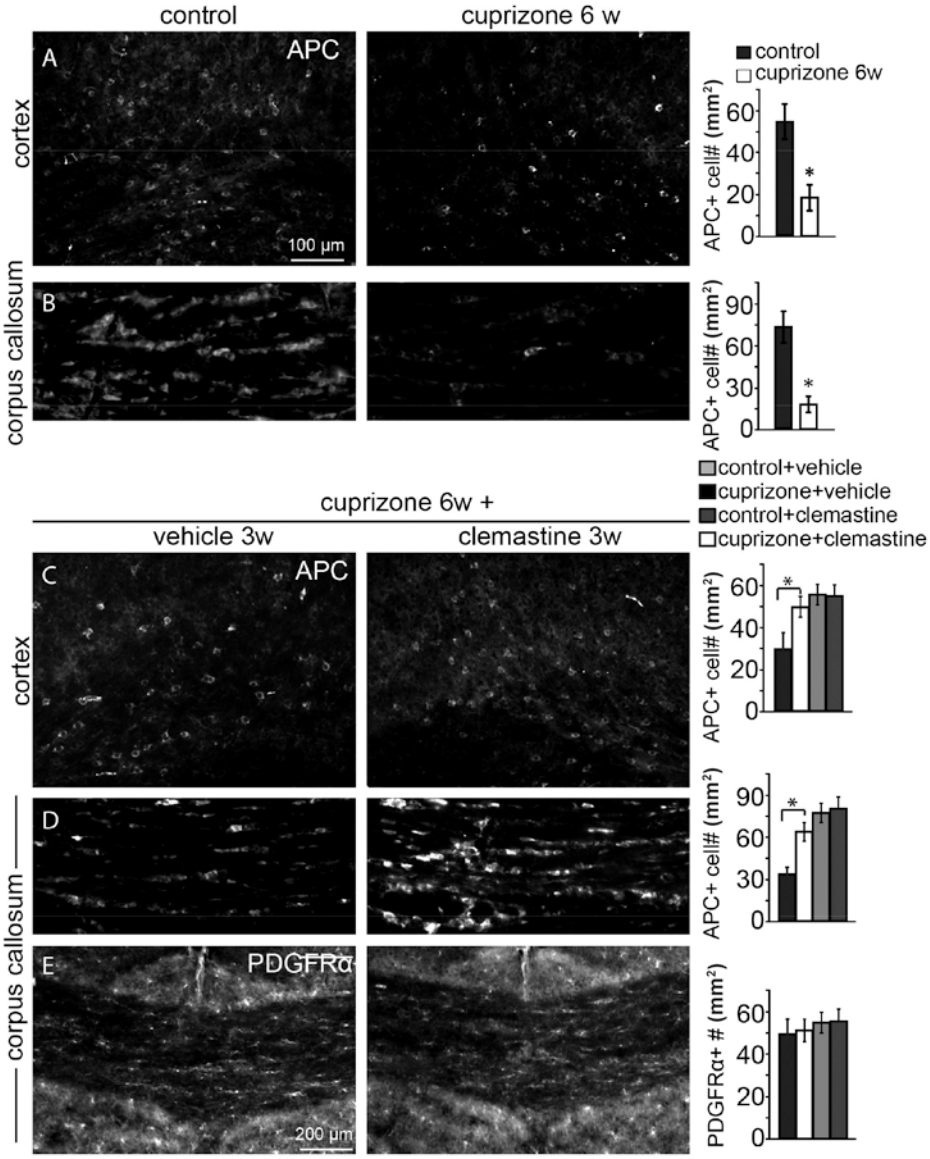

Fig. 2. Clemastine promotes the differentiation of oligodendroglia as assessed by immunostaining for mature oligodendrocytes (APC+) and OPCs (PDGFRα+). (A, B) Numbers of mature OLs (APC+) decreased to 30% after exposure to cuprizone for 6 weeks in the cortex (A) and corpus callosum (B). (C, D) Numbers of APC+ cells greatly recovered after 3 weeks of clemastine treatment in the cortex (C) and corpus callosum (D). (E) Number of PDGFRα+ cells was unchanged after treatment with clemastine for 3 weeks. *$P < 0.05$, $n = 5$ animals/group.

vehicle controls without changing the total distance traveled (Fig. 3C), suggesting that clemastine decreases anxiety-like behavior by promoting remyelination.

Impairments of spatial working memory have been reported in the cuprizone model and in schizophrenic patients[18,20,30]. To measure the spatial working memory in the cuprizone model, we counted the arm entries. As expected, the mice with cuprizone-induced demyelination had significantly increased arm entries and decreased alternations, suggesting impaired spatial memory at the end of six weeks of cuprizone exposure caused by demyelination (Fig. 4). Interestingly, clemastine treatment

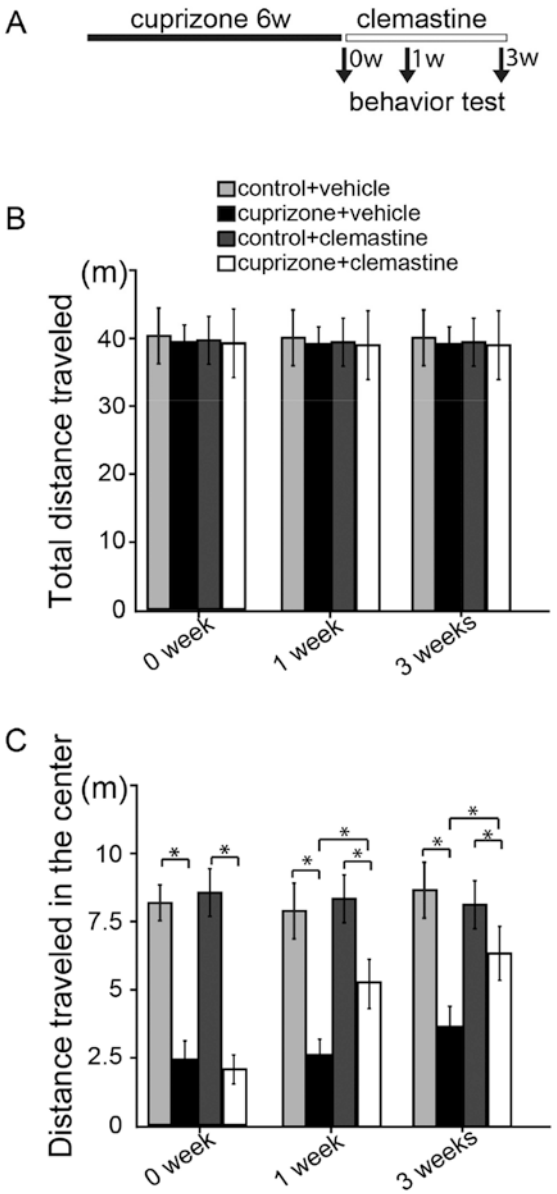

Fig. 3. Clemastine treatment rescues exploratory activity in the open field. (A) Schematic diagram of time-course of behavioral tests. (B,C) Analysis indicates the total distance was unchanged between cuprizone-exposed mice with or without clemastine treatment (B), but the distance in the central field decreased upon 6-week exposure to cuprizone, while clemastine gradually rescued the behavioral change (C). *P <0.05, n = 10 animals/group.

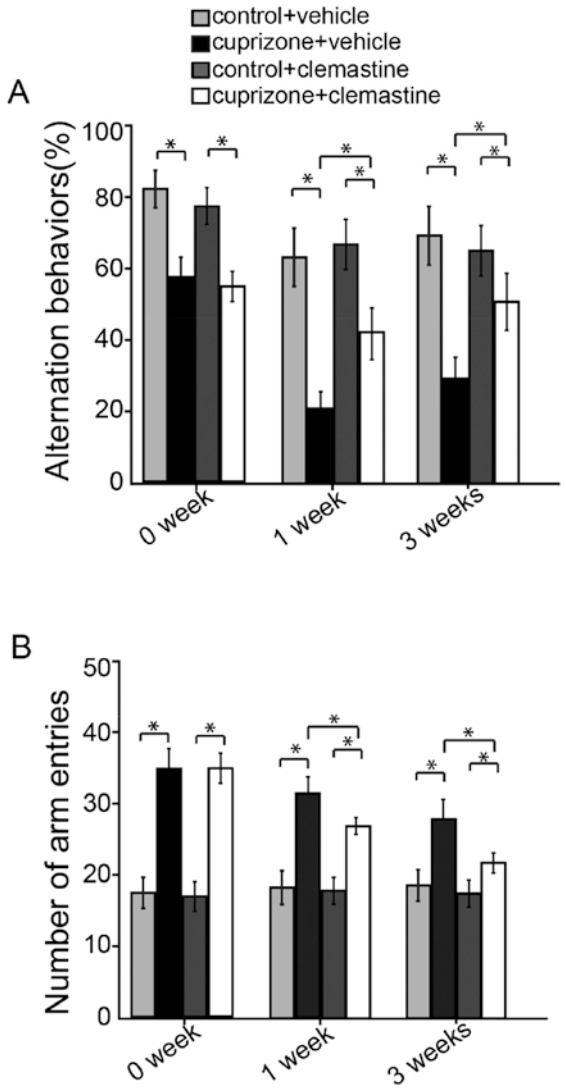

Fig. 4. Clemastine promotes recovery of cuprizone-induced behavioral change in the Y-maze task. (A) Clemastine rescued the cuprizone-induced decrease in the alternation of behavior. (B) Clemastine promotes recovery of the cuprizone-induced increase of arm entries. *P <0.05, n = 10 animals/group.

for 3 weeks significantly decreased the arm entries (Fig. 4A) and increased behavioral alternations (Fig. 4B), suggesting that clemastine rescues the spatial memory impairments by enhancing remyelination.

Myelin sheaths play an important role in insulating axons and propagating electrical signals rapidly. Recent studies have shown that myelination is essential for social experience-based cognition and motor skill learning[31–33], suggesting that myelin is an important component for the proper functioning of neuronal circuits, that is, properly

myelinated nerve fibers are essential for the information flows within and between neuronal circuits. Given that myelin formation is actively involved in the plasticity of neuronal functions in adult animals[34-36], abnormalities of the myelin sheath may contribute to defects of neuronal function. Increasing evidence has shown that the impairment of white matter integrity is prominent in schizophrenic brains[10,11,14,15] and further underscores the importance of the myelin sheath in schizophrenia, pointing to myelin repair as a potential therapeutic strategy for schizophrenia. In support of this notion, quetiapine, an atypical anti-schizophrenic drug, has been found to be potent in promoting the differentiation of OLs *in vitro* and enhancing remyelination in mouse models of demyelination[17–20]. In the present study, we used clemastine as the reagent to promote remyelination in the cuprizone-induced demyelination model, given that it is potent in promoting remyelination and also passes the blood-brain barrier. As expected, clemastine was capable of promoting remyelination, suggesting that it may be used for this purpose.

Cuprizone induces global demyelination in the brain and has been widely used to investigate the mechanisms of remyelination. A number of reports have indicated that this demyelination model also displays abnormal behaviors and cognitive defects that are believed to be a direct result of demyelination[28,29,37,38]. The behavior changes may reflect neuronal abnormalities, like anxiety and cognitive impairment, and mimic, at least partially, schizophrenia symptoms in humans. It has been well documented that quetiapine rescues the behavioral changes in the cuprizone-induced animal model of demyelination, presumably by promoting remyelination or protecting myelin from breakdown[17,18]. Quetiapine targets multiple receptors, including dopaminergic, serotoninergic, adrenergic, and histaminergic receptors[20]. Given the non-specific binding affinity, it is possible that the effect of quetiapine on behavioral changes is independent of the enhanced remyelination. Different from quetiapine, clemastine has a high affinity for muscarinic and histaminergic receptors. Our results indicate that clemastine treatment is beneficial for rescuing schizophrenia-like behaviors and spatial memory impairment in cuprizone-treated mice. We propose that the effects of clemastine on the behavioral changes may directly result from enhanced remyelination, although a possibility of the contribution of muscarinic antagonism cannot be excluded in this case. Nonetheless, muscarinic antagonism is a potential approach for schizophrenia therapy.

Altogether, our results indicate that clemastine treatment enhances myelin repair in the demyelinated regions with an increase of mature OLs and up-regulation of MBP. More importantly, clemastine treatment rescued the schizophrenia-like behavioral changes in the open field and Y-maze tests as compared to vehicle, suggesting a beneficial effect on rescuing schizophrenia-like behavioral changes *via* promoting myelin repair. These findings, for the first time, suggest that enhancing remyelination may be a potential therapy for schizophrenia.

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