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Research Article

Spontaneous remyelination following dimethyl sulfoxide-induced demyelination is accompanied by behavioral and neurological alteration in mice

Nikita V. Kudryashov[1], Alexander A. Gorbunov[1], Nadezhda B. Sviridkina[2], Sergey E. Mironov[1], Dmitriy A. Tikhonov[1], Andrey A. Nedorubov[1], Vladimir P. Fisenko[1]

1 Sechenov First Moscow State Medical University (Sechenov University), 8-2 Trubetskaya St., Moscow 119991 Russian Federation
2 OOO Research Registration Center Biolife, 34 1st Kuryanovskaya St., Moscow 109235 Russian Federation

Corresponding author: Nikita V. Kudryashov (kudryashov_n_v@staff.sechenov.ru)

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Abstract

Introduction: Dimethyl sulfoxide (DMSO) is a commonly used solvent that can be applied in experimental studies for preparation of hydrophobic solutions as well as in capacity of a cryopreservative in transplantology. According to modern data acquired from *in vitro* experiments, DMSO is able to change the structure of myelin by decreasing synthesis of its main components and inhibiting oligodendrocyte genesis.

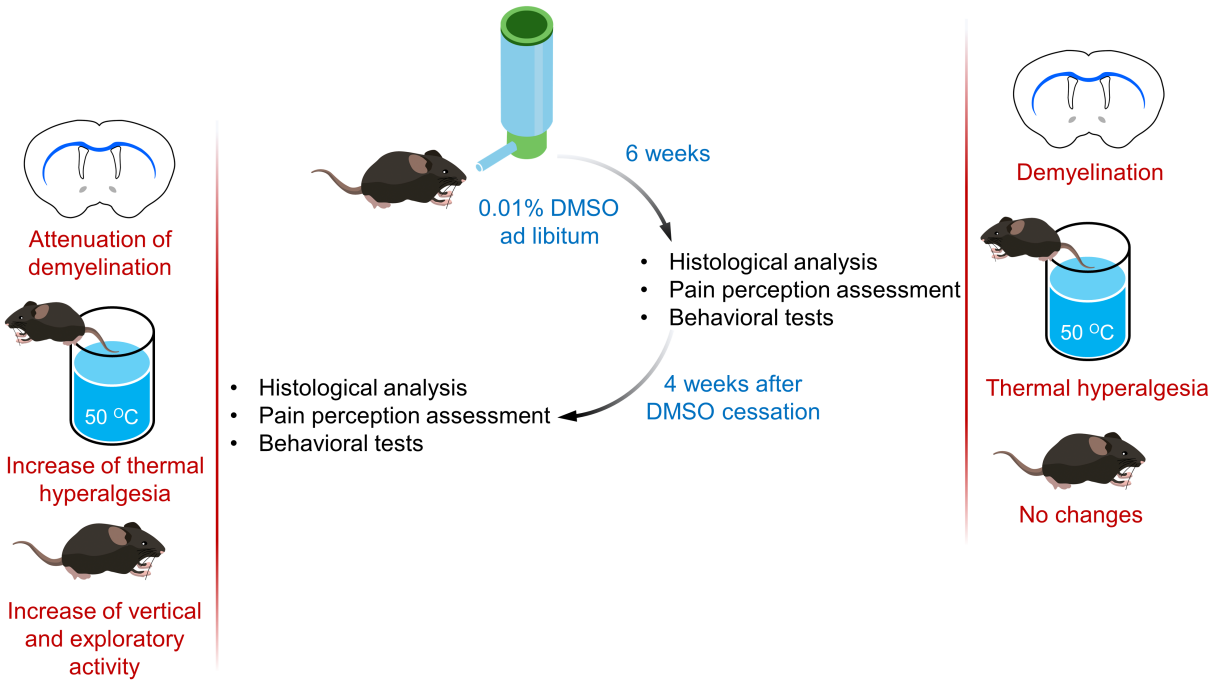
Aim of the study: We studied influence of DMSO on anxiety and compulsive-like behavior, pain perception, motor coordination and myelin quantity in the *corpus callosum* of the C57BL/6 mice brain after prolonged oral administration of the solvent and 4 weeks after administration was stopped.

Materials and Methods: All the experiments were conducted on male inbreed C57BL/6 mice. DMSO was added to drinking water to achieve 0.01% concentration, and the obtained solution was administered *ad libitum* for 6 weeks. After 6 weeks of administration of DMSO and 4 weeks after administration of DMSO was stopped, anxiety-like behavior in open field test, compulsive-like behavior in marble burying test, motor coordination in rotarod test, pain perception in tail-immersion test, as well as myelin quantity in the *corpus callosum* were evaluated.

Results: It was established that DMSO consumed for 6 weeks was associated with decrease in the myelin quantity in the *corpus callosum* and thermal hyperalgesia in tail-immersion test. During 4-week period after DMSO administration was stopped, attenuation of demyelination was observed, followed by an increase in thermal hyperalgesia in tail-immersion test, as well as vertical locomotion and exploratory activity in open field test.

Conclusion: 6-week *ad libitum* administration of 0.01% DMSO solution was associated with demyelination in *corpus callosum* of C57BL/6 mice, followed by thermal hyperalgesia. Cessation of DMSO led to spontaneous remyelination with an increase in thermal hyperalgesia, vertical locomotion and exploratory activity of mice.

Graphical Abstract



Keywords

demyelination, dimethyl sulfoxide, hyperalgesia, mice, remyelination

Introduction

Dimethyl sulfoxide (DMSO) is widely used in experimental studies for preparation of hydrophobic solutions as well as in capacity of a cryopreservative in transplantation (Tamagnini et al. 2014). Most common adverse effects of DMSO reported after organ transplantology include dysfunction of cardiovascular and respiratory systems, as well as central nervous system – amnesia and seizures (Abdelkefi et al. 2009). Moreover, in experimental studies prolonged administration of low concentrations DMSO has shown its ability to induce anxiety-like and compulsive-like behavior in mice (Kudryashov et al. 2022).

Neurotoxicity of DMSO can be explained by its influence on neuronal myelin sheath. According to modern data acquired from in vitro experiments, DMSO is able to change the structure of myelin, decreasing synthesis of its main components and inhibiting oligodendrocyte genesis (O'Sullivan et al. 2019; Sutrina et al. 1987). At the same time there is no data about DMSO influence on structure of myelin sheath after systematical prolonged administration.

In our experimental work, we focused on studying the influence of DMSO on anxiety reactions, compulsive-like behavior, motor coordination, pain perception and myelin quantity in the *corpus callosum* of the C57BL/6 mice brain right after systematical oral administration of DMSO and 4 weeks after the administration was stopped.

Materials and Methods

Animals

All the experiments were conducted on 27 male inbreed C57BL/6 mice with the body mass of 23-25 grams (breeding station “Andreevka” of Federal Publicly Funded Institution of Science “Science center of Biomedical Technology” of Federal Medico-Biological Agency of Russia). The animals were kept under standard conditions of the vivarium with natural ‘day/night’ light cycle and free access to drinking water and food. Animal care was organized according to the principles of Good Laboratory Practice established and approved by Council of the Eurasian Economic Commission (Resoluton № 81 of 03.11.2016) and Directive 2010/63/EU of the European Parliament and of the European Council of 22.09.2010. All the procedures with animals were considered and approved by the Local Ethics Committee of Sechenov First Moscow State Medical University (Minutes No. 25-22, December 8, 2022).

Compounds under study

In our study, we used DMSO (concentrate for solution 99%, Tatchempharmpreparaty JSC, Russia), added to drinking water to achieve 0.01% concentration. Obtained solution was administered *ad libitum* for 6 weeks. Mice in control group were given pure water without DMSO. Dose and regimen were chosen according to the results of preceding studies (Kudryashov et al. 2022). Equivalent dose for 0.01% solution accounts for 0.024 mg/kg.

Experimental design

Animals were divided into 3 groups: (1) control group, animals received pure drinking water during the whole experimental period – for 10 weeks; (2) DMSO group, animals received pure drinking water for 4 weeks and then 0.01% DMSO solution for 6 weeks; (3) post-DMSO group, animals received 0.01% DMSO solution for 6 weeks and then pure drinking water for 4 weeks (Fig. 1). Behavioral tests, evaluation of pain perception and quantity of myelin in the *corpus callosum* of the mice brain were conducted after 6 weeks of DMSO administration and then 4 weeks after administration was stopped.

Open field test

The experimental unit had the form of a round stage of 63 cm in diameter with walls of 32 cm in height (OpenScience, Russia) The arena was illuminated at 300 lx and the mice were kept in the dark – a location with illumination of 5 lx – for 30 min before being placed in the arena. During 2-minute period, peripheral and central horizontal activity, vertical activity and the number of holes explored in the stage floor were recorded. Total distance travelled was calculated as a sum of all locomotion activities (Kudryashov et al. 2022). After every animal, the stage was cleaned with 70% ethanol solution and paper towels.

Marble burying test

For the test, we used individual polycarbonate boxes for rodents (400 x 230 x 150 mm) with caps, filled with 5 cm of hard-packed corn flooring. Before the experiment, the mice were kept for 30 min in the box with corn flooring for habituation. After preliminary habituation, the flooring was rammed down and 20 glass marbles of 16 mm in diameter were placed on it. The mice were placed in the boxes with glass marbles for 30 min, and the number of buried marbles was counted afterwards (the marbles were considered buried if they were embedded in the flooring by two-thirds) (Kudryashov et al. 2022).

Rota-rod test

Motor coordination was evaluated with a specific mouse rotating rod (Ugo Basile, Italy) of 3 cm in diameter divided into 5 tracks, each 5.7 cm wide. The initial rotating speed was 4 r.p.m., maximal – 40 r.p.m., and the rate of rotation was accelerated by 1 rotation every 8 sec. The time the animal remained on a rotating rod before falling was measured (Jakkamsetti et al. 2021).

Tail-immersion test

Pain perception was evaluated by inducing heat painful stimuli after putting the mouse tail into a reservoir with hot water (constant temperature 50.0±1.0°C). Tail flick latency response was registered (Udell et al. 2021).

Histological analysis

Brain samplings were fixated in 2.5% glutaraldehyde solution for 7 days with subsequent slicing using a vibratome Leica VT 1000S into frontal sections of 13 µm thick in a way that every section contained splenium of *corpus callosum*. After drying on polylysine-coated glass slides, all samplings were stained with Luxol fast blue according to Kluwer-Barrera (Bio-Optica) to detect myelin and Nissl substance and covered by cover slip. The prepared samplings were examined by using Olympus BX1 microscope equipped with Olympus U-TV0.5XC-3 photo camera (magnification x20). The overall area of splenium of *corpus callosum* caught on camera with magnification x20 was detected with Olympus cellSens software, with subsequent measurement of the damaged area – off-color zones lacking myelin. Demyelination was identified by the ratio between the sum of damaged areas to overall area in percentage (Yu et al. 2017).

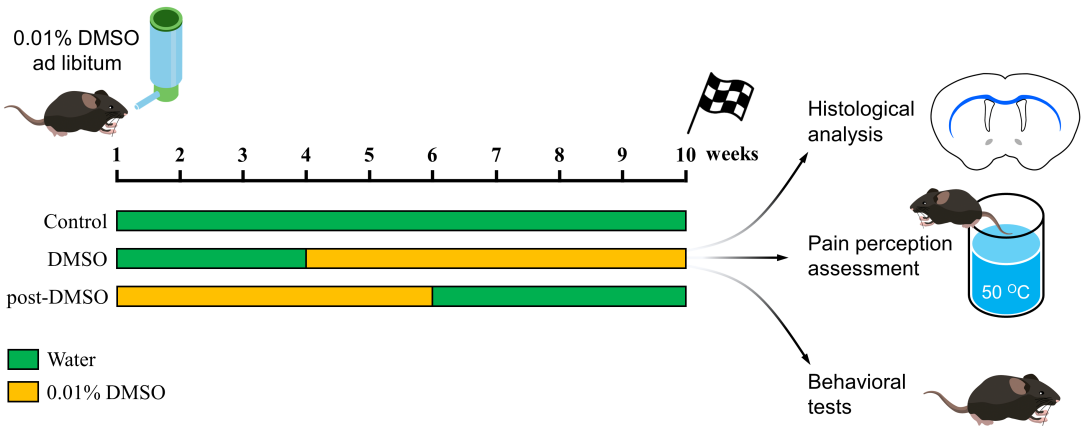


Figure 1. Experiment design.

Statistical analysis

Statistical analysis was performed with GraphPad Prizm 8.0 (GraphPad Software, Inc. USA) software. Normality of distribution was evaluated by Shapiro–Wilk test, and the acquired data were represented as M±SEM. Differences between the experimental groups were determined by ANOVA with post-hoc Tukey HSD. The results were considered as significant if the p-value was lower than 0.05 ($p<0.05$).

Results and Discussion

After histological analysis of c*orpus callosum* slices of C57BL/6 mouse brain, one-way ANOVA revealed significant influence of DMSO and its withdrawal on demyelination area to overall area of the slice ratio ($F_{2, 24} = 12.38$, $p = 0.0002$) (Fig. 2). Post-hoc Tukey test showed differences between the mean values of the areas of demyelination to the overall slice area in the DMSO (3.4±0.6) and control groups (0.4±0.1, $p = 0.0001$), DMSO (3.4±0.6) and post-DMSO (1.8±0.5, $p = 0.032$) groups.

In the tail-immersion test, one-way ANOVA revealed significant influence of DMSO and its withdrawal on tail flick latency response ($F_{2, 24} = 70.78$, $p<0.0001$) (Fig. 2E). Post-hoc Tukey test showed differences in the mean values of tail flick latency response in DMSO (0.9±0.1) and control groups (1.9±0.1, $p<0.0001$), post-DMSO (0.6±0.1) and control groups (1.9±0.1, $p<0.0001$), and DMSO (0.9±0.1) and post-DMSO groups (0.6±0.1, $p=0.0256$).

The open field test revealed significant changes in vertical activity after a 4-week period without solvent administration ($F_{2, 24} = 7.826$, $p = 0.0024$) and the number of holes explored ($F_{2, 24} = 20.37$, $p<0.0001$), while post-hoc Tukey test showed differences in the mean values of vertical activity in the post-DMSO (7.3±0.8) and control groups (2.5±0.5, $p = 0.0017$), and the mean values of the number of holes explored in the post-DMSO (9.2±1.0) and control groups (3.4±0.6, $p<0.0001$), post-DMSO (9.2±1.0) and DMSO groups (3.0±0.7, $p<0.0001$) (Fig. 3). At the same time, there were no signs of influence of DMSO or the withdrawal of the solvent either on peripheral ($F_{2, 24} = 0.6043$, $p = 0.5546$) and central ($F_{2, 24} = 1.722$, $p = 0.2$) activity, or on the total distance traveled ($F_{2, 24} = 1.766$, $p = 0.1925$).

One-way ANOVA did not reveal any significant influence of DMSO or its withdrawal on locomotion activity in rota-rod test ($F_{2, 24} = 0.1352$, $p = 0.8742$) and compulsive-like behavior in marble burying test ($F2, 24 = 0.3595$, $p = 0.7017$).

An increase in the demyelination area to the overall area of the *corpus callosum* slice ratio observed after 6-week administration of DMSO to C57BL/6 mice indicates development of demyelination. A decrease in this parameter after 4-week period without DMSO administration demonstrates development of spontaneous remyelination. The acquired data is corresponding to dynamics of morphological changes observed on the animal model of demyelination following 5-week *ad libitum* administration of cuprizone (0.2% solution) and spontaneous remyelination 2 weeks after its withdrawal (Palavra et al. 2022).

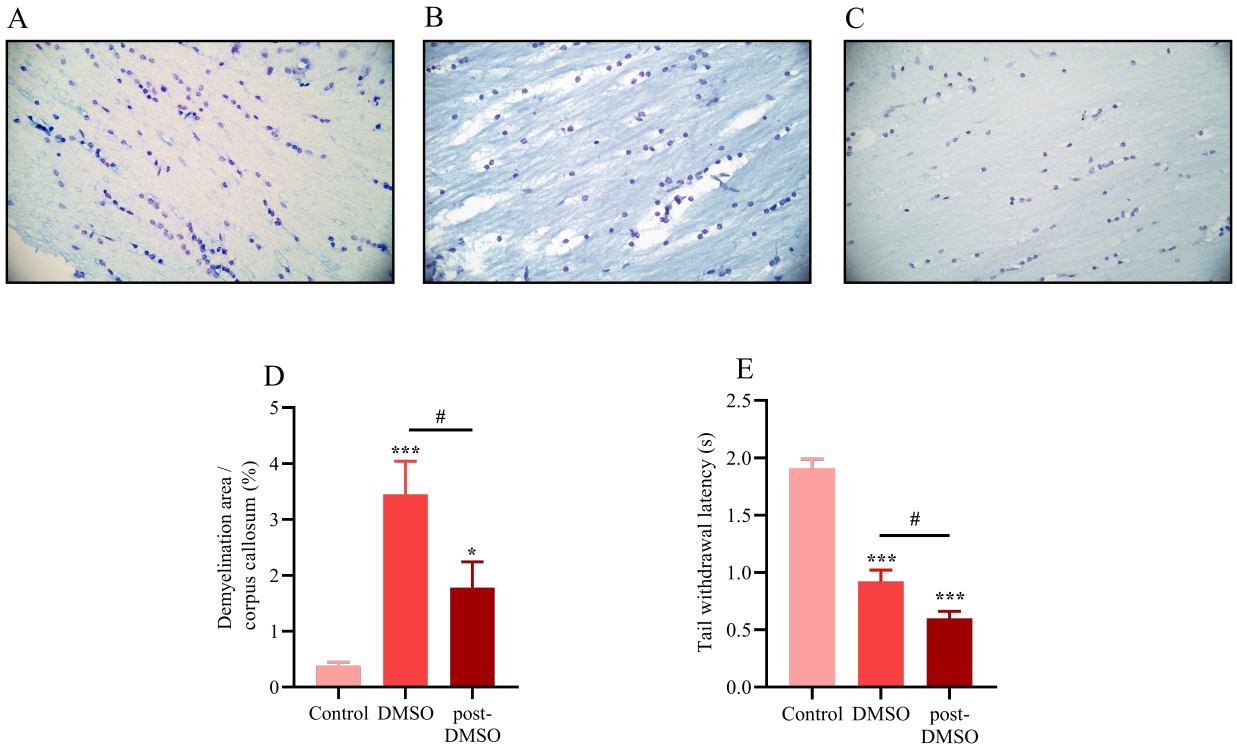


Figure 2. Influence of prolonged DMSO administration and its withdrawal on quantity of myelin in the *corpus callosum* and pain perception of C57BL/6 mice. ***Note:*** **A** – Control, **B** – DMSO, **C** – post-DMSO, **D** – influence of prolonged DMSO administration on quantity of myelin in the *corpus callosum* of C57BL/6 mice, **E** – influence of prolonged DMSO administration on pain perception of C57BL/6 mice in tail-immersion test. Luxol fast blue according to Kluwer-Barrera, x40. * – $p<0.05$, *** – $p<0.001$ compared to control, # – $p<0.05$ compared to DMSO.

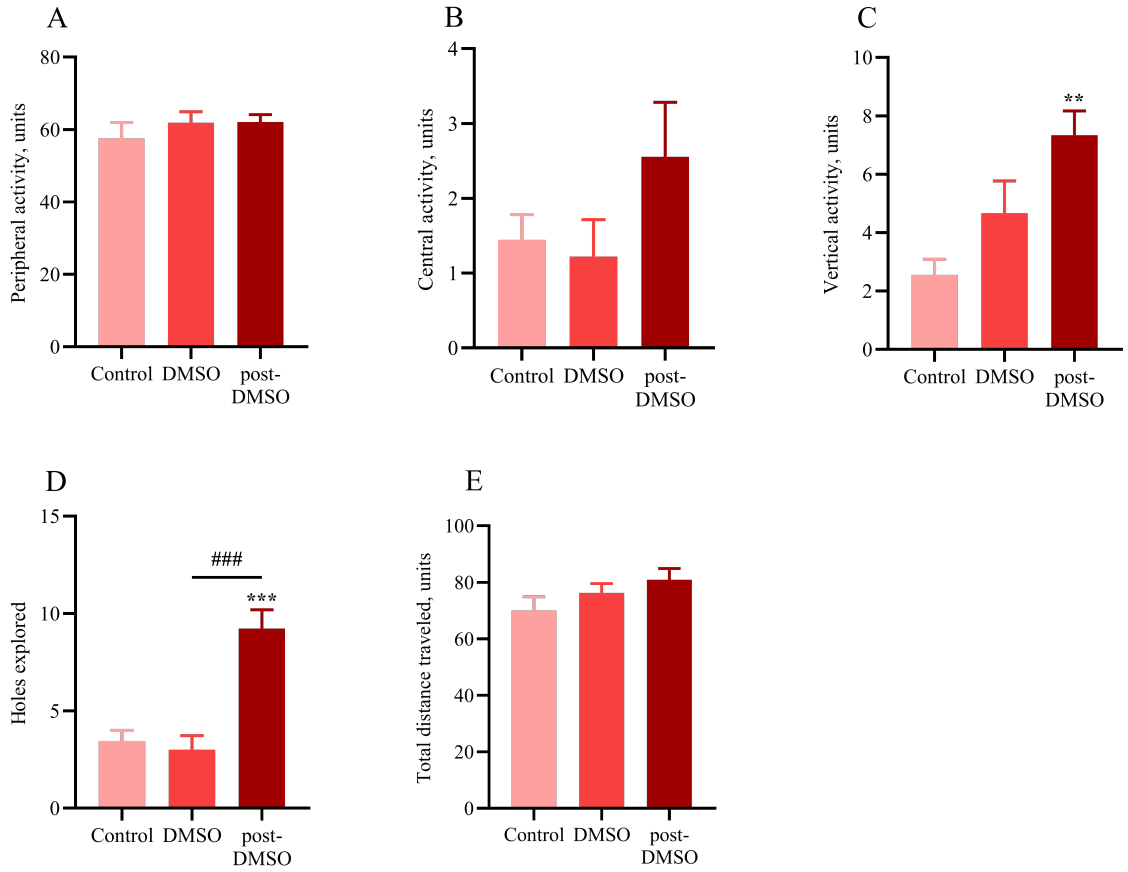


Figure 3. Influence of prolonged DMSO administration and its withdrawal on anxiety reactions of C57BL/6 mice in open field test. ***Note:*** **A** – peripheral horizontal activity, **B** – central horizontal activity, **C** – vertical activity, **D** – number of holes explored, **E** – total distance traveled. ** – $p<0.01$, *** – $p<0.001$ compared to control, ### – $p<0.001$ compared to DMSO.

Demyelination after DMSO consuption was accompanied with changes in pain perception as well. A decrease in the tail flick latency response in the tail-immersion test after the 6-week administration of DMSO corresponds to hyperalgesia that accords with data showing hyperalgesia development on the animal models of demyelination – cuprizone model (Tsukahara et al. 2018) and experimental autoimmune encephalomyelitis (Thorburn et al. 2016). However after a 4-week period of DMSO withdrawal, hyperalgesia following the decrease in demyelination in *corpus callosum* became even more intense. There is no literature data about enhancement of hyperalgesia following remyelination on the experimental models of demyelination. It seems that this phenomenon is associated with pharmacokinetic particularities of DMSO in mice, especially with its ability to distribute rapidly from blood flow to tissues (Kaye et al. 1983). It can be suggested that after withdrawal, DMSO was redistributed from CNS to peripheral tissues with low vascularization with consequent prolongation of its damaging influence on peripheral neuronal fibers that finally led to an increase in hyperalgesia.

An increase invertical locomotion activity 4 weeks after DMSO withdrawal is likely associated with remyelination linked with changes in dopaminergic system in mice brain. On the one hand, an increase in the dopamine level in prefrontal cortex of C57BL/6 mice is observed after 3-4 weeks of cuprizone administration, which is associated with disturbance of the function of monoamine oxidase, dopamine β-hydrolase and dopamine transporter (Chang et al. 2017; Xu et al. 2010). These neurochemical changes are followed by vertical locomotion activity of mice. On the other hand, dopamine can play important role in processes of de- and remyelination (Ding et al. 2020), which is proved by less active remyelination in the knock-out mice with D_3-receptor deficiency (Richter 2013), as well as by participation of dopaminergic neurons of the midbrain in innervation of oligodendrocyte progenitor cells in the *corpus callosum* of C57BL/6 mice (Caldwell et al. 2023). The increase in the number of holes explored probably pointing at an increase in the exploratory activity of mice, which can likewise be the result of enhancement of dopaminergic transmission in CNS (Shieh and Yang 2020).

At the same time, there were no changes in compulsive-like behavior of mice in the marble burying test after both 6-week administration of DMSO and 4 weeks after its withdrawal. It is consistent with the data obtained earlier showing that 0.01% DMSO had no effect on compulsive-like behavior in mice after 6 weeks of administration (Kudryashov et al. 2022). Absence of changes in locomotor activity in the rotarod test accords to the data proving that demyelination caused by prolonged administration of cuprizone does not always lead to disturbances of motor activity in C57BL/6 mice (Lubrich et al. 2022).

Conclusion

Therefore 6-week *ad libitum* consumption of DMSO is associated with demyelination in *corpus callosum* of C57BL/6 mice, which is followed by thermal hyperalgesia. Withdrawal of DMSO shows signs of attenuation of potency of morphological disturbances after a 4-week period, which probably points at spontaneous remyelination, which, in turn, can be accompanied with an increase in thermal hyperalgesia, vertical locomotor activity and exploratory behavior of mice.

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Conflict of interests

The authors declare no conflict of interests.

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Author Contributions

- **Nikita V. Kudryashov,** PhD in Biology, Associate professor at the Department of Pharmacology of the Institute of Biodesign and Complex System Modelling, Sechenov First Moscow State Medical University (Sechenov University), 8-2 Trubetskaya str., Moscow, Russian Federation, 119991, e-mail: kudryashov_n_v@staff.sechenov.ru, **ORCID ID** https://orcid.org/0000-0002-1819-1867, (development of the idea of the study, carrying out behavioral tests, writing the manuscript).

- **Alexander A. Gorbunov,** PhD in Biology, Associate professor at the Department of Pharmacology of the Institute of Biodesign and Complex System Modelling, Sechenov First Moscow State Medical University (Sechenov University), 8-2 Trubetskaya str., Moscow, Russian Federation, 119991, e-mail: gorbunov_a_a@staff.sechenov.ru, **ORCID ID** https://orcid.org/0000-0002-5773-5177, (pain perception assessment, writing the manuscript).

- **Nadezhda B. Sviridkina**, PhD in Biology, Head of the vivarium, OOO Research Registration Center Biolife, 34 1st Kuryanovskaya str., Moscow, Russian Federation, 109235, e-mail: mag115@list.ru, (histological analysis).

- **Sergey E. Mironov,** PhD in Pharmacy, Associate professor at the Department of Pharmacology of the Institute of Biodesign and Complex System Modelling, Sechenov First Moscow State Medical University (Sechenov University), 8-2 Trubetskaya str., Moscow, Russian Federation, 119991, e-mail: mironov_s_e@staff.sechenov.ru, (DMSO administration, rota-rod test, editing the manuscript).

- **Dmitriy A. Tikhonov**, PhD in Medicine, Associate professor at the Department of Pharmacology of the Institute of Biodesign and Complex System Modelling, Sechenov First Moscow State Medical University (Sechenov University), 8-2 Trubetskaya str., Moscow, Russian Federation, 119991, e-mail: tikhonov_d_a@staff.sechenov.ru, (statistical analysis, editing the manuscript).

- **Andrey A. Nedorubov,** Head of the Center for Preclinical Studies of the Institute for Translational Medicine and Biotechnology, Sechenov First Moscow State Medical University (Sechenov University), 8-2 Trubetskaya str., Moscow, Russian Federation, 119991, e-mail: nedorubov_a_a@staff.sechenov.ru, **ORCID ID** https://orcid.org/0000-0002-5915-7999, (DMSO administration, editing the manuscript).

- **Vladimir P. Fisenko,** Doctor Habilit. of Medicine, Professor, RAS academician, Head of the Department of Pharmacology of the Institute of Biodesign and Complex System Modelling, Sechenov First Moscow State Medical University (Sechenov University), 8-2 Trubetskaya str., Moscow, Russian Federation, 119991, e-mail: vpfisenko@mail.ru, (development of the idea of the study, editing the manuscript).