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EXPERIMENTAL ARTICLES

The Effect of Omega-3 Polyunsaturated Fatty Acids on Neuroinflammation in the Hippocampus

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Abstract—We studied the effects of oral administration of ω-3 polyunsaturated fatty acids (PUFAs) on behavioral characteristics and immunohistochemical, morphological, and biochemical parameters in hippocampi of mice with experimental neuroinflammation. We found that animals with neuroinflammation that were treated with ω-3 PUFAs had higher parameters of locomotor activity and working memory compared to the vehicle-treated group. The immunohistochemical analysis showed that activation of glial cells was less significant in ω-3 PUFA-treated animals than in vehicle-treated animals with neuroinflammation. An increase in the production of pro-inflammatory cytokine IL-1β and malonic dialdehyde, a marker of lipid peroxidation, in the hippocampus was less pronounced than in the vehicle-treated group. Thus, we assume that the normalization of locomotor activity and working memory after PUFA administration reduce microglial and astroglial activation, the intensity of neuroinflammation and oxidative stress and, as a consequence, prevent changes in the physicochemical characteristics of cellular and mitochondrial membranes.

Keywords: hippocampus, neuroinflammation, microglia, polyunsaturated fatty acids, docosahexaenoic acid
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INTRODUCTION

Psychoneurological disorders, including cognitive and emotional disorders, frequently result from organic lesions of the central nervous system (CNS) [1]. According to the World Health Organization, 47.5 million people worldwide have dementia [2]. Given the high prevalence of central nervous system diseases accompanied by cognitive deficits, the maintenance of patients and symptomatic methods of their treatment require considerable expenses [3]. The use of existing drugs for the pharmacotherapy of cognitive disorders can only slow the development of neuropsychiatric symptoms of neurodegenerative pathology slightly [4]. Development of new drugs for the treatment and prevention of dementia requires detailed studies on the mechanisms of development of cognitive disorders and the identification of promising therapeutic targets.

The process of neuroinflammation, which accompanies the majority of CNS diseases and is recognized as a key component of the development of cognitive disorders, is a promising target for therapeutic treatment of these pathologies [5].

Long-chain ω-3 polyunsaturated fatty acids (PUFAs) are a promising group of compounds that may influence the intensity of neuroinflammation and its cognitive consequences. It is known that this class of compounds plays a role in the normal functioning of the central nervous system [6]. Current evidence suggests that consumption of ω-3 PUFAs, in particular docosahexaenoic (DHA) and eicosapentaenoic (EPA), reduces the risk of development of dementia [7, 8] and contributes to the improvement of cognitive functions [9]. However, a decrease in the content of ω-3 PUFAs in the brain, which accompanies the aging process, is often associated with cognitive deficits and the development of neurodegenerative diseases [10]. It is known that ω-3 PUFAs and their metabolites have anti-inflammatory characteristics, hence, they are considered as potential means of prevention and treatment of neurological diseases accompanied by neuroinflammation [11]. Despite the active study of the pharmacological properties of ω-3 PUFAs, the mechanisms of their positive effect on cognitive functions in pathologies associated with neuroinflammation are poorly understood. The establishment of mechanisms of the influence of ω-3 PUFAs on the functions of higher nervous activity is an important stage in the search for pharmacological agents for the correction of cognitive disorders accompanying CNS diseases. Given the important role of the hippocampus in cognitive functions, a detailed study of the changes that occur in this part of the brain after the exogenous administration of ω-3 PUFAs during

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neuroinflammation will form the basis for further clinical use of this class of compounds.

The purpose of this work was to investigate the effect of oral administration of ω-3 PUFAs on a number of behavioral indices, as well as immunohistochemical, morphological, and biochemical changes in the hippocampus of mice with experimentally induced neuroinflammation.

MATERIALS AND METHODS

Characteristics of the experimental material. The experiments were performed with 80 male CD1 mice at an age of 3 months. The animals were housed four per cage, had free access to food and water, and were kept at a constant temperature (23 ± 2°C) and humidity (55 ± 15%) with a 12-hour day/night cycle. A preparation based on DHA (38%) and EPA (46%) (Omacor, Abbott Products GmbH, Germany) as an emulsion with milk was administered intragastrically to half of the animals at a dosage of 300 mg/kg/day (114 mg/kg DHA and 138 mg/kg EPA) for 7 weeks. The control group received milk in the same volume (200 μL). During the last week of administration of the PUFA mixture, animals were injected intraperitoneally with a solution of LPS (*Escherichia coli* 0111: B4, Sigma Aldrich) at a dosage of 200 μg/kg/day. The control group received a 0.9% NaCl solution. PUFA and LPS injections were administered once a day between 9:00 and 11:00 a.m. Thus, the experiment involved the following four groups of mice:

(1) Control (administration of milk and 0.9% NaCl solution);

(2) LPS (administration of milk and the LPS solution);

(3) PUFA (administration of a PUFA mixture and a 0.9% NaCl solution);

(4) PUFA + LPS (administration of a PUFA mixture and the LPS solution).

On day 7 of LPS administration 3 hours after injection, the animals were divided into two subgroups: the first subgroup was subjected to behavioral tests followed by perfusion and immunohistochemical examination (40 mice); the second subgroup was removed from the experiment to determine the concentration of malonic dialdehyde (MDA) in tissues of the hippocampus and the fatty acid composition of the phospholipids of the brain.

Behavioral studies. Evaluation of spontaneous locomotor activity. Spontaneous locomotor activity was evaluated in an automated open field, which is a 43.2 × 43.2 × 20 cm acrylic glass chamber equipped with infrared detectors located in three planes (Med Associates Inc, St. Albans, VT). The cameras were housed in soundproof boxes with built-in fans to create background noise (65 dB) and bright illumination. For testing, the animal was placed in the center of the test arena, where it was allowed to move freely for 10 minutes. The activity was monitored using an automated tracking system (Med Associates Activity Monitor, version 5.93.773). During the testing, the following parameters, which characterize the locomotor activity, were monitored automatically: the distance traveled, the number of jumps, and the number of vertical episodes [12].

Evaluation of working memory and locomotor activity in the test of spontaneous alternation in a Y maze. At 3–4 hours after LPS injection, the motor activity and working memory were evaluated in a Y-maze. The Y-maze is a device made of plexiglass with three identical arms, whose length is 30 cm and width is 10 cm. The height of the maze walls was 20 cm. At the beginning of the test, the mouse was placed in the center of the maze and left for 5 minutes. A video was recorded and the number and sequence of entries to the maze arms were recorded in order to calculate the coefficient of spontaneous alternation of the arms and evaluate motor activity [13]. The criterion for arm entry in the maze was the mouse position, when all four paws were inside the arm. To calculate the coefficient of spontaneous alternation of arms, the following formula was used:

$$ks = R/A, \quad (1)$$

where ks is the coefficient of spontaneous alternation of the arms; R is the number of alternations, and A is the total number of possible alternations.

Evaluation of the function of long-term memory. Long-term memory was studied using the Novel-object recognition test following the Bevins and Besheer protocol [14]. The testing chamber was a black rectangular box made of acrylic glass (31 × 24 cm, height 27 cm). Before the test, the animals were placed in the chamber for 10 minutes for 2 consecutive days. Testing was performed under dimmed light. The activity of the mice was recorded using a video camera. The study included two sessions: habituation and testing. During the habituation session, two identical objects were placed in diagonally opposite corners of the camera at a distance of 8–9 cm from the walls. The mouse was placed in the middle of the chamber between the objects and left for 10 minutes. After 24 hours, one of the objects in the chamber was replaced with a new object that was similar in height and volume but with a different form and appearance. The mouse was again placed in the chamber and allowed to examine the objects within 5 minutes. When analyzing the records, the total time of contact with each object was determined. As contact criteria, we used the contact of the animal's paws with the object or the approach of the nose to a distance of closer than 2 cm. The recognition coefficient was calculated using the formula:

$$R = T_{nov}/T_{tot} \times 100\%, \quad (2)$$

where R is the recognition factor; T_{nov} is the time of contact with a novel object during the second session;

and T_{tot} is the total time of contact with the new and old object during the second session.

In animals without long-term memory disturbances, the duration of contact with the novel object is higher than with the familiar one and, as a consequence, the value of the recognition index is higher.

Immunohistochemical studies. At 3 hours after the injection of LPS, the animals were anesthetized by intraperitoneal injection of sodium thiopental (3%, 60 mg/kg); a transcardial perfusion was then performed with a 4% paraformaldehyde solution (PFA) prepared on 0.1 M phosphate buffered saline with pH 7.4 (PBS). After perfusion, the brain was removed and placed in 4% PFA solution for 1 day. In 24 hours, the material was thoroughly washed with PBS and immersed for 48 hours in 30% sucrose solution (Sigma Aldrich). After this, the material was placed for 24 hours in a Nego-50 medium (Thermo Scientific) in a cryostat and 30-μm thick free-floating sagittal sections were made using a microtome (HM525, Thermo Scientific). For the morphological and quantitative evaluation of glial cells, as well as for the evaluation of the expression of neuroinflammation markers in the hippocampus, we used the immunoperoxidase reaction method. The immunohistochemical method used in the work consisted of the following steps: (1) preincubation in a solution that blocks endogenous peroxidase activity (0.3% H_2O_2 solution); (2) preincubation in a solution that blocks the non-specific binding of antibodies (5% skim milk in PBS); (3) incubation of sections with primary antibodies (4°C, 24 h); (4) incubation of sections with secondary antibodies labeled with horseradish peroxidase: PI-1000 (anti-rabbit); PI-2000 (anti-mouse), 1 : 100 (Vector Laboratories, United States); (5) ImmPACT™ DAB Peroxidase Substrate (SK-4105, Vector Laboratories, United States) was used for the immunoperoxidase reaction; (6), the stained sections were carefully washed in 0.1 M phosphate buffer (pH 7.4), dried, and mounted in VectaMount Permanent Mounting Medium (H-5000, Vector laboratories) according to a standard procedure. Primary polyclonal rabbit anti-Iba-1 antibodies (ab107159, Abcam, United States, 1 : 500), GFAP (Abcam ab7260, United States, 1 : 1000), IL-1β (ab9722, Abcam, United States, 1 : 100) were used.

From each animal, five sections were taken for analysis, which composed 50 slices for each group.

The images were taken using a Zeiss AxioScope A1 microscope equipped with an AxioCam 503 video camera and AxiosVision (Zeiss) software. The images were saved in TIF format; their size was 1080 × 800 pixels. Images were processed and analyzed using ImageJ software (NIH, United States). The processing of each photomicrograph included the following steps: conversion to black and white, background subtraction, contrast enhancement, binarization, noise reduction, and measurement. In addition, the primary processing included image calibration, i.e., the conversion of pixels into square micrometers. Only the cells with visible nuclei were used to count Iba-1, GFAP, and IL-1β-immunopositive cells. The area of the CA1 region was multiplied by the thickness of the slice and the result was expressed in cubic millimeters. The number of immunopositive cells per mm^3 was calculated using the formula: $d = (10^6 n)/(S \times l)$, where d is the number of cells; S is the area of the analyzed area (μm^2); l is the section thickness, and 10^6 is a factor for the conversion of μm^3 in mm^3.

Homogenization of tissue samples. After behavioral experiments, a subgroup of 40 animals was used to determine the concentration of malonic dialdehyde in the tissues of the hippocampus and the fatty acid composition of the brain phospholipids. The animals were decapitated after preliminary intraperitoneal administration of sodium thiopental (3%, 60 mg/kg). The extraction of the brain and the release of the hippocampus and cortex was rapidly performed at a temperature of 4°C. After isolation, the material was frozen in liquid nitrogen and the samples were then stored at −70°C.

Samples of the hippocampus and cerebral cortex obtained from each animal were homogenized using a buffer of the following composition: 250 mM sucrose; 10 mM Tris-HCl buffer, pH 7.4; mM EDTA; and 10 mM HEPES. One tablet of protease inhibitor (cOmplete, Roche) was dissolved in 10 mL of a homogenization buffer. The tissue fragments to be analyzed were transferred to test tubes and weighed; homogenization buffer at a concentration of 5 mL/g was added to them. Homogenization was performed in two stages: manually using homogenizer tubes and ultrasonic homogenization using an ultrasonic Q125 sonicator (Qsonica). The homogenates obtained were centrifuged and the supernatant was collected and stored at −70 for subsequent use. The supernatant of the hippocampus was subsequently used to determine the protein content and MDA concentration. The Pierce™ BCA Protein Assay Kit was used to determine the total protein content. Experimental procedures and calculation of protein concentration were performed strictly following the instructions. To measure the optical density of the obtained dilutions, a BioMark xMark (BioRad) plate spectrophotometer was used. According to the calculated concentrations, all samples were adjusted to a protein concentration of 1 mg/mL.

Determination of the concentration of malonic dialdehyde in the hippocampus. The concentration of MDA in the hippocampus was measured according to the method of Wasowicz et al. [18]. The method is based on the reaction of MDA with thiobarbituric acid at a temperature of 95°C.

Determination of the fatty acid composition of brain phospholipids. The homogenized tissue obtained after the extraction of the complex of water-soluble compounds was used for the subsequent extraction of the

lipid fraction. Extraction of lipids from the tissues of the hippocampus and cortex was performed according to the method of Folch et al. [15]. The obtained samples were homogenized with a mortar and pestle for 1 minute with the addition of methanol at a ratio of 10 mL/1 g tissue. Chloroform was added at a ratio of 20 mL/1 g tissue and homogenized for another 2 minutes. The resulting homogenate was filtered and the tissue remaining after filtering was used for repeated extraction. The extracts were transferred to a graduated cylinder, mixed with 0.9% NaCl solution in a volume equal to ¼ of the volume of the extract obtained, and transferred to a centrifuge tube. The tubes were centrifuged for 5 minutes at 2000 rpm. The upper phase was taken by a pipette and washed twice with small volumes of chloroform, which was then added to the lower phase. The lower phase was transferred to a round-bottomed flask and evaporated with a vacuum evaporator. The resulting lipid precipitate was dissolved in 5 mL of chloroform and stored at –20°C. The next step was the preparation of methyl esters of lipids for the subsequent separation of the fraction of phospholipids by thin layer chromatography. The resulting chloroform extracts were evaporated using a vacuum evaporator; 1 mL of benzene was added to the samples and they were completely transferred to test tubes. Next, 50 μL of 0.7 M NaOH in 70% methanol were poured into the tubes and incubated in a water bath for 30 minutes at 50°C. The mixture was neutralized by the addition of 250 μL of a solution of HCl in methanol. The resulting mixture was evaporated and 1 mL of hexane was added to the precipitate. The solution was applied to a 10 × 10 cm G60 silica gel plate (Merck) for analysis by thin layer chromatography. The chromatogram was visualized by spraying a thin strip of the plate with 50% sulfuric acid and then heating to 135°C. The fraction of methyl esters of fatty acids was removed from the plate, extracted with chloroform, and analyzed by gas chromatography (GC). A Shimadzu GC-17A gas chromatograph (Shimadzu, Japan) with a flame ionization detector and a Supelcowax capillary column 10, 30 m × 0.25 mm (Sigma, United States) was used for the analysis. During the analysis, the column temperature was 190°C and the injector and detector temperature was 240°C. Helium was used as a carrier gas. The peaks of methyl esters of fatty acids were identified on the basis of retention time which was compared with the retention times of the standards (47015-U SUPELCO, Sigma, United States).

Statistical processing of results. For statistical analysis and processing of the results of the study, mean ($\bar{x}$) and standard deviations (σ) were calculated. All parameters were checked for normality of distributions using the Kolmogorov–Smirnov test. The significance of differences in the results of experimental observations for behavioral, immunohistochemical studies and determination of MDA content was evaluated using a one-way analysis of variance. The Tukey criterion was used for a posteriori comparisons of averages in the groups. The significance of the differences in the results of determination of fatty acid composition of brain phospholipids was evaluated using the Student's t test. The significance level was $p < 0.05$. Statistical analysis was performed using Microsoft Excel software (United States).

RESULTS

The effect of polyunsaturated fatty acids on the behavioral characteristics of mice with neuroinflammation. The working memory and locomotor activity were evaluated using a Y-maze. The working memory was evaluated using the spontaneous alternation coefficient. Locomotor activity was evaluated both in the Y-maze (the number of entries in the arms) and in the automated open field, where in addition to the distance traveled, the numbers of jumps and vertical episodes were counted. According to the results of testing in the automated open field, in the LPS group the distance traveled was reduced by 36% compared with the Control group. However, in the PUFA + LPS group this indicator was 27% higher than in the LPS group ($p < 0.05$), $F(3,36) = 6.47$ (Fig. 1a). As a result of induction of neuroinflammation, the parameter number of jumps in the LPS group decreased by 6 times ($p < 0.001$), in the PUFA + LPS group, by 2.5 times ($p < 0.01$), $F(3,36) = 9.73$. There were no significant differences between the PUFA + LPS and LPS groups (Fig. 1b). When analyzing the Number of vertical episodes parameter, there were no significant differences between the LPS and PUFA + LPS groups. In the LPS group, there was a significant decrease in the parameter compared to the Control group ($p < 0.05$), $F(3,36) = 6.78$, while there was no significant difference between the Control and PUFA + LPS groups (Fig. 1c).

The determination of locomotor activity in the Y-maze showed a 2-fold decrease in the number of entries in the arms in the LPS group compared to the Control group ($p < 0.001$). In the PUFA + LPS group, this indicator was significantly higher than in the LPS group ($p < 0.05$), $F(3,36) = 17.02$ (Fig. 1d). Analysis of functioning of working (operative) memory in the Y-maze demonstrated the absence of a significant decrease in the spontaneous alternation coefficient in the PUFA + LPS group, while in the LPS group the average coefficient was 18% lower than the Control and PUFA + LPS groups ($p < 0.05$), $F(3,36) = 6.48$ (Fig. 1e).

The study of long-term memory in the Novel object recognition test revealed no significant decrease in the recognition index in the LPS and PUFA + LPS groups compared to the control group (Fig. 1f).

According to the results of behavioral studies, in animals with neuroinflammation who received ω-3

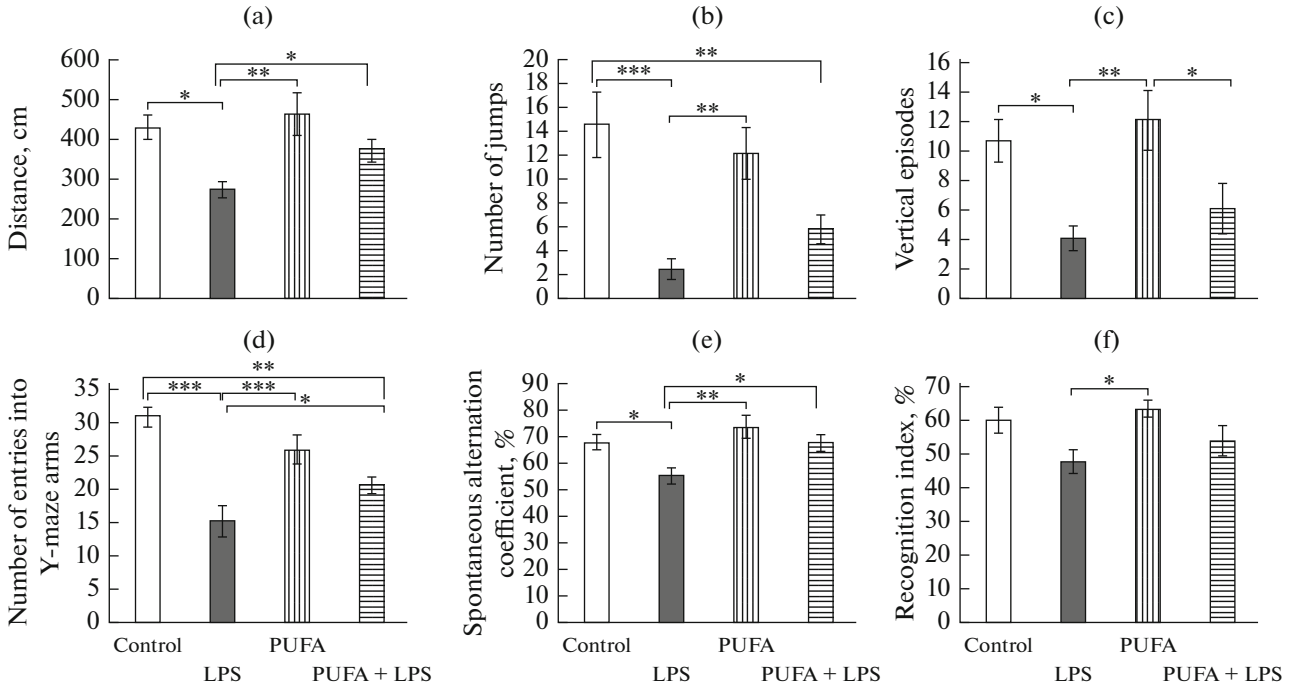

Fig. 1. The results of behavioral tests. (a) The average distance traveled in an automated open field; (b) the number of jumps; (c) number of vertical episodes; (d) the number of entries in the Y-maze arms, which reflects the locomotor activity; (e) the coefficient of spontaneous alternations in the Y-maze, which reflects the state of the working memory; (f), recognition index in the Novel object recognition test, which reflects the state of long-term memory; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

PUFAs, the parameters of locomotor activity and working memory were significantly higher than values in the group that did not receive fatty acids.

Analysis of the fatty acid composition of mouse brain phospholipids after administration of polyunsaturated fatty acids. It is known that ω-3 PUFAs have low bioavailability for the brain. Graf et al. showed that only 0.07–0.67% of radioactively labeled DHA, which was administered orally at a dosage of 2 mg/kg of brain weight, was detected in the rat brain in 24 h [16]. According to Rapoport et al., only 1% of the intravenously administered dose of DHA or AA was detected in the phospholipids of the brain in 15 minutes [17]. In the framework of this study, we performed a quantitative analysis of the fatty acid content of the brain phospholipids. This analysis allowed us to determine the degree of accumulation of DHA and EPA in the phospholipids of the membranes of hippocampal cells and the cerebral cortex.

The fatty acid composition of the phospholipids of the hippocampus and the cerebral cortex is shown in Fig. 2. In PUFA-treated animals, we observed an increase in the content of docosahexaenoic acid (22:6n-3) in both the hippocampus and the cortex. In the hippocampus, the DHA concentration increased by 45%, while in the cortex the concentration increase was only 13% of the DHA content in the control group. The most important index that determines the degree of accumulation of ω-3 or ω-6 PUFAs in the brain is the ratio between the DHA concentration and the concentration of arachidonic acid (AA). According to the results of our experiments, the DHA/AA ratio in the cerebral cortex in the control group was 2.03, and in the PUFA-treated group it was 2.29; in the hippocampus, 1.25 and 1.63, respectively.

Expression of neuroinflammation markers in the hippocampus during consumption of ω-3 polyunsaturated fatty acids. Next, we studied the intensity of expression of several neuroinflammation markers to assess the morphological changes that accompany the behavioral effects caused by the administration of PUFAs during neuroinflammation.

Immunohistochemical staining of microglia marker Iba-1 in mice treated with PUFAs showed that the average number of microglial cells per 1 mm^3 after LPS administration was 26% lower than the number in the group of mice not treated with PUFAs ($p < 0.01$), $F(3,36) = 5.63$ (Fig. 3b). When performing the morphometric analysis, we found an increase in the average area of microglial cells by 38% in the LPS group. In the PUFA + LPS group , there were no significant differences in the average area of microglial cells compared with the Control group. In the mice of PUFA + LPS group, the average area of the microglial cells was 30% lower than in the LPS group ($p < 0.001$), $F(3,115) = 8.74$ (Fig. 3c).

Peripherally induced neuroinflammation was accompanied by a change in the morphology and

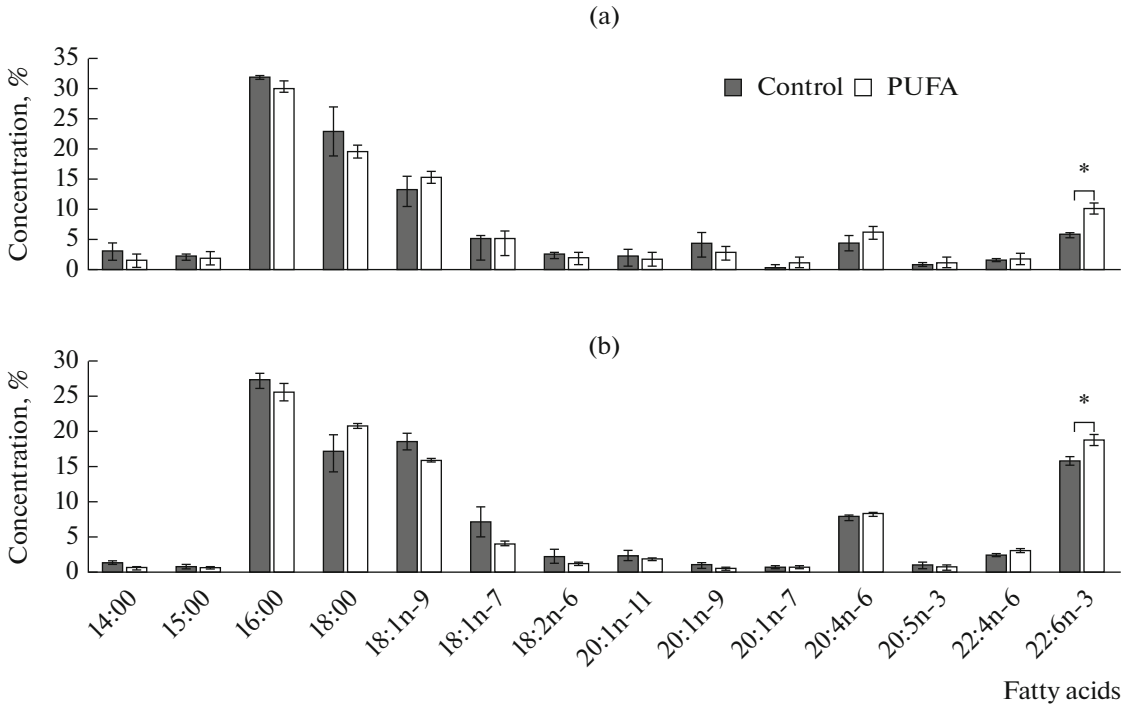

Fig. 2. The relative contents of fatty acids in the phospholipids of the brain regions, C% ± σ, $n = 10$. (a) The content of fatty acids in the phospholipids of the hippocampus; (b) fatty acid contents in the phospholipids of the cerebral cortex, * $p < 0.01$.

number of astrocytes. In the LPS group, there was an increase in the number of astrocytes in the CA1 region of the hippocampus by 55% compared to the control group ($p < 0.001$), $F(3,36) = 57.56$ in the absence of significant changes in the number of cells in the PUFA + LPS group (Fig. 4b). During neuroinflammation in the PUFA + LPS group, the average area of astrocyte bodies was less than the area in the LPS group, on average, by 2 times ($p < 0.001$), $F(3,76) = 25.25$ (Fig. 4c). The mean length of the astrocyte processes in the LPS group with neuroinflammation was 20% lower than in the PUFA + LPS group ($p < 0.01$), $F(3,81) = 25.6$ (Fig. 4d).

According to the results of the experiment, the number of IL-1β-positive cells in the hippocampus in the LPS group increased by 6 times ($p < 0.001$), while in the PUFA + LPS group the number of cells increased by 3 times ($p < 0.001$), $F(3,36) = 98.29$ (Fig. 5).

The data from immunohistochemical studies indicate that in the presence of ω-3 PUFAs the degree of activation of glial cells in the hippocampus with neuroinflammation is lower than in the absence of additional sources of DHA and EPA. A decrease in the degree of activation of microglia and astrocytes after administration of PUFAs leads to a less-pronounced increase in the production of the pro-inflammatory cytokine IL-1β in the hippocampus than in the group not treated with PUFAs.

The content of malonic dialdehyde in the hippocampus of mice with neuroinflammation in the presence of polyunsaturated fatty acids.

Numerous studies have shown that DHA and EPA have antioxidant activities and reduce lipid peroxidation (LP) [18]. Neuroinflammation is characterized by an elevated level of reactive oxygen species (ROS) in tissues, which leads to the development of oxidative stress and the intensification of LP. One of LP products is MDA, whose concentration in tissues grows with the intensification of LP [19]. In this study, we evaluated the antioxidant properties of the injected preparation of ω-3 PUFAs by determining the concentration of MDA in tissues.

Our results suggest that in the LPS group the concentration of malonic dialdehyde in the tissues of the hippocampus during neuroinflammation increased by a factor of 2.4 ($p < 0.001$) and by 37% ($p = 0.03$) in the PUFA + LPS group. In mice treated with PUFAs, the MDA concentration in the hippocampus during neuroinflammation was 33% higher than in mice that did not receive PUFAs ($p = 0.03$), $F(3,36) = 16.79$ (Fig. 6).

DISCUSSION

The need to find new drugs for the treatment and prevention of dementia has led to an increase in the number of studies on the mechanisms of development of cognitive disorders. The main goal of these studies was the search for promising therapeutic targets for the

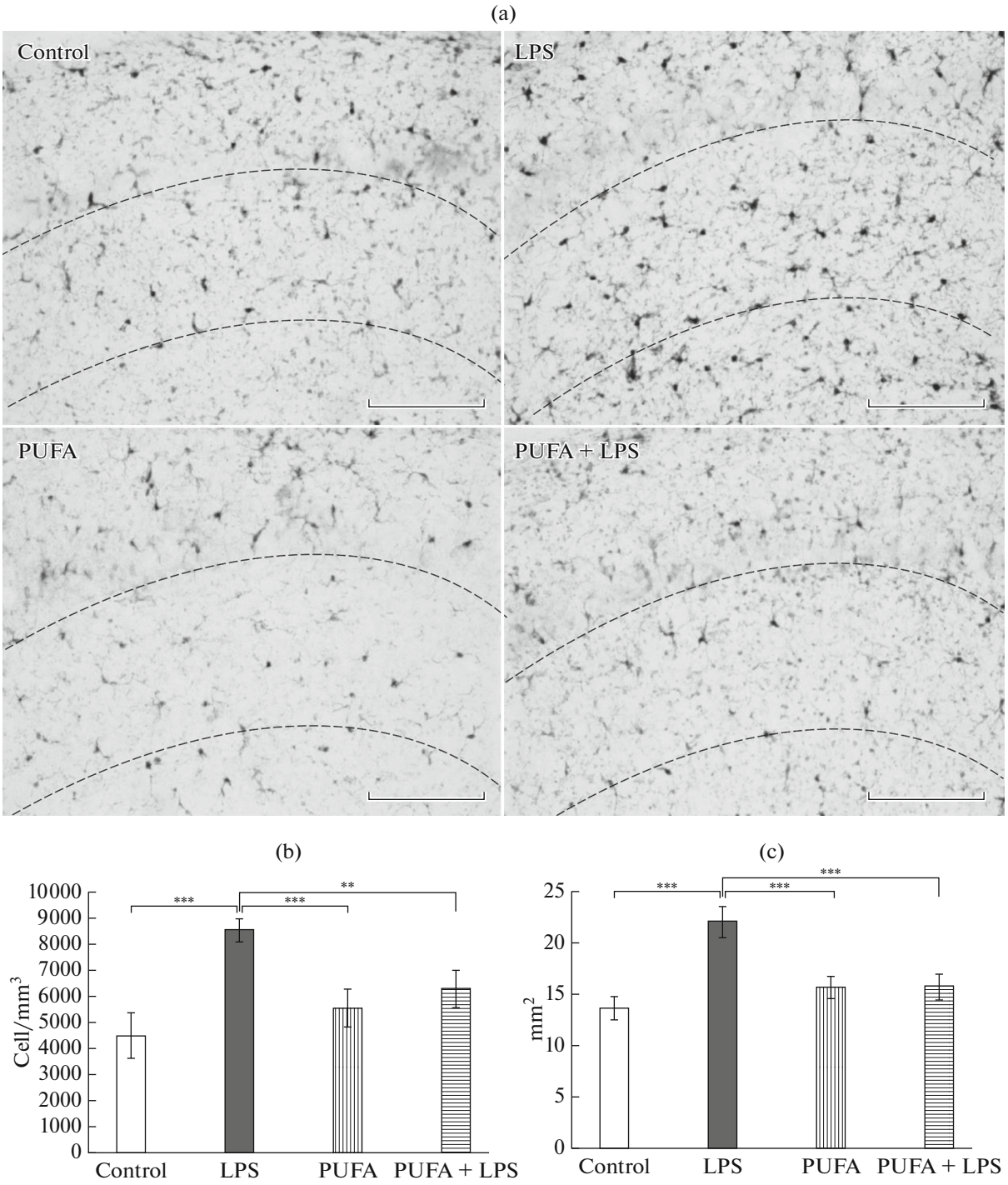

Fig. 3. Iba1-positive microglia in the hippocampus. (a) Typical images of immunohistochemical staining of the CA1 region of the hippocampus; (b) average number of Iba1-positive microglial cells; (c) average areas of microglial cells, ** $p < 0.01$; *** $p < 0.001$.

treatment of neurological diseases with symptoms of cognitive deficits. As a result of numerous studies, a common component of various neurological and, in particular, neurodegenerative diseases, the neuroinflammation reaction was discovered. Although the causal relationship between neuroinflammation and the development of neurodegenerative diseases requires more detailed study, neuroinflammation is considered a neurodestructive factor. Mediators of neuroinflammation in addition to a direct neurotoxic effect have indirect effects, such as brain edema, ischemia, and angiospasm. In this regard, modern neuro-

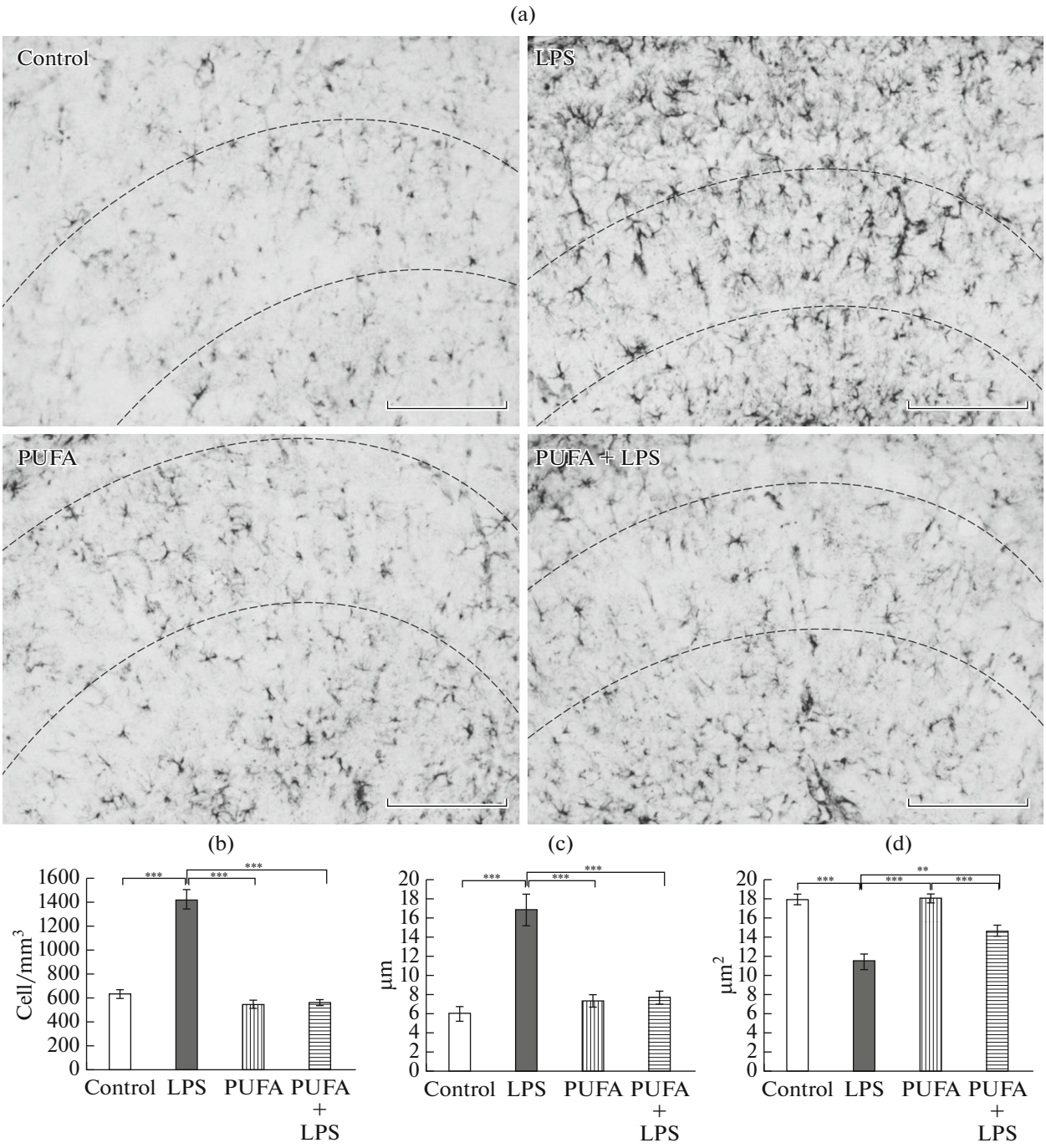

Fig. 4. GFAP-positive astrocytes in the hippocampus. (a) Typical images of immunohistochemical staining of CA1 region of the hippocampus; scale, 100 μm; (b) average number of GFAP-positive astrocytes; (c) mean lengths of astrocyte processes; (d) mean areas of astrocyte bodies; ** $p < 0.01$, *** $p < 0.001$.

protection strategies suppress the neuroinflammatory response. Nevertheless, the complete blocking of the molecules necessary to initiate neuroinflammation leads to negative consequences. As an example, in mice deficient in tumor necrosis factor-α gene, acute traumatic brain injury is characterized by a decrease in microglia activation and a high degree of neuronal death [20]. This result clearly demonstrates the idea that an inflammatory reaction is a complex program necessary for normal recovery after exposure to a damaging factor [21, 22]. Thus, the goal of the therapeutic effect should not be a complete blockage of the neuroinflammation reaction, but reprogramming of this process to accelerate the resolution of the inflam-

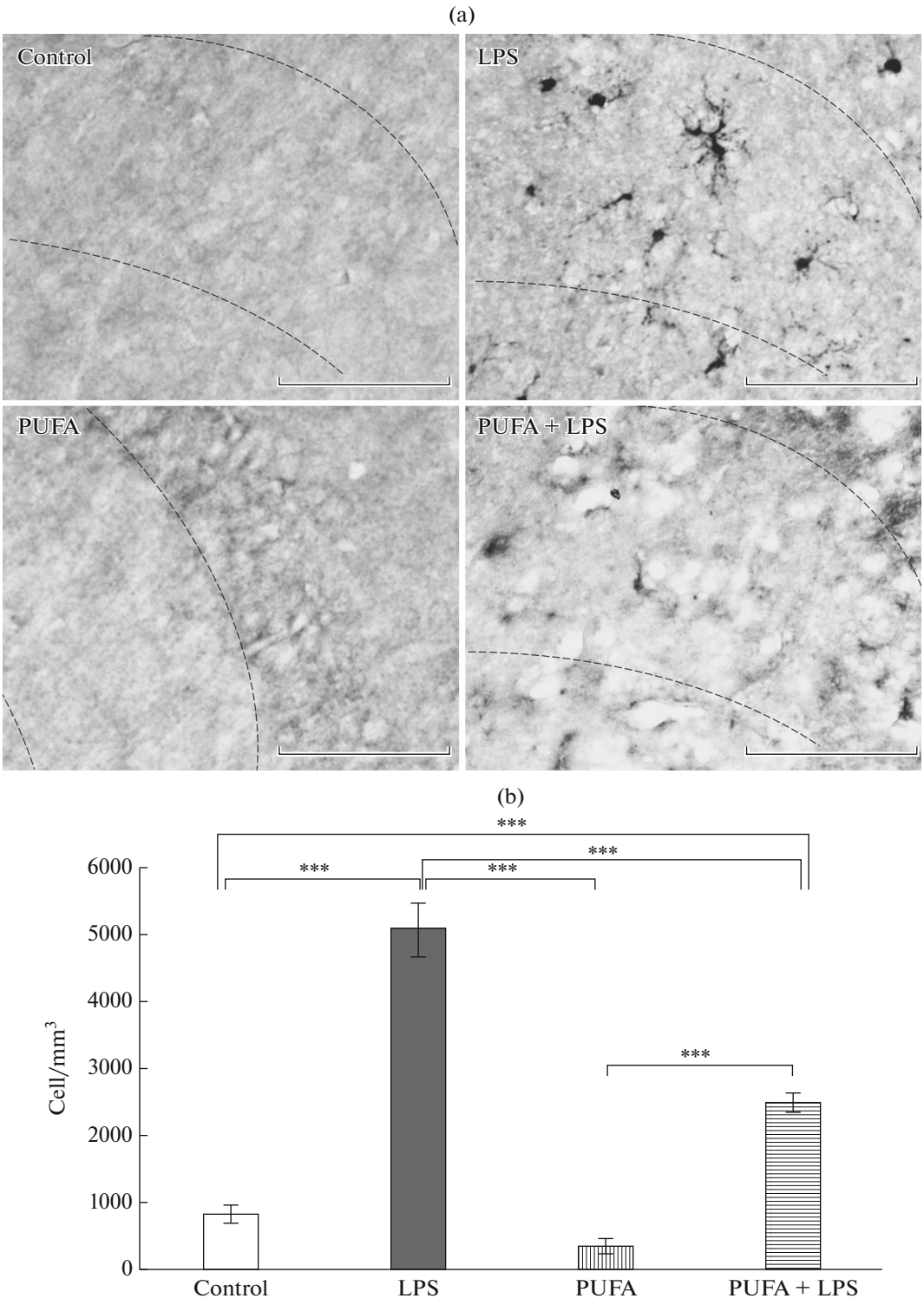

Fig. 5. Expression of IL-1β in the hippocampus. (a) Typical images of immunohistochemical staining of CA1 region of the hippocampus; scale, 400 μm; (b) average number of IL-1β-positive elements; *** $p = 0.001$.

matory reaction, restore damaged tissues and cognitive functions. In this respect, ω-3 PUFAs, in particular DHA and EPA are a promising group of compounds. A number of studies have linked the consumption of marine products rich in ω-3 PUFAs to a lower incidence of neurological diseases. A large quantity of data that confirm the anti-inflammatory properties of ω-3 PUFAs in diseases of peripheral tis-

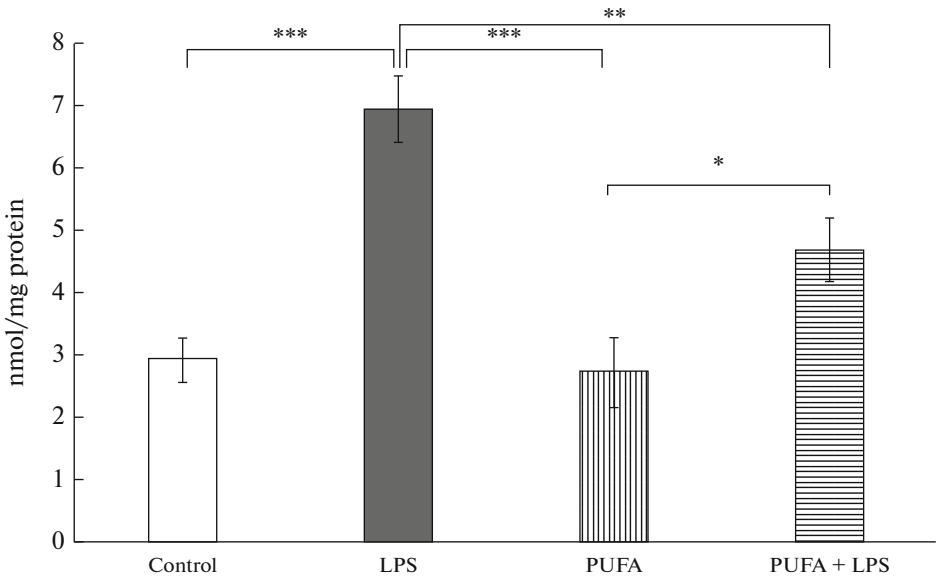

Fig. 6. The concentration of malonic dialdehyde in the hippocampus, nmol/mg protein, $x \pm \sigma$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

sues has been obtained [23]; however, their participation in neuroinflammatory processes has been less studied. In this study, we demonstrated a decrease in the intensity of neuroinflammation after oral administration of a DHA- or EPA-containing preparation. Administration of ω-3 PUFAs inhibited the development of micro- and astrogliosis in the hippocampus with neuroinflammation, while maintaining normal morphological characteristics of microglia and astrocytes. However, in the group that did not receive ω-3 PUFAs, LPS administration resulted in an increase in the number of glial cells, an increase in the areas of their bodies and a retraction of the processes. Activation of microglia is a key stage in the development of neuroinflammation, hence, a decrease in the degree of its activation limits the production of a large number of pro-inflammatory cytokines, hinders chronic inflammation, and facilitates the resolution of this process. Thus, in the presence of fatty acids, the increase in the number of IL-1β-positive cells in the hippocampus was less pronounced than in the group not treated with PUFAs. We note that IL-1β-positive cells had morphological signs of microgliocytes, which indicates the activation of the microglia of the proinflammatory (M1) phenotype. This indicates the ability of ω-3 PUFAs to suppress the inflammatory response at the early stages, when activated microgliocytes begin to synthesize proinflammatory cytokines.

However, glia are known to be the main source of ROS during oxidative stress [24]. ROS excess inevitably leads to LP activation, which, in turn, contributes to disruption of the integrity of cell membranes and destruction of membrane proteins [25]. In this study, induction of neuroinflammation led to a significant increase in the MDA concentration in the hippocampus, which reflects an increase in the LP intensity. The administration of PUFAs prevented an increase in MDA, which points to the antioxidant activity of the compounds administered. An increase in the LP intensity in the central nervous system may lead to a number of negative consequences that contribute to the disruption of synaptic plasticity and the development of cognitive deficits. As an example, an increase in the ROS concentration and the LP intensity may lead to the inactivation of membrane receptors and enzymes involved in cellular homeostasis, such as glucose-6-phosphatase and Na^+/K^+-ATPase. In mitochondria, both matrix enzymes and respiratory chain components may be affected. Damaged membranes lose their energy potential, excitability and the ability to control ion currents, which causes pathological changes. In particular, intensive Ca^{2+} transport into the cell may be a consequence of oxidative stress and reflects disruption of membrane integrity, as well as abnormalities in the functioning of the plasmalemma Na^+/Ca^{2+}-exchanger and Ca^{2+}-ATPase in the internal mitochondrial membrane [26]. An increase in intracellular Ca^{2+} concentration, in turn, is the cause of the activation of neuronal NO-synthase, phospholipase A2 and calpains (a family of Ca^{2+}-dependent proteinases), which leads to the destruction of the cytoskeleton and other membrane proteins. Activation of A2 phospholipase leads to an excess release of arachidonic acid from the phospholipids of cell membranes, thereby causing a change in the physical and chemical properties of membranes and leading to a disruption in long-term potentiation and, as a consequence, memory and learning ability [27]. Changes in synaptic plas-

ticity during neuroinflammation can develop as a result of disturbance of the acid–base balance of the nervous tissue and a decrease in intracellular pH resulting from the development of oxidative stress, disruption of functioning of mitochondria and transmembrane ion carriers [28]. Thus, we assume that the normalization of locomotor activity and working memory in the presence of PUFAs is due to reduced micro- and astroglial activation, a decrease in the intensity of neuroinflammation and oxidative stress and, as a consequence, prevention of changes in the physicochemical properties of cellular and mitochondrial membranes.

The study of the fatty acid composition of the cell membranes of the hippocampus and the cerebral cortex of experimental animals suggests that the chosen fatty acid dosage regimen allows optimal DHA accumulation in the brain. The most important index that determines the degree of accumulation of ω-3 or ω-6 PUFAs in the brain is the ratio between the DHA concentration and the concentration of arachidonic acid (AA). Researchers have demonstrated a positive correlation between the DHA/AA ratio and cognitive functions and a negative correlation between this ratio and the formation of apoptosis products after administration of β-amyloid [29]. The increase in the DHA/AA ratio in our studies reflects partial replacement of arachidonic acid with docosahexaenoic acid in the phospholipids of the brain. However, DHA and EPA serve as substrates for cyclooxygenases and lipoxygenases, which reduce the intensity of oxidation of arachidonic acid and the formation of proinflammatory mediators such as prostaglandins and thromboxanes [30]. However, the intensity of formation of anti-inflammatory mediators, oxidized derivatives of DHA and EPA, protectines and resolvins increases. Oxidized derivatives of DHA are able to reduce the expression of CD14 and TLR4 on the surface of cells, thereby suppressing the inflammatory response.

CONCLUSIONS

Our data help to explain the behavioral changes observed during neuroinflammation and substantiate the effects observed upon the administration of ω-3 PUFAs. The study showed that drug based on DHA and EPA may effectively prevent the development of neuroinflammation and normalize cognitive functions.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of interest. The authors declared no conflict of interest.

Ethical approval. All experimental manipulations with animals have been approved by the commission on biomedical ethics of the National Scientific Center of Marine Biology FEB RAS.

Informed consent. This article does not contain any studies with human participants performed by any of the authors.

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