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·Original Article·

Locomotor activity and anxiety status, but not spatial working memory, are affected in mice after brief exposure to cuprizone

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ABSTRACT

Chronic long-term exposure to cuprizone causes severe brain demyelination in mice, which leads to changes in locomotion, working memory and anxiety. These findings suggest the importance of intact myelin for these behaviors. This study aimed to investigate the possible behavioral changes in mice with mild oligodendrocyte/myelin damage that parallels the white matter changes seen in the brains of patients with psychiatric disporders. We used the cuprizone-treated mouse model to test both tissue changes and behavioral functions (locomotor activity, anxiety status, and spatial working memory). The results showed that mice given cuprizone in their diet for 7 days had no significant myelin breakdown as evaluated by immunohistochemical staining for myelin basic protein, while the number of mature oligodendrocytes was reduced. The number and length of Caspr protein clusters, a structural marker of the node of Ranvier, did not change. The locomotor activity of the cuprizone-treated mice increased whereas their anxiety levels were lower than in normal controls; spatial working memory, however, did not change. These results, for the first time, link emotion-related behavior with mild white matter damage in cuprizone-treated mice.

Keywords: myelination; oligodendrocyte; locomotor activity; anxiety; spatial working memory; cuprizone; mouse

INTRODUCTION

Recently, studies have found that oligodendrocyte/myelin alterations show consistent pathological changes in several mental disorders, including schizophrenia, mood disorders, and attention deficient hyperactivity disorder[1-6]. These findings highlight the important role of oligodendrocytes/myelin in the behavioral abnormalities and treatment of mental disorders. Animal models with altered oligodendrocytes/myelin can provide valuable information about the relationship between these alterations and disease-related behavioral changes[6].

Bis-cyclohexylidenehydrazide (cuprizone) is a copper chelator that has been shown to specifically damage the white matter of the brain[7, 8]. In recent animal studies, abnormal cognitive and emotion-related behaviors were reported in cuprizone-exposed mice with demyelination. For example, after exposure to 0.2% cuprizone for three and four weeks, mice exhibited an increase in brain activity and

a reduced anxiety response to a novelty challenge test[9]. In another study, C57BL/6 mice given 0.2% cuprizone for two to six weeks showed a variety of abnormal behaviors at various time points. Mice exposed to cuprizone for two and three weeks displayed more climbing behavior and reduced prepulse inhibition; mice exposed to cuprizone for four to six weeks had less social interaction, along with severe brain demyelination, myelin breakdown, and loss of oligodendrocytes. At all these time points, the cuprizone-exposed mice spent more time in the open arms of an elevated plus-maze and exhibited spatial working memory impairment[10].

Although behavioral changes are associated with profound demyelination in cuprizone-treated mice, no efforts have been made to investigate the behaviors of mice with mild oligodendrocyte/myelin damage that parallels the white matter changes seen in the brains of schizophrenic patients. We therefore set out to determine whether mice with mild white matter damage have abnormalities in emotion-related behaviors and spatial working memory.

MATERIALS AND METHODS

Animals and Experimental Procedures

Eight-week old male C57BL/6 mice were purchased from Central Animal Care Services, University of Manitoba (Winnipeg, MB, Canada). The animal facility maintained a 12 h/12 h light-dark cycle, a constant temperature of 21 ± 1°C, and 60% humidity. After acclimatization for one week, mice were divided into two groups: control (n = 12) and curprizone groups (n = 16). Cuprizone was from Sigma-Aldrich (St. Louis, MO) and mixed into milled LabDiet rodent chow (PMI Nutrition International LLC, Brentwood, MO) at 0.2% (*w*/*w*). The control group received the rodent chow without cuprizone, while those in the cuprizone group consumed the cuprizone-containing diet for 7 days. Starting from the eighth day, behavioral tests evaluating locomotor activity, spatial working memory, and anxiety status were carried out in order, with an interval of 24 h between each test. The tests were performed from 09:00 to 12:00 each day, and before each test the mice were allowed to adapt to the test room for one hour. The mice were then sacrificed (details are in the Immunohistochemical and Immunofluoresence part below) for immunohistochemical and immunofluorescent staining to examine the white matter status. During the experimental period, food and water were provided *ad libitum*. All procedures were in accordance with the guidelines set by the Canadian Council on Animal Care and approved by the University of Manitoba Committee on Animal Care and Supply.

Behavioral Tests

Locomotor activity test Locomotor activity was measured in a transparent Plexiglas activity box (40 × 40 × 23 cm^3) using the Animal Activity Monitor System (Columbus Instruments, Columbus, OH). This system sent out 12 × 12 horizontal crossed infrared sensor beams which covered the whole arena of the box. An event was automatically counted once the beams were broken by a mouse moving around inside the box and the number of times the beams were broken represented the horizontal activity of the mouse. In the test, a mouse was placed in one corner of the box and allowed to move freely for 10 min. The horizontal movement was measured by counting the number of times the horizontal infrared sensor beams were broken.

Elevated plus-maze test The anxiety level of mice was assessed in an elevated plus-maze in a 5-min session. The maze consisted of two opposite open and closed arms (25 × 5 cm^2) connected by a central area (5 × 5 cm^2) 40 cm above the ground. A mouse was placed in the central area facing an open arm at the beginning of the test and allowed to move freely in the maze during the 5-min test session. A mouse's entry into any of the four arms was counted when all four paws entered the arm and evaluated by ANY-maze software (Version 4.70; Stoelting Co., Wood Dale, IL).

Y-maze test The spatial working memory of mice was assessed with the Y-maze test. The maze was made of grey-painted wood. Each arm was 40 cm long, 12 cm high, 3 cm wide at the bottom, and 10 cm wide at the top. The arms converged in an equilateral triangular central area. An individual mouse was placed at the end of one arm and allowed to move freely in the maze during an 8-min session. An arm entry was counted when all four paws were in the arm. Alternation was defined as successive entries into the three arms, on overlapping triplet sets. Spontaneous alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus 2) and expressed as a percentage. The alternating behavior of rodents in a Y-maze has been used to evaluate spatial working memory in previous

studies[14-16]. The test was recorded by a camera above the apparatus and analyzed by ANY-maze software.

Immunohistochemistry and Immunofluorescence

C57BL/6 mice were deeply anesthetized with isoflurane and perfused intracardially with phosphate-buffered saline (PBS; pH 7.4). The brains were removed and fixed in 4% paraformaldehyde in PBS at 4°C overnight, then cryoprotected in 30% sucrose at 4°C for 24–48 h. Serial coronal sections (15 μm) were cut on a sliding microtome (Thermo, Kalamazoo, MI). The free-floating sections were pretreated with 3% hydrogen peroxide in PBS for 20 min, washed with PBS, and incubated with a blocking solution composed of 0.3% Triton X-100 and 5% normal rabbit serum at 22°C for 30 min. Alternate sections were subsequently incubated with primary antibody to myelin basic protein (MBP) (1:500; Santa Cruz Biotechnology, CA), which is a primary structural protein of the myelin sheath, or antibody to the pi form of glutathione-S-transferase (GST-π) (1:500; BD Biosciences, Missisauga, ON, Canada), a marker for mature oligodendrocytes, in a blocking solution at 4°C overnight. After rinsing in PBS, the sections were incubated in a solution of biotinylated secondary antibody (1:1 000) at 22°C for 2 h. The immunoreactive product was labeled by incubation in an avidin-biotin-horseradish peroxidase solution of Vectastain (Elite) (ABC kit; Vector Labs, Burlingame, CA) at 22°C for 30 min. Finally, the sections were developed with a VIP kit (Vector Labs). For immunofluorescence, free-floating sections were blocked with a blocking solution composed of 0.3% Triton X-100 and 5% normal goat serum. The sections were then incubated with the primary antibody to contactin-associated protein (Caspr) (1: 2 000; Abcam, San Francisco, CA) in the blocking solution at 4°C overnight. Caspr expression is restricted to the paranodal junctions and is used as a marker of nodes of Ranvier[17]. After rinsing in PBS, the sections were incubated with Alexa Fluor®594 goat anti-rabbit IgG (H+L) at 22°C for 1 h. After rinses in PBS, the sections were mounted and protected from quenching using Fluoromount (Sigma), then observed under a Nikon TE 2000-E fluorescence microscope (Nikon Instruments Inc., Melville, NY).

Image Analysis

Coronal sections between 1.98 mm and 0.74 mm from bregma were photographed and the targets measured/counted with Image-Pro Plus (version 4.1, Fryer, Huntley, IL). The results were expressed as the percentage of MBP-positive area over the examined area and the number of GST-π positive cells/mm^2. The paired Caspr-positive staining (Caspr clusters) was expressed as the Caspr cluster number/mm^2. In addition, the length of Caspr clusters was measured and presented as the frequency in each subgroup.

Statistical Analysis

The two-tailed Student's *t*-test was performed to determine differences between the control and cuprizone groups. The results are expressed as mean ± SEM. When a *P* value was <0.05, the difference was considered significant.

RESULTS

Behavioral Outcomes of Cuprizone-exposure for Seven Days

The cuprizone group showed more horizontal activity in the locomotor activity test than the control group (1269 ± 62 *versus* 969 ± 91, the number of times beams were broken, *P* <0.01; Fig. 1C). In the Y-maze test, the cuprizone group had spontaneous alternation and total arm entries comparable to the control group (Fig. 1D, E). In the elevated plus-maze, mice in the cuprizone group spent more time in the two open arms and more frequently entered the open arms than the control group (Fig. 1F, G). Treated mice traveled a distance in the two closed arms of the maze comparable to that of the control group (Fig. 1H).

GST-π-positive Cells Decreased in Cuprizone-exposed Mice

GST-π is a myelin- and oligodendrocyte-associated enzyme used to identify mature (myelin-forming) oligodendrocytes in mice[18]. In sections labeled with the specific antibody to GST-π, cuprizone exposure for 7 days reduced the number of GST-π positive cells (Fig. 2A–D). Statistical analysis showed a significant difference in the number of GST-π positive cells in a segment of the corpus callosum between the cuprizone-fed group and the normal control group (Fig. 2E).

No Significant Myelin Sheath Damage Was Seen in Cuprizone-exposed Mice

In sections labeled with the specific antibody to MBP,

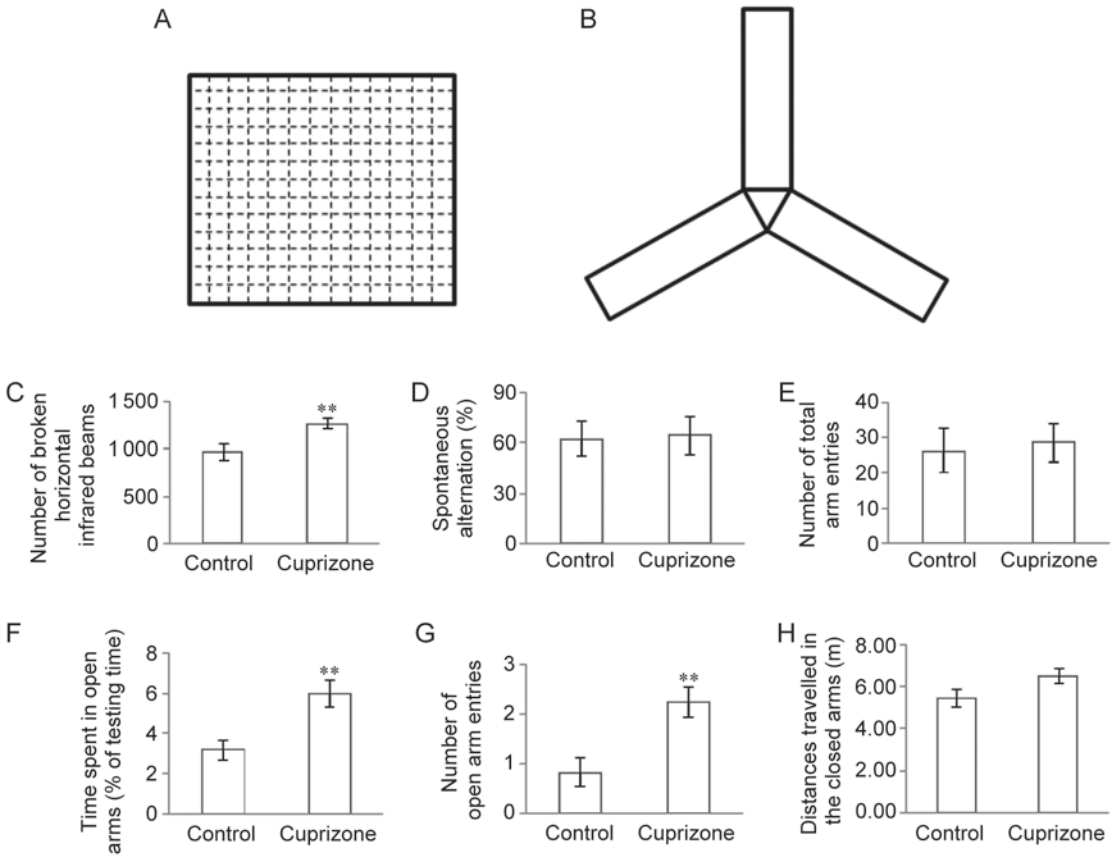

Fig. 1. Locomotor activity, spatial working memory, and anxiety status of C57BL/6 mice fed a cuprizone-containing diet for 7 days. A: Plexiglas activity box; dashed lines (12 × 12) indicate the infrared sensor beams. B: Y-maze apparatus. C: Mice in the cuprizone group showed an increased horizontal locomotor activity during a 10-min test session as shown by the number of broken horizontal beams. D–E: In the Y-maze test, mice in the cuprizone group showed spontaneous alternation (C) and total arm entries (D) comparable to the control group. F–H: In the elevated plus maze test, mice in the cuprizone group spent more time on open arms (F) and more frequently entered the open arms of the maze (G). Mice in the cuprizone group traveled a distance in the two closed arms comparable to the control group (H). Data are expressed as mean ± SEM (*n* = 12–16/group). **P* <0.05; ***P* <0.01.

a structural protein specifically expressed in the myelin sheath, cuprizone exposure for 7 days did not cause evident demyelination in the frontal cortex and corpus callosum, as the control and cuprizone groups showed similar MBP staining (Fig. 3A–D). There was no statistically significant difference between the cuprizone-fed and the normal control groups (Fig. 3E).

The nodes of Ranvier are delicate structures arranged regularly along myelinated axons to allow the efficient conduction of action potentials. Caspr is a structural protein important for maintaining the nodes of Ranvier intact[17]. In sections with the specific antibody to Caspr, it was hard to differentiate the cuprizone-fed mice from normal controls, as they showed similar Caspr-positive clusters (Fig. 4A and B). Counts of the Caspr-positive clusters and measurements of their lengths showed that the cuprizone-fed mice and the normal controls had comparable numbers of clusters (Fig. 4C) and the same frequencies of cluster lengths (Fig. 4D).

DISCUSSION

In previous studies, mice consuming cuprizone for three weeks showed a tendency to increase locomotor activity in the open-field test[9] and more climbing behavior[9, 10]. Meanwhile, these mice showed clear demyelination in

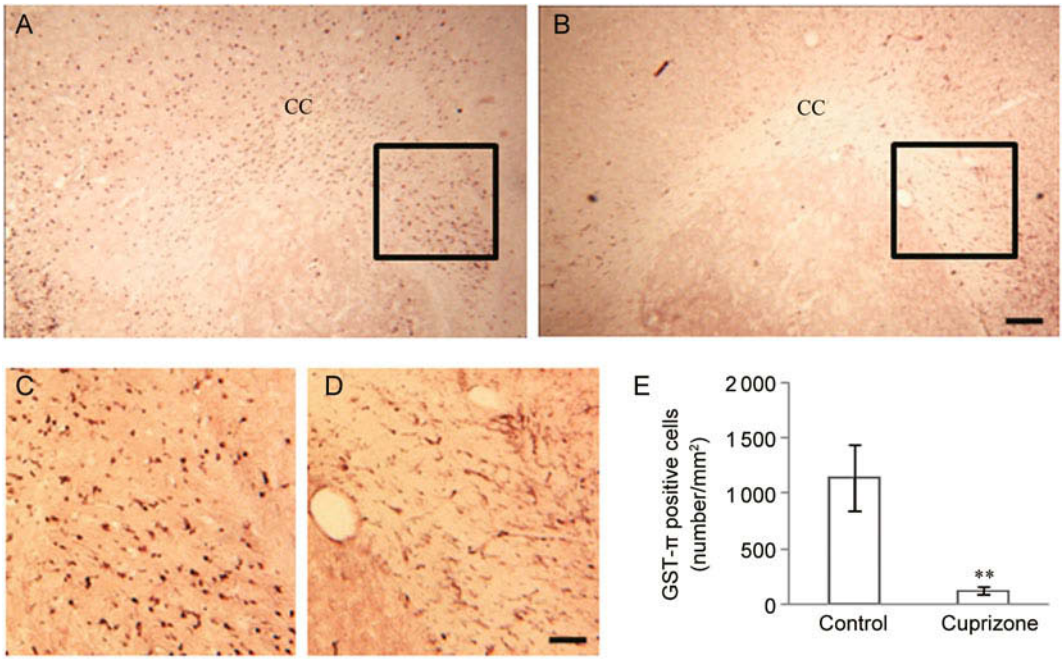

Fig. 2. Loss of mature oligodendrocytes in C57BL/6 mice fed a cuprizone-containing diet for 7 days. A–D: Representative images showing GST-π positive cells in sections from control (A) and cuprizone-challenged mice (B). Framed regions in A and B magnified in C and D. E: Quantitative analysis of GST-π-positive cells in the corpus callosum (CC). Data are expressed as mean ± SEM (*n* = 6/group). ***P* <0.01. Scale bars: A and B,125 µm; C and D, 50 µm.

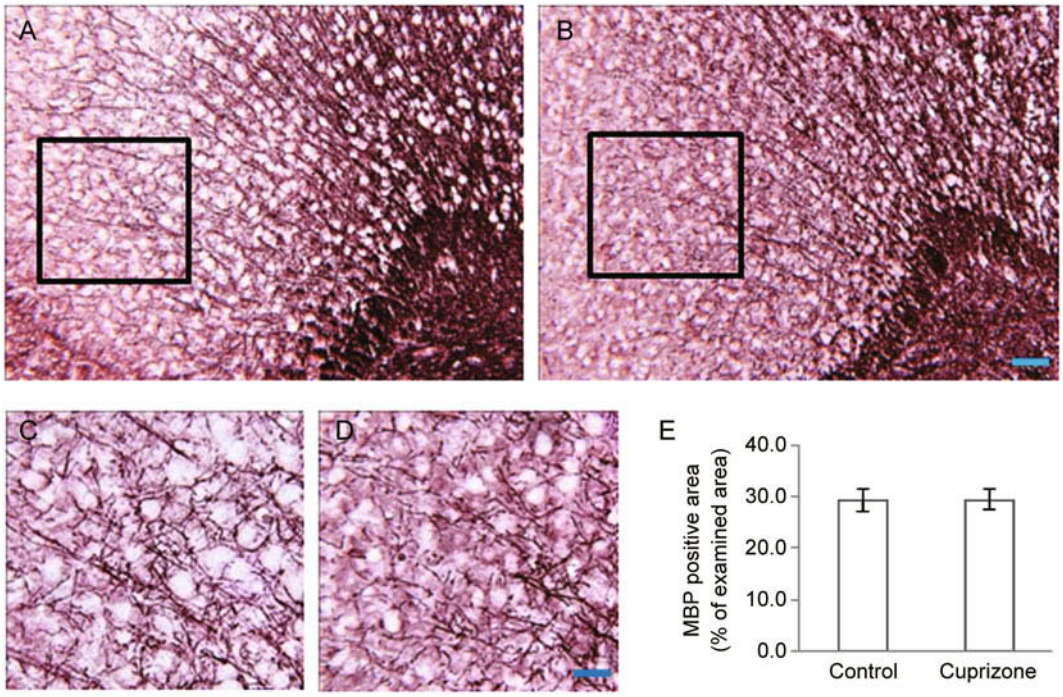

Fig. 3. No demyelination in C57BL/6 mice fed a cuprizone-containing diet for 7 days. A–D: Representative images showing MBP-like immunoreactivity in sections from control (A) and challenged mice (B). Framed regions in A and B magnified in C and D. E: Quantitative analysis of MBP staining area in the frontal cortex. Data are expressed as mean ± SEM (*n* = 6/group). Scale bars: A and B, 100 µm; C and D, 50 µm.

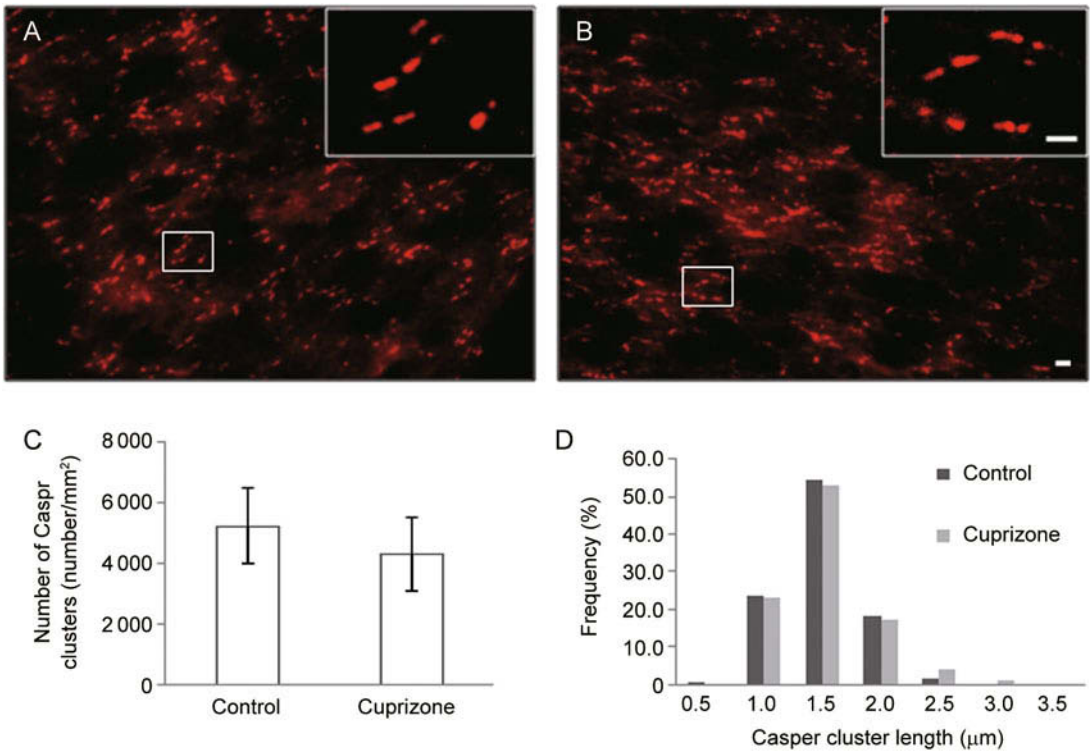

Fig. 4. No change in Caspr clusters in C57BL/6 mice fed a cuprizone-containing diet for 7 days. A and B: Representative images showing Caspr-positive clusters in sections from control (A) and challenged mice (B). Inserts in A and B are higher-magnification images of the selected areas. C: Quantitative analysis of the number of Caspr clusters in frontal cortex. Data are expressed as mean ± SEM (*n* = 6/group). D: Histogram showing the frequency distribution of the length of Caspr clusters in frontal cortex (*n* = 400–500/group). Scale bars, 5 μm (B) and 2.5 μm (inserts).

the brain. However, a definitive correlation between white matter damage and increased locomotor activity could not be reached until the same behaviors were assessed in cuprizone-exposed mice without demyelination. For the first time, we measured the locomotor activity of mice treated with cuprizone for only 7 days. Compared to normal controls, the cuprizone-treated mice showed increased locomotor activity, comparable immunostaining of MBP, normal number and distribution of Caspr (a node of Ranvier marker), but reduced numbers of mature oligodendrocytes. There are two potential explanations for the increased locomotor activity seen in the cuprizone-fed mice. One is that the increased locomotor activity is independent of intact myelin sheaths. This implies that the increased locomotor activity is related to increased brain activity, rather than myelin sheath damage. In support of this notion, increased locomotor activity and climbing behavior disappear in mice consuming a cuprizone-containing diet for 4, 5, or 6 weeks, which causes marked demyelination[9, 10]. Another explanation is that the increased locomotor activity seen in cuprizone-fed mice may be a consequence of mild myelin damage, as indicated by the reduced number of mature oligodendrocytes; the essential function of mature oligodendrocytes is to produce and maintain the integrity of myelin sheaths.

In contrast to locomotor activity, the spatial working memory of mice fed a cuprizone diet for 7 days was comparable to that of normal controls, suggesting that spatial working memory remains normal until the myelin sheath damage reaches a certain extent. In support of this view, decreased spontaneous alternation is seen in mice exposed to cuprizone for 2–6 weeks[10], which sufficiently damages white matter[7, 8, 12]. This decrease is also seen in mice with white matter changes caused by a non-toxic paradigm using methamphetamine[19]. Moreover, the cuprizone-induced spatial memory impairment is

significantly improved when the white matter damage spontaneously repairs after cuprizone withdrawal[20]. More supporting evidence for the essential role of intact white matter in spatial working memory includes: transgenic mice with impaired myelin/oligodendrocyte structure caused by blockade of erbB signaling[21] exhibit cognitive dysfunction; in rhesus monkeys, the number of myelinated axons is correlated with cognitive function between years 5 and 20[22]; and patients with multiple sclerosis exhibit cognitive impairments[23, 24]. Finally, myelin integrity has been correlated with cognitive processing speed in humans[25].

Mice exposed to cuprizone for 7 days spent longer periods in the open arms and more frequently entered the arms in the elevated plus-maze test, suggesting a lower anxiety level than controls. This finding is in accordance with the results of our recent study in which mice fed a cuprizone-containing diet for 2–6 weeks spent longer periods in the open arms of the elevated plus-maze[10]. As presented in this study, cuprizone feeding for 7 days did not severely damage the myelin sheath in the brains of mice as evaluated immunohistochemically. However, it is possible that 7-day treatment with cuprizone caused mild damage that was not severe enough to be detected by the immunohistochemical staining used in the present study. Therefore, it appears that abnormal performance in the elevated plus-maze test either is independent of intact myelin sheaths or is related to mild damage of sheaths caused by short-term cuprizone treatment. A recent study reported complete recovery of mouse performance in the elevated plus-maze in the second week after cuprizone withdrawal, when white matter damage was still evident[20], which did not support an association between the myelin sheath and anxiety. Definitely, further study using electron microscopy would be helpful for detecting possible damage caused by short-term cuprizone treatment.

It has been demonstrated in mice that demyelination occurs in the third week of cuprizone-feeding and thereafter[7, 11, 26]. Recent studies have investigated the white matter of mice exposed to cuprizone for one and two weeks. Cuprizone exposure for two weeks significantly decreased the expression of MBP mRNA in the corpus callosum[13]. In another study[11], two weeks of cuprizone feeding caused a significant decrease in the number of GST-π-positive cells in the corpus callosum, suggesting a decrease in mature oligodendrocytes. This is in line with the increased apoptosis seen in mice treated with cuprizone for two weeks[11]. In the same study[11], one week of cuprizone feeding caused no changes in the number of GST-π positive cells, in contrast to the report by Hesse *et al*.[27], which found decreased Nogo-A-positive oligodendrocytes in mice that consumed a cuprizone-containing diet for 4 or 6 days. The discrepancy in mature oligodendrocyte numbers after cuprizone exposure for a short time may be due to the different brain regions examined in different studies, as we found more severe loss of mature oligodendrocytes in the anterior than the posterior part of brain (data not shown). Consistent with Hesse's study, we found a significant decrease in the number of GST-π-positive cells in the corpus callosum and cerebral cortex, but no changes in MBP-like immunoreactivity and in Caspr clusters, suggesting that the cell bodies of oligodendrocytes may be more sensitive than their processes in response to cuprizone challenge. Alternatively, myelin pathology and mature oligodendrocyte death may be independent processes[28].

The data presented in this study expand the current understanding of the influence of oligodendrocyte/myelin changes on behavior. However, future studies investigating specific brain regions such as prefrontal cortex are warranted as distinct brain regions relate to different behavioral changes. The mechanisms underlying hyperactivity and decreased anxiety levels in short-term cuprizone-exposed mice were not addressed in this study either. Although oligodendrocyte loss is associated with abnormal emotional behavior, we do not know the underlying molecular mechanism. The only clue is the findings from one of our recent studies in which mice fed a cuprizone-containing diet for two weeks showed higher dopamine and lower norepinephrine in the frontal cortex, along with a lower anxiety level and increased climbing behavior[10]. These findings suggest an association between abnormal emotional behaviors and altered levels of dopamine and norepinephrine in the brain, which is independent of the condition of myelin sheaths, but can nevertheless be induced by cuprizone treatment. In addition, normal MBP immunostaining and Caspr clusters after short-term cuprizone exposure do not necessarily mean that action potential conduction along axons is unaffected in these animals. Examining the electrophysiological properties of the short-term cuprizone-

exposed mice will be an interesting and challenging task for future studies.

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REFERENCES

[1] Takahashi N, Sakurai T, Davis KL, Buxbaum JD. Linking oligodendrocyte and myelin dysfunction to neurocircuitry abnormalities in schizophrenia. Prog Neurobiol 2011, 93: 13–24.

[2] Mahon K, Burdick KE, Szeszko PR. A role for white matter abnormalities in the pathophysiology of bipolar disorder. Neurosci Biobehav Rev 2010, 34: 533–554.

[3] Tham MW, Woon PS, Sum MY, Lee TS, Sim K. White matter abnormalities in major depression: evidence from post-mortem, neuroimaging and genetic studies. J Affect Disord 2011, 132: 26–36.

[4] Thomason ME, Thompson PM. Diffusion imaging, white matter, and psychopathology. Annu Rev Clin Psychol 2011, 7: 63–85.

[5] D'Agati E, Casarelli L, Pitzianti MB, Pasini A. Overflow movements and white matter abnormalities in ADHD. Prog Neuropsychopharmacol Biol Psychiatry 2010, 34: 441–445.

[6] Xu H, Li XM. White matter abnormalities and animal models examining a putative role of altered white matter in schizophrenia. Schizophr Res Treatment 2011, 2011: 826976.

[7] Hiremath MM, Saito Y, Knapp GW, Ting JP, Suzuki K, Matsushima GK. Microglial/macrophage accumulation during cuprizone-induced demyelination in C57BL/6 mice. J Neuroimmunol 1998, 92: 38–49.

[8] Morell P, Barrett CV, Mason JL, Toews AD, Hostettler JD, Knapp GW*, et al.* Gene expression in brain during cuprizone-induced demyelination and remyelination. Mol Cell Neurosci 1998, 12: 220–227.

[9] Franco-Pons N, Torrente M, Colomina MT, Vilella E. Behavioral deficits in the cuprizone-induced murine model of demyelination/remyelination. Toxicol Lett 2007, 169: 205–213.

[10] Xu H, Yang HJ, Zhang Y, Clough R, Browning R, Li XM. Behavioral and neurobiological changes in C57BL/6 mice exposed to cuprizone. Behav Neurosci 2009, 123: 418–429.

[11] Liu L, Belkadi A, Darnall L, Hu T, Drescher C, Cotleur AC*, et al.* CXCR2-positive neutrophils are essential for cuprizone-induced demyelination: relevance to multiple sclerosis. Nat Neurosci 2010, 13: 319–326.

[12] Mason JL, Jones JJ, Taniike M, Morell P, Suzuki K, Matsushima GK. Mature oligodendrocyte apoptosis precedes IGF-1 production and oligodendrocyte progenitor accumulation and differentiation during demyelination/remyelination. J Neurosci Res 2000, 61: 251–262.

[13] Binder MD, Cate HS, Prieto AL, Kemper D, Butzkueven H, Gresle MM*, et al.* Gas6 deficiency increases oligodendrocyte loss and microglial activation in response to cuprizone-induced demyelination. J Neurosci 2008, 28: 5195–5206.

[14] Hughes RN. The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. Neurosci Biobehav Rev 2004, 28: 497–505.

[15] Maurice T, Hiramatsu M, Itoh J, Kameyama T, Hasegawa T, Nabeshima T. Behavioral evidence for a modulating role of sigma ligands in memory processes. I. Attenuation of dizocilpine (MK-801)-induced amnesia. Brain Res 1994, 647: 44–56.

[16] Sarter M, Bodewitz G, Stephens DN. Attenuation of scopolamine-induced impairment of spontaneous alteration behaviour by antagonist but not inverse agonist and agonist beta-carbolines. Psychopharmacology (Berl) 1988, 94: 491–495.

[17] Einheber S, Zanazzi G, Ching W, Scherer S, Milner TA, Peles E*, et al.* The axonal membrane protein Caspr, a homologue of neurexin IV, is a component of the septate-like paranodal junctions that assemble during myelination. J Cell Biol 1997, 139: 1495–1506.

[18] Mason JL, Toews A, Hostettler JD, Morell P, Suzuki K, Goldman JE*, et al.* Oligodendrocytes and progenitors become progressively depleted within chronically demyelinated lesions. Am J Pathol 2004, 164: 1673–1682.

[19] Yang HJ, Wang L, Cheng Q, Xu H. Abnormal behaviors and microstructural changes in white matter of juvenile mice repeatedly exposed to amphetamine. Schizophr Res Treatment 2011, 2011: 542896.

[20] Xu H, Yang HJ, Rose GM, Li XM. Recovery of behavioral changes and compromised white matter in C57BL/6 mice exposed to cuprizone: effects of antipsychotic drugs. Front Behav Neurosci 2011, 5: 31.

[21] Roy K, Murtie JC, El-Khodor BF, Edgar N, Sardi SP, Hooks BM*, et al.* Loss of erbB signaling in oligodendrocytes alters myelin and dopaminergic function, a potential mechanism for neuropsychiatric disorders. Proc Natl Acad Sci U S A 2007, 104: 8131–8136.

[22] Sandell JH, Peters A. Disrupted myelin and axon loss in the

anterior commissure of the aged rhesus monkey. J Comp Neurol 2003, 466: 14–30.

[23] Peyser JM, Edwards KR, Poser CM, Filskov SB. Cognitive function in patients with multiple sclerosis. Arch Neurol 1980, 37: 577–579.

[24] Rao SM, Leo GJ, Bernardin L, Unverzagt F. Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction. Neurology 1991, 41: 685–691.

[25] Liston C, Watts R, Tottenham N, Davidson MC, Niogi S, Ulug AM*, et al*. Frontostriatal microstructure modulates efficient recruitment of cognitive control. Cereb Cortex 2006, 16: 553–560.

[26] Linares D, Taconis M, Mana P, Correcha M, Fordham S, Staykova M*, et al*. Neuronal nitric oxide synthase plays a key role in CNS demyelination. J Neurosci 2006, 26: 12672–12681.

[27] Hesse A, Wagner M, Held J, Bruck W, Salinas-Riester G, Hao Z*, et al*. In toxic demyelination oligodendroglial cell death occurs early and is FAS independent. Neurobiol Dis 2010, 37: 362–369.

[28] Mason JL, Ye P, Suzuki K, D'Ercole AJ, Matsushima GK. Insulin-like growth factor-1 inhibits mature oligodendrocyte apoptosis during primary demyelination. J Neurosci 2000, 20: 5703–5708.