Behavioural Brain Research 331 (2017) 143–150

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Increased central dopaminergic activity might be involved in the behavioral abnormality of cuprizone exposure mice

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ARTICLE INFO

Keywords:
Cuprizone
Behavior
Demyelination
Dopaminergic activity

ABSTRACT

The neurotoxican cuprizone (CPZ, a copper chelator) has been used extensively to create a mouse model of demyelination. However, the effects on behavior of CPZ treatment have not been reported in C57BL/6 mice given a diet containing 0.4% CPZ within 3 weeks. Behavioral abnormalities were assessed using a range of test: Y-maze, spontaneous locomotor activity, rota-rod test, novel object recognition, climbing. Mice exposed to CPZ displayed more arm entrance, locomotor movements, and climbing behavior, suggesting an increase in central nervous system activity. However, no significant differences either in spontaneous alternation or latency to fall from the rotating drum were observed, demonstrating that spatial working memory or motor coordination and balance didn't impair by CPZ short-term exposure. In addition, they showed higher dopamine levels and dopamine transporter expression in the cortex. Our findings indicate that increased central dopaminergic activity may relate to the behavioral abnormality in mice, and this CPZ short-term exposure with higher dose may offer a model to study some aspects of biology relevant to schizophrenia and other related disorders.

1. Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS), which leads to myelin sheath breakdown and neuronal loss [1]. It is believed the MS autoimmune attack is orchestrated against antigens of myelin and oligodendrocytes. Recently, studies have found that myelin/oligodendrocytes alterations show consistent pathological changes in several mental disorders, including schizophrenia, mood disorders, and attention deficient hyperactivity disorder [2–4]. These findings highlight the important role of myelin/oligodendrocytes in the behavioral abnormalities. Animal models with altered myelin can provide valuable information about the relationship between these alterations and disease-related behavioral abnormality.

Traditionally used as a model of MS, cuprizone (CPZ, bis-(cyclohexanone)oxaldihydrazone) is a copper chelator that has been shown to specifically damage the oligodendrocytes of the brain [5]. C57BL/6 mice given 0.2% CPZ for three or four weeks showed a variety of abnormal behaviors, including deficits inhibition (PPI), reduced social interaction (SI), and impaired spatial working memory [6]. Several hypotheses have been proposed, but it is still unclear why CPZ, a copper chelator molecule, could induce behavior abnormality.

The physiopathology of CPZ model varied extensively in different CPZ treatment time and dose. In the present study we therefore determine to evaluate whether the behaviors of C57BL/6 mice changed after feeding with 0.4% CPZ during demyelinating process. We used Y-maze, spontaneous locomotor activity, rota-rod test, novel object recognition (NOR), and climbing standard models to measure spatial working memory, locomotor activity, motor coordination and balance, exploration, and climbing activity respectively. Our results demonstrate that the behavioral abnormality in mice treated with CPZ may relate to the increased central dopaminergic function.

2. Materials and methods

2.1. Animals and CPZ administration

Male C57BL/6 mice aged 6-week-old weight ranging between 17 g and 20 g were purchased from the Beijing Vital River Laboratory

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http://dx.doi.org/10.1016/j.bbr.2017.05.045
Received 22 January 2017; Received in revised form 11 May 2017; Accepted 17 May 2017
Available online 23 May 2017

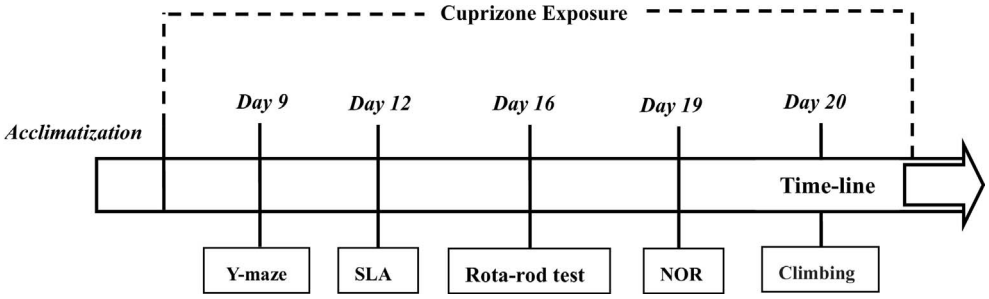

Fig. 1. Time-line and scheme for the behavioral tests in experiment. Control group was fed with normal chow (0% cuprizone), cuprizone exposure group was fed a diet containing 0.4% cuprizone, and all mice were sacrificed on day 21th. Behavioral assessments were conducted by one or two trained observers on the indicated day throughout the entire study. SLA, spontaneous locomotor activity; NOR, novel object recognition.

Animal Technology Co. Ltd. (Beijing, China), and housed in groups of 5 mice per cage under standard laboratory conditions with a 12/12 h light-dark cycle at a constant room temperature of 24 ± 1 °C and 50 ± 5% humidity. Food and water were available *ad libitum*. After an acclimatization period of one week, the mice from the cuprizone (CPZ) group were fed with 0.4% (w/w) CPZ (bis(cyclohexanone)oxaldihydrazone, Sigma-Aldrich, St. Louis, MO, USA) mixed in a standard ground rodent chow for 3 weeks. The control group was fed with the same standard ground chow without CPZ addition. Mixed chows were produced by Academy of Military Medical Sciences. Mice were weighed and examined daily. All procedures in this protocol were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and were approved by the Bioethics Committee of Xuan Wu Hospital of Capital Medical University.

2.2. Behavioral assessment

Behavioral studies were carried out by one or two trained observers throughout the entire study on scheme day of the experimental protocol (Fig. 1). All tests were administered in the 12-h light cycle in an isolated room. The following studies were performed: Y-maze, spontaneous locomotor activity, rota-rod test, novel object recognition (NOR), climbing and to measure the spatial working memory, locomotor activity, motor coordination and balance, exploration, and climbing activity, respectively. After each experiment, all devices were wiped clean to remove traces of the previous assay.

2.2.1. Y-Maze test

In order to test spatial working memory, the Y-maze (30 cm long, 15 cm high and 8 cm wide, with arms positioned 120° apart) was used according to a published protocol [7]. An individual mouse was placed at the end of one arm and allowed to move freely through the maze for a 10 min period. 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 Y-maze and all tests were recorded by a camera above the apparatus and analyzed by a video analysis system. The total distance, speed, rest time and the number of arm entries that mice explored and moved were all recorded.

2.2.2. Spontaneous locomotor activity

The spontaneous locomotor activity was measured using a locomotion detective system as previously described [8]. Mice were individually placed in a non-transparent cylinder cage (25 cm diameter × 13 cm height) for 6 min: 1 min was for adaptation and 5 min for recording activity. Frequency of activities was recorded by the total number of movements, including both horizontal and vertical movements.

2.2.3. Rota-Rod test

The rota-rod test was performed to examine motor coordination and balance [11]. The experiment was performed using an accelerating rota-rod (YLS-4C) where a mouse was placed on the rotating drum (3 cm diameter). One day before the test session, each mouse was trained in the drum for 3 min, which was accelerated gradually from a speed of 4–35 rpm. Training improves animals' skill and avoids fortuitous falling. After 24 h, each mouse was tested in the drum with the same speed over 5 min. The time each animal was able to maintain its balance on the rotating drum was recorded and presented as latency to fall.

2.2.4. Novel object recognition (NOR) test

The novel object recognition (NOR) test was conducted according to the experimental protocol as described previously [9]. Briefly, mice were habituated to an open field box (65 cm × 40 cm × 20 cm) for 10 min without any objects. An acquisition phase was conducted 24 h later, mice were allowed to explore for 10 min in the arena which containing two identical objects. A recognition phase was conducted 24 h later and one of the objects was replaced by a novel object. During recognition, two identical objects (a familiar object and a novel object) were placed in the arena and the animal was allowed to explore for 10 min. Exploratory behaviors were defined as the mice licking, sniffing, biting or touching the objects in a distance of approximately 2 cm. The familiar and novel objects were 10 cm high, being too heavy to be displaced by mouse and distinct in shape, color and texture. The total exploration time for novel and familiar objects of each mouse was recorded manually by use of two stopwatches. The discrimination index (DI) was calculated as the ratio of the time spent exploring the novel object (Tn) subtracting the time spent exploring the familiar objects (Tf) over the total exploring time, DI (%) = (Tn-Tf)/(Tn + Tf) × 100. The recognition index (RI) was defined as the ratio of the time spent exploring the novel object over the time spent exploring the novel and the familiar objects, RI (%) = Tn/(Tn + Tf) × 100 [10].

2.2.5. Climbing behavior test

Climbing activity is an index of central dopaminergic function [12]. Each mouse was placed into a cylinder wire mesh cage (15 cm diameter × 25 cm height) and observed for 5-min during which time spent on climbing was recorded. All four paws of mouse on the wire mesh were defined as climbing behavior.

2.3. Histological and immunohistochemical staining

All mice were sacrificed on day 21 following CPZ-exposure. Animals were anesthetized with chloral hydrate (400 mg/kg) by intraperitoneal injection and perfused intracardially with normal saline followed by 4% paraformaldehyde (PFA) in phosphate buffer (3.12 g/L of NaH_2PO_4, 28.64 g/L of $Na_2HPO_4{\cdot}12H_2O$, 30 g/L sucrose, pH-7.4) for fixation. The whole brain was removed and post-fixated in 4% PFA in phosphate buffer saline (PBS) at 4 °C overnight, followed by cryoprotection in 30%